On marginal bone resorption around oral implants

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To Victoria, Ivar, Adrian, and Ebba, to my Mother and Father

This thesis is number 57 in a series of investigations on implants, hard tissues and the loco-motor apparatus originating from the Department of Biomaterials, University of Gothenburg, the Department of Prosthodontics/Material Sciences, University of Gothenburg, the Department of Prosthetic Dentistry/Material Sciences and Department of Oral & Maxillofacial Surgery and Oral Medicine, Malmö University.

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On marginal bone resorption around oral implants

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ABSTRACT

The etiology of marginal bone loss (MBL) around osseointegrated implants remains controversial. Despite extensive research, the initiating causes of MBL are unclear. Long-term clinical studies challenge the notion that peri-implantitis, an infectious condition resulting in accelerating MBL over time, is the primary driver, as most implant failures occur early and decline over time. This thesis investigates alternative etiological factors for MBL, focusing on immune-mediated and iatrogenic causes.

Study I systematically reviewed animal models for peri-implantitis, resulting in a qualitative synthesis of 133 studies and a meta-analysis of 35 studies. Plaque accumulation alone did not induce relevant MBL after up to 1.5 years. When artificial ligatures were added, as in 119 studies, MBL varied significantly depending on ligature materials and ligature application methods. The possibility that the ligature itself, due to its provocation of the immune system, could induce MBL, although refuted by some authors, was not tested in any of the reviewed ligature studies.

Studies II and III examined possible immune-mediated MBL from aseptic ligatures in rabbit femurs and tibia using histology and qPCR, comparing silk and cotton ligatures to controls over 8 weeks (silk and cotton) and 12 weeks (silk). The studies confirmed significant MBL from ligatures at 8 weeks ($p=0.007$), with partial recovery at 12 weeks. At both time points, inflammatory infiltrates dominated by macrophages and foreign body giant cells surrounded all ligatures and were separated from resorbed bone surfaces by fibrous enclosure. M2 macrophages balanced inflammatory responses.

Study IV compared chair-side cement-fitted (CR) and screw-fitted (SR) implant restorations over 1 year in 24 patients in a cross-over RCT design divided into three periods (P) of 16 + 16 + 20 weeks. Patients were randomly assigned to SR (group 1) or CR (group 2) at baseline. Restoration type was then swapped during P2, and during P3, both groups had SR. Radiographic MBL and clinical peri-implant indices were measured at baseline, 16, 32, and 52 weeks. The immunological response was assessed by qPCR on peri-implant soft tissue biopsies at 32 weeks. MBL was observed during periods with CR in both groups, and bone gain was observed during periods with SR. Differences in MBL were significant between groups during P1 ($p = 0.006$) and P2 ($p < 0.001$) and within group 2 during P1 ($p = 0.002$). Further, a 7-fold upregulation of IL-6 was found from CR after P2. Excess cement was found in 75% of all CR cases upon removal.

In summary, this thesis demonstrates that immune-material interactions beyond the presence or absence of infection regulate MBL. Further, MBL recovery may occur after removing iatrogenic factors such as excess cement.

Keywords: biological complications, marginal bone loss, foreign body reaction, ligature-induced peri-implantitis, aseptic loosening

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SAMMANFATTNING PÅ SVENSKA

Benförlust runt tandimplantat är ett omdebatterat ämne inom tandvården. Trots omfattande forskning är orsakerna fortfarande inte helt klarlagda. En vanlig uppfattning är att benförlust främst beror på periimplantit – en infektionssjukdom som leder till en gradvis accelererande benförlust. Samtidigt visar långtidsstudier att bennivåerna kring implantat kan variera över tid, att de flesta implantatförluster sker första åren och att risken minskar med tiden. Detta tyder på att andra faktorer kan spela en betydande roll.

För att undersöka alternativa orsaker till benförlust genomfördes fyra studier. Studie I är en systematisk litteraturöversikt av 133 djurstudier om periimplantit. Där framkom att plackansamling runt implantat inte orsakade mätbar benförlust ens efter 1,5 år. Däremot uppstod omfattande benförlust när bomulls- eller silkestrådar (ligaturer) placerades i implantatens motsvarighet till tandköttsfickor – en metod som användes i 89 % av studierna. Trots att flera forskare kopplade benförlusten till plack snarare än ligaturerna, fann vi inga undersökningar som bekräftade detta. Det gick därför inte att utesluta att ligaturerna i sig triggade benförlust genom en främmandekroppsreaktion.

För att testa detta genomfördes två djurstudier (Studie II och III) där sterila ligaturer av bomull och silke applicerades runt implantat i lår och underben på kaniner, utan plackansamling. Efter 8 och 12 veckor observerades både benförlust och inflammatoriska reaktioner, men även viss spontan benåterväxt vid 12 veckor. Resultaten ifrågasätter tillförlitligheten i studier där ligaturer används för att simulera periimplantit.

I Studie IV, en klinisk studie med 24 patienter, jämfördes cementerade och skruvförankrade implantatkronor under ett års tid. Samtliga patienter testade båda krontyperna i slumpmässig ordning. Under perioder med cementerade kronor uppstod benförlust, men vid byte till skruvförankrade kronor observerades istället benåterväxt. När de cementerade kronorna avlägsnades noterades cementrester i 75 % av fallen. Studiens resultat antyder att benförlust kan orsakas av inflammatoriska reaktioner mot cementöverskott i vävnaderna.

Sammantaget visar projektet att benförlust kring implantat kan orsakas av inflammatoriska reaktioner mot olika material, att bennivåerna kan variera över tid och att benet ofta återhämtar sig efter avlägsnande av cementrester.

LIST OF PAPERS

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

I. **Reinedahl D**, Chrcanovic B, Albrektsson T, Tengvall P, Wennerberg A. Ligature-Induced Experimental Peri-Implantitis-A Systematic Review. J Clin Med 2018; 7(12):492.

II. **Reinedahl D**, Galli S, Albrektsson T, Tengvall P, Johansson CB, Hammarström Johansson P, Wennerberg A. Aseptic Ligatures Induce Marginal Peri-Implant Bone Loss-An 8-Week Trial in Rabbits. J Clin Med 2018; 8(8):1248

III. **Reinedahl D**, Galli S, Albrektsson T, Tengvall P, Wennerberg A. Aseptic Silk Ligatures Induce Bone Resorption Around Titanium Implants: A 12-Week Pilot Study in Rabbits. Int J Oral Maxillofac Implants. 2024 Oct 16;39(5):755-764.

IV. **Reinedahl D**, Gjelvold B, Chrcanovic B, Galli S, Wennerberg A, Naenni N. Clinical and immunological effects of cemented and screw retained implant restorations - A prospective crossover study. In Manuscript. (List of authors preliminary)

CONTENTS

ABBREVIATIONS

16S rRNA	16S Ribosomal RNA
ACTβ	Beta actin
ALPL	Alkaline phosphatase
ANOVA	Analysis of variance
ARG1	Arginase 1
BMU	Basic multicellular unit
BOP	Bleeding on probing
C5aR1	Complement C5a Receptor 1
CAL	Clinical attachment level
CD4	T-cell surface glycoprotein CD4
CD8	T-cell transmembrane glycoprotein CD8
CD11β	Cluster of differentiation 11 beta
CD19	Transmembrane glycoprotein CD19
CR	Chair side cemented implant restoration
CTSK	Cathepsin K
DAMP	Damage-associated molecular pattern
ELISA	Enzyme-linked immunosorbent assay
FBR	Foreign body reaction
FGF2	Fibroblast growth factor 2
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase

IFN-γ	Interferon gamma
IL1β	Interleukin 1 beta
IL6	Interleukin6
IL8	Interleukin 8
IL10	Interleukin 10
LDHA	Lactate dehydrogenase A
LPS	Lipopolysaccharide
M1	Pro-inflammatory macrophage
M2	Anti-inflammatory/pro-reparative macrophage
MAC1	Macrophage-1 antigen
MBL	Marginal bone loss
MCP1	Monocyte chemoattractant protein-1
MNGC	Multinucleated giant cell
mRNA	Messenger ribonucleic acid
NCF1	Neutrophil cytosolic factor 1
NOX2	Nicotinamide adenine dinucleotide phosphate oxidase 2
OC	Osteocalcin
OPG	Osteoprotegerin
OsteoMacs	Osteal macrophages
P	Period
PI	Plaque index

PCR	Polymerase chain reaction
PPD	Pocket probing depth
qPCR	Quantitative polymerase chain reaction
RANK	Receptor activator of nuclear factor-κB
RANKL	Receptor activator of nuclear factor-κB ligand
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SR	Screw retained implant restoration
TGFβ	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
TRAP	Triiodothyronine receptor auxiliary protein
VEGF	Vascular endothelial growth factor

1 INTRODUCTION

1.1 THE HISTORY OF OSSEOINTEGRATION

Osseointegration was discovered by Brånemark [1], a late professor of anatomy at the University of Gothenburg, after a series of experiments with long-term in vivo microscopy of bone and marrow. It was discovered that the implanted titanium chambers used for the microscopy integrated with rabbit bone and could not be removed without causing bone fracture. Initially, his novel finding was applied to anchor bone grafts to reconstruct segmental osseous bone defects in a closed (sterile) environment. Successful transmucosal experiments ensued in a series of dogs with oral implants, with maintained osseointegration over 10 years [1]. In 1965, Gösta Larsson of Gothenburg, Sweden, became the first human treated with Brånemark's implants for edentulism. This treatment did necessitate some exchanged implants, but it remained fully functional until he passed away in 2006.

Brånemark's invention was initially met with staunch resistance [2] due to its transmucosal application, which exposes implants to the contaminated oral cavity. Previous failures of other transmucosal devices—including subperiosteal implants, vitreous carbon implants, and blade-vent implants—likely fueled skepticism due to their high failure rates and complications, such as progressive marginal bone loss (MBL), osteomyelitis, and sensory nerve disturbances [3].

It was not until 1982, after a congress in Toronto where Brånemark presented his clinical results to an audience of about 100 clinicians from North American university clinics, that his implant treatment concept reached global acceptance.

Today, the tide has shifted, with osseointegrated implants commonly regarded as the state-of-the-art choice for rehabilitating edentulism. The treatment's popularity has grown so significantly that some dental professionals refer to themselves as "implantologists" or "specialists in implantology." This is a remarkable development, as it reflects a focus on a product rather than an organ or disease, which is otherwise customary in healthcare professions.

1.1.1 ORIGINAL INTERPRETATION OF BIOLOGICAL MECHANISMS

Following its discovery, the process of osseointegration was rigorously studied using the methods available at the time. Since the implants were not rejected, it was concluded that they must be bioinert. For several decades, this unexpected tissue response was widely regarded as a natural bone-healing process. It was attributed to the presumed bioinert properties of osseointegrating materials, especially Ti, which was believed to evade cellular detection and systemic effects [4].

In line with the assumed bioinert host-implant relationship, signs of inflammation in the transmucosal tissues were exclusively attributed to infectious mechanisms [5].

Since its discovery, numerous research advances have significantly evolved our understanding of osseointegration. Two of the most critical realizations are that bone is an integral immune system component and that no biomaterial is truly bioinert [6]. The following sections will review both of these concepts.

1.2 BONE TISSUE IN HEALTH AND DISEASE

A TISSUE WITH MANY FUNCTIONS

Bone serves many functions: it maintains our body shape, protects critical organs, and enables us to move and walk upright. It is also integral to our hormonal and immune systems. Further, it serves as the development site for all cells in the peripheral bloodstream, including white blood cells for our immune system, red blood cells for oxygen transport, and platelets essential for blood clotting. Hence, bone is itself a vital organ. Given this, it is unsurprising that bone tissue responds rapidly to fend off threats that could compromise its integrity.

BONE MAINTENANCE IN HEALTH

In a healthy adult, bone resorption and formation are balanced, resulting in an annual turnover of approximately 8% in cortical bone and 18% in cancellous

bone [7]. This balance, known as osteoblast-osteoclast coupling, ensures bone formation matches resorption during homeostasis [8].

Coupling occurs within 1–2 million basic multicellular units (BMUs) distributed throughout the skeleton [9]. Within each unit, osteoclasts resorb bone, which takes approximately three weeks. Osteoblasts then form new bone, which takes 3 to 4 months to complete [8].

This dynamic process is essential for regulating blood calcium levels, maintaining bone strength and structure, and allowing the bone to adapt to mechanical stress while replacing old or damaged tissue [8].

SYSTEMIC AND LOCAL REGULATION OF BONE METABOLISM

The growing research field of osteoimmunology in the twenty-first century has significantly advanced our understanding of the immunological processes involved in bone homeostasis, pathology, and fracture healing [10].

Bone metabolism is regulated at both systemic and local levels. Systemically, various hormones influence bone turnover. For example, the loss of estrogen can lead to osteoporosis by increasing bone resorption through multiple pathways [11]. Locally, BMUs operate autonomously and are regulated by direct cell-to-cell interactions between osteoblasts and osteoclasts and cytokines shared with the immune system [8].

Beyond specific bone cells, mounting evidence shows that macrophages, innate immune cells, directly regulate bone metabolism. These macrophages are present within BMUs as tissue-resident osteal macrophages (OsteoMacs) [12]. During bone fracture healing, OsteoMacs and recruited macrophages are critical for the inflammatory phase and subsequent healing. Their depletion has been shown to completely inhibit callus formation, highlighting their essential role [13,14]. Notably, macrophages are also key cells for regulating foreign body reactions (FBRs) to biomaterials [15].

On a molecular level, bone remodeling is tightly regulated through the RANK-RANKL-OPG axis. RANKL, a pro-inflammatory cytokine in the TNF family, promotes osteoclast formation and activation by binding to the RANK receptor on monocyte/macrophage progenitor cells. To counteract excessive bone resorption, OPG binds to RANKL and prevents its interaction with RANK [8,16]. An increased RANKL/OPG ratio indicates disrupted bone metabolism,

as observed in patients with rheumatoid arthritis experiencing active bone erosion, and in osteoporotic patients with pathological bone fractures [17,18].

LOCAL BONE RESORPTION

Inflammation and local bone resorption are interconnected through shared signaling pathways and molecular mediators [19]. Notably, RANKL is produced not only by osteoblasts but also by B- and T-cells, as well as gingival and synovial fibroblasts, in response to various stimuli [20-22]. As a result, local bone resorption may occur when inflammation is sustained in its immediate vicinity, potentially serving an evolutionary purpose to protect bone from danger [23,24].

Bone resorption can be triggered by various factors, including autoimmune diseases, infections, wear particles, specific materials, or damage-associated molecular patterns (DAMPs) released from damaged cells or tissue [6,23]. The immunological response typically follows a similar pattern regardless of the triggering agent. Inflammation is initiated and sustained through a cascade of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, which, in turn, activate RANKL and can ultimately lead to bone resorption (Figure 1) [23,24].

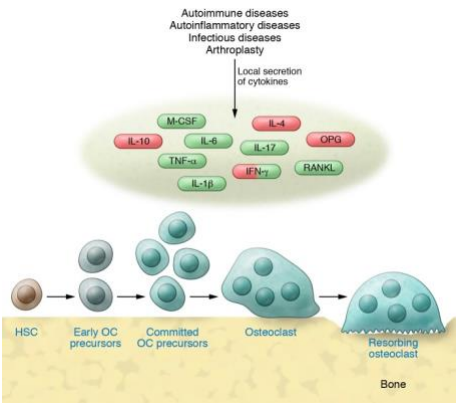

Figure 1. Regardless of the triggering agent, sustained inflammation near bone can result in bone resorption when the effects of pro-inflammatory cytokines (green) outweigh those of anti-inflammatory cytokines (red). This figure was originally published by Mbalaviele et al. (2017) and is used here with written permission courtesy of Dr. Teitelbaum.

When inflammation becomes excessive, bone resorption can progress rapidly. Under these conditions, overexpression of RANKL can drive OsteoMacs and

recruited macrophages to fuse quickly into osteoclasts, significantly accelerating bone destruction (Figure 2) [25].

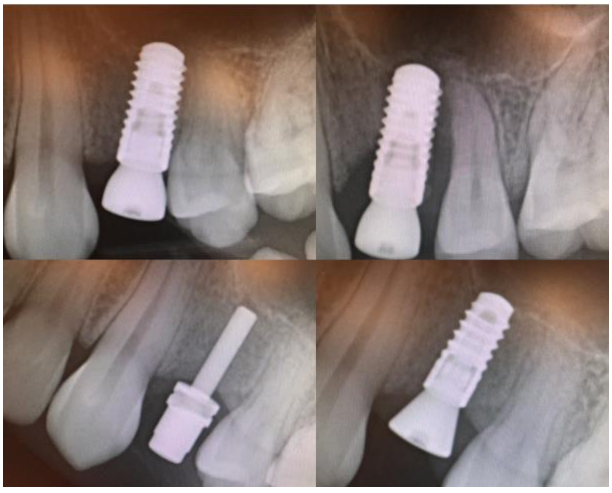

Figure 2. A healthy 24-year-old patient presented with early implant failure and significant bone resorption 4 weeks after insertion, likely due to suboptimal placement involving the periodontal ligament of the adjacent tooth. The referral suggested removal of the implant and tooth 25, followed by bone grafting. The patient reported pain but showed no clinical signs of infection. The implant was removed without debridement to preserve the root cementum of the adjacent tooth. After 3 months of spontaneous healing, a new implant was placed optimally. This case highlights bone's rapid resorption in response to threats and its remarkable healing capacity.

Once a threat has been eliminated or a soft tissue capsule shields the resorbed bone surface, local bone resorption typically ceases. At the molecular level, anti-inflammatory cytokines, such as IL-10 and IL-4, are released by anti-inflammatory/pro-reparative macrophages (M1), while osteoclasts secrete TGF-β and IGF-1 as part of a negative feedback loop. Together, these signals promote the upregulation of OPG production in osteoblasts, thereby haltering further bone resorption by osteoclasts [23,26]. On the clinical level, consider a chronic periapical lesion where bone resorption may be arrested long-term due to a dense fibrous capsule that separates the bone from the infiltrate [27].

1.3 OSSEOINTEGRATION FROM AN IMMUNOLOGICAL PERSPECTIVE

FUNDAMENTAL PUBLICATIONS FOR A SHIFTED PERSPECTIVE

It was not until 2014 that Albrektsson et al. [28] began investigating the immunological mechanisms underlying osseointegration and its complications. In a self-critical review, they assessed osseointegration in the context of up-to-date knowledge about foreign body responses from other areas of biomaterials research. They also revisited overlooked observations from implant studies conducted by Donath et al. in the 1990s [15,29,30].

Based on the reviewed literature [28], the authors concluded that the thick, cortical, and nearly avascular bone that forms around implants over time is likely an immune-mediated condition, functioning to isolate the implant from the bone marrow for protective purposes. Conversely, they suggested that marginal inflammatory reactions may vary over time, driven by immunological responses to implants or restorative materials and influenced by changes in both local and systemic conditions [28].

Their findings align with Karl Donath, the pioneer of the sawing-grinding technique, which remains a standard method for histological observation of undecalcified bone [31]. In their review of FBRs to materials ranging from wartime shrapnel to oral implants, Donath et al. [29] observed that all the materials they investigated triggered FBRs of various types. Although the specific manifestations of these reactions differed, they consistently appeared to function as defense mechanisms, aiming to either reject, dissolve, or resorb the materials. Alternatively, the body would isolate the material by encasing it in bone or a dense fibrous capsule, depending on the type of adjacent tissue. They found that implants and other foreign materials were often in direct contact with bone tissue (i.e., osseointegrated). At the same time, macrophages and MNGCs were present in areas devoid of bone [29]. In another review, Donath [30] found that marginal bony pocket formations around implants were sometimes not associated with inflammation and, in other cases, involved granulomatous infiltrates similar to those found in FBRs in closed soft tissue environments [30].

The work of James M. Anderson's group has also been fundamental in shaping the approach to immunological investigations of osseointegration. In an exhaustive review, cited over 5,000 times, Anderson et al. [15] provided

substantial evidence that FBRs are initiated immediately upon material interaction with tissue, regardless of the material type. Moreover, they observed that the FBR persists in various forms for the entire duration of the implant's presence in situ. Macrophages, whether acting alone or fused into MNGCs, along with their interactions, were found to play a crucial role in the FBR. Additionally, the characteristics of the implant's surface significantly influenced the nature and extent of the response [15].

In the same review, the authors provided an intriguing example illustrating how pacemakers failed due to the long-term consequences of FBRs when the plastic used to insulate the leads cracked after a few years due to immune-mediated degradation. This highlights the importance of thoroughly investigating and accounting for FBRs in response to different materials [15].

MACROPHAGES – KEY CELLS FOR HEALING AND FBR

Macrophages are derived from differentiating monocytes. They are part of the innate immune system and are key regulators of wound healing, bone fracture healing, and FBRs in the short and long term [14,15,32].

In response to DAMPs from a fresh wound, neutrophils and monocytes of the innate immune system will rapidly invade the wound site. Monocytes then differentiate into pro-inflammatory (M1) macrophages, which work alongside neutrophils to clear out debris via phagocytosis and ROS/RNS degradation.

Macrophages further regulate inflammation and recruit additional immune cells by secreting pro-inflammatory cytokines such as IL-1β, IL-6, IFN-γ, and TNF-α [33]. The intensity and duration of inflammation correlate with the extent of surgical trauma—more traumatic procedures trigger more potent and prolonged inflammatory responses [34].

At the end of the inflammatory phase, M1 macrophages transition to M2 macrophages in response to cytokines such as IL-4, IL-13, IL-10, and TGF-β. In this new role, they regulate early healing by secreting factors like VEGF, which promotes angiogenesis, and TGF-β, which activates fibroblasts—leading to callus formation in bone and granulating soft tissue healing [33,35]. A timely transition is crucial for effective healing. Recent studies suggest neutrophils are also involved in the early switching phase [36]. When switching is impaired, chronic wounds may develop, as seen in diabetic ulcers (Figure 3) [37].

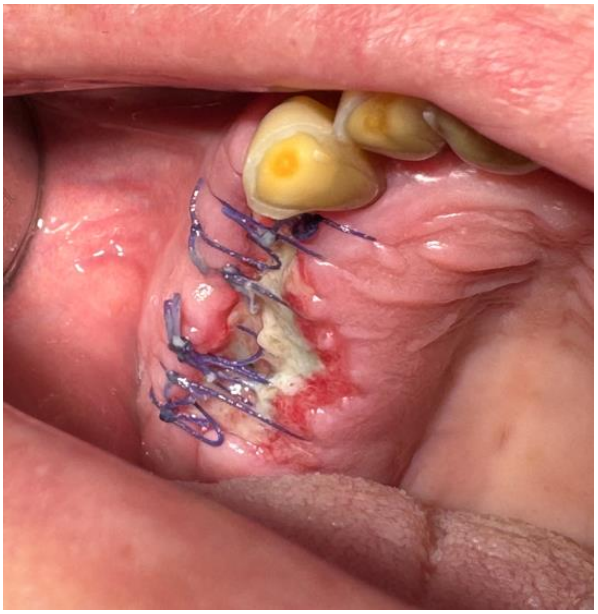

Figure 3. A wound dehiscence was detected one week after primary closure over implants. The consultation request, sent via SMS, proposed wound revision and closure with a mobilized flap. However, a medical evaluation was advised instead, revealing poor diabetes control. Following medication adjustment and suture removal, secondary healing was achieved within a few weeks.

During bone maturation, M1 and M2 macrophages work together to remove and replace callus tissue with mature bone [13].

When a biomaterial is added, the sequential M1/M2 switch is also regulated by the material's chemistry and surface characteristics [15]. Hence, a material that promotes inflammation will prolong the M1 response, possibly leading to frustrated phagocytosis, tissue damage, and implant failure. Conversely, excessive M2 activity can result in excessive fibrotic encapsulation [38]. In contrast to normal wound healing, regulating macrophages will remain in contact with implanted materials throughout their in situ lifetime [15].

IMMUNE REGULATION OF OSSEOINTEGRATION

The foreign body reaction to implant surfaces during osseointegration of oral implants is a new area of study. Nonetheless, interesting findings have emerged. For instance, Hotchkiss et al. [39] observed differences in immune responses to six implant surface types in vitro. When the implants were exposed to macrophages and mesenchymal stem cells (MSCs), a low-

inflammatory (M1) and high anti-inflammatory (M2) response from macrophages was positively associated with more significant osteoblast differentiation in MSCs at seven days, indicating that surface-dependent immunological reactions are involved in early osseointegration events [39].

Interestingly, a study on dogs found that the implant surface associated with the lowest M1/M2 ratio and highest osteoblast differentiation in Hotchkiss et al.'s study [39] also facilitated complete bone healing of artificial buccal bone defects within three months of implantation. In contrast, a surface eliciting a higher M1/M2 response resulted in dense fibrous tissue healing instead of bone regeneration [40].

Recent in vivo studies further underscore the role of the innate immune system in osseointegration. For example, Wang et al. found that macrophage depletion delayed osseointegration of Ti implants in rats [41].

Trindade et al. [42] found distinct immunological patterns around Ti implants compared to empty implant sites with selected qPCR markers and histology in experiments investigating the reactions to different implants compared to sham site healing [42]. They further observed different histological responses for other materials, each associated with distinct immunological reactions. Four weeks after implantation, Ti implants were osseointegrated, copper implants were fibrously encapsulated, and PEEK implants were embedded in fat tissue [43].

In a similar study with an extended healing period of 12 weeks, Reinedahl et al. [44] observed an anti-inflammatory response to titanium (Ti) implants and two modified PEEK surfaces compared to sham sites. The anti-inflammatory (M2) markers ARG1 and IL-10 were upregulated for all implants, while cytokine markers associated with M1 macrophages, T, and B cells were consistently downregulated around the implants. These findings suggest an M2-dominated immune response involved in osseointegration, effectively shielding the implants from excessive inflammation [44].

Notably, IL-10 production was also detected in circulating monocytes from patients with healthy implants, whereas no production was observed in a control group without implants. This finding further demonstrates long-term M2-associated regulation of osseointegration [45].

In light of the emerging evidence, Albrektsson et al. [6] recently proposed that osseointegration should be viewed as a foreign body equilibrium, emphasizing the role of constant and sustained innate immune regulation in its maintenance. Given that innate immunity is characterized by its rapid response and that its primary function is protecting the body rather than the implant or its osseointegrated state, the immunological research approach can hopefully help us better understand why bone is sometimes gained and sometimes lost [6].

1.4 CLINICAL IMPLANT TREATMENT

1.4.1 SUCCESS CRITERIA

IMPLANT LEVEL

In 1986, Albrektsson et al. [3] suggested that for an implant to be successful, it should not be mobile, have any radiographic peri-implant radiolucency, have an annual MBL of less than 0.2 mm after the first year of loading, and not cause persistent pain, infection, or paresthesia. Back then, a success rate of 85% and 80% at 5 and 10 years was considered acceptable in cohorts [3].

PATIENT LEVEL

Papaspyridakos et al. [46] identified four primary levels of success criteria for implant treatments reported in the literature. These were implant level, peri-implant soft tissue level, prosthetic level, and patient satisfaction level [46]. Most frequently reported outcomes in the literature were at the implant level, such as mobility and pain, followed by peri-implant soft-tissue parameters like suppuration and pocket probing depth (PPD). Few studies reported prosthetic and patient satisfaction factors. Therefore, the authors concluded that the implant prosthetic complex should be viewed as a whole [46].

Neglecting to report prosthetic and patient satisfaction factors, such as prosthetic failure and the ability to chew, may divert the results of scientific reports away from patient needs. For example, the loss of a single implant may be detrimental to the patient and clinically challenging in the single crown esthetic zone case or have little to no effect in the full arch case if the remaining implants can carry the prosthesis load. Likewise, a history of MBL may cause severe dissatisfaction with the appearance in the esthetic zone, but often not in

the posterior regions. Hence, prosthetics and patient satisfaction parameters may better identify treatment needs than those related to implants or peri-implant soft tissue.

PERI-IMPLANT HEALTH-RELATED CRITERIA

According to recent guidelines from the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [47], peri-implant health is established if: 1. Bleeding in probing (BOP) is absent, except traumatic bleeding dots caused by excessive probing force. 2. Pocket probing depth is ≤ 5 mm in general, and pocket depth does not increase over time. 3. Radiographic MBL is absent following initial bone remodeling and/or ≤ 2 mm after loading. 4. There are no visual signs of inflammation [48]. The authors recommended that implants be visually inspected and probed at least once yearly to ensure continuous health [47,48].

In the same conference, inflammation in the peri-implant mucosa, verified by BOP^+ or suppuration, was considered a sole diagnostic criterion for peri-mucositis, or peri-implantitis if associated with progressive loss of supportive marginal bone, verified by MBL following initial bone remodeling [47,49].

In clinical outcome studies, peri-implant soft tissue parameters retrieved from probing, like PPD, BOP, clinical attachment level (CAL), and suppuration, are often reported [46,50] as diagnostic markers for peri-mucositis (inflammation limited to the peri-implant mucosa) and peri-implantitis (peri-mucositis and MBL) [51].

The interpretation of clinical findings from probing is heavily debated in the literature. For example, in an exhaustive review, Coli and Sennerby [52] identified several problems related to the registration, interpretation, and prognostic value of probing findings [52]. First, probing is technique-sensitive, with the outcome depending on the amount of pressure applied [53]. Further, a pathological cut-off value for PPD is hard to establish since the mucosal thickness varies profoundly from the outset of implant treatment, and the fact that pockets up to 9 mm have been found at long-term stable implant sites after 18 years of function [54,55].

For BOP, Lekholm et al. [56] found no association between BOP^+ and histological signs of infection in subsequent biopsies [56]. Further, Weber et al. [57] found that the cumulative power of 6 clinical parameters to predict

MBL, including suppuration, CAL, BOP, PPD, and plaque index (PI), was only 2,8% at 1 year, 4.4% at 3 years, and 14.3 % at years 5 in a prospective study on 112 tissue level implants. They concluded that "low levels of correlation between the individual and cumulative clinical parameters with radiographically measured bone loss suggests that these parameters are of limited clinical value in assessing and predicting future peri-implant bone loss" [57].

In their review, Coli and Sennerby [52] found some scientific support for profuse bleeding or suppuration as risk factors for future MBL based on a retrospective study with 4,500 implants followed for up to 10 years [58]. Hence, they suggested that pus drainage from bi-digital palpation around implant sites and visual signs of redness and swelling may be more valuable predictors of future MBL than probing parameters [52].

1.4.2 CLINICAL OUTCOME

PATIENT SATISFACTION

Based on patient VAS satisfaction questionnaires, two studies reported that patients are generally very satisfied with their implant treatments. The studies reported >80% and >90% complete treatment outcome satisfaction at 1 and 10 years, respectively [59,60]. When matched with clinical parameters, one study found that experiencing implant failure and mechanical complications was most negatively associated with satisfaction [60].

IMPLANT SURVIVAL AND MBL

Long-term studies have shown excellent implant survival rates with modern, moderately rough surfaces, achieving 95–100% survival over 10 years or more [61]. These surfaces outperform turned surfaces, particularly in the maxilla, where moderately rough surfaces significantly reduce the risk of early and late failures [62,63].

Studies with very long follow-up periods show that most implant failures occur early, within the first years of function, and that the risk of failure decreases over time [64-66]. In a large dataset of 4,585 consecutively treated full arches with up to 30 years of follow-up, Jemt [64] reported that 43% of all implant

failures occurred during the first year and 16% during the second year, followed by a reduced rate [64].

This pattern was also observed in a study of 2,915 consecutively treated partially edentulous jaws with 8,645 implants, where 45% of all failures were recorded in the first year, 29% during years 1-5, and 25% during years 5-30 [65]. Similarly, Chrcanovic et al. [66] reported that 35% of implant failures occurred during the first year and 26.8% during the second and third years in a cohort of 227 patients with 1,045 implants followed for ≥20 years. Only 17.8% of failures occurred after 10 years, and 2.4% were recorded between 20-35 years [66].

While implant survival is a critical outcome, its relationship with MBL is nuanced. For example, a systematic review of 62 publications found that one moderately rough surface type had more MBL than all others but the best survival rate after 10 years or longer. Conversely, turned implants had less MBL than all others but the highest failure rate [62].

Another systematic review of 31 publications observed no relevant differences in MBL between turned and modified surfaces over 5-30 years. Instead, observed differences were within the expected crestal resorption patterns of the reviewed implant types [67].

Interestingly, some data suggest that late implant failures from progressive MBL are quite rare. For example, one retrospective study found that only 30% of failed implants exhibited severe MBL in the last radiogram before failure in patients followed for 20 years or more [66]. Additionally, peri-implantitis treatment was found to have limited effectiveness in preventing further bone loss or improving survival in the long run [68].

While MBL may differ between implant systems and treatment protocols, its extent does not necessarily predict failure. Further, suggestions that moderately rough surfaces are more prone to developing progressive MBL from harboring more plaque than smooth surfaces have not stood the test of time [69].

1.5 MARGINAL BONE LOSS

According to the World Health Organization (WHO) International Classification of Diseases, 10th Revision, 2nd ed. (ICD-10), both early and late infections, along with inflammatory reactions to implanted devices, are classified under code T85, “Complications of other internal prosthetic devices, implants, and grafts”. Progressive MBL with peri-implant inflammation falls under subcategory T85.6, "Infection and inflammatory reaction due to other internal prosthetic devices, implants, and grafts” [70].

By categorizing implant-related adverse conditions as complications instead of diseases, the focus shifts to the original treatment provided. Using this universal classification may encourage clinical self-reflection when addressing biological complications. Such consideration is crucial for revision treatments to prevent repeating the suboptimal aspects of the initial procedure. Moreover, it would align oral implant diagnostics with broader Swedish and international healthcare practices.

Further, adhering to the ICD-10 system would support a multifactorial approach to MBL, acknowledging that its causes are not always clear. Jemt [71] identified 86 scientifically discussed factors that may cause or contribute to MBL. These factors encompass a wide range of aspects, from material characteristics and treatment execution to host factors and patient compliance [71]. The following sections will explore some of these factors.

1.5.1 MBL FROM IMMUNOLOGICAL REACTIONS AGAINST MATERIALS

ASEPTIC LOOSENING OF ORTHOPEDIC IMPLANTS

In a review, Albrektsson et al. [72] found that oral implants may be more similar to orthopedic implants than natural teeth. Indeed, they are both conceived from engineering and surgical trauma rather than evolution. Further, they both lack a periodontal ligament [73].

Orthopedic implant failure most often results from aseptic loosening, as seen in 56.0% of all hip revision surgeries in Sweden from 1999 to 2019 [74]. Aseptic loosening results from inflammatory responses to wear particles generated from materials used in implants such as polyethylene, Ti, cobalt-

chromium alloys, or cementum. These small particles are phagocytosed by macrophages, triggering the release of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, followed by up-regulation of RANKL and bone resorption [75,76].

PARTICLE AND MATERIAL-INDUCED MBL AROUND ORAL IMPLANTS

Even though aseptic loosening is a non-infectious complication, it may still prove relevant for oral implants in a contaminated environment. Wear debris's potent capacity to induce inflammation and bone resorption in the orthopedic setting motivates the question of whether it can do the same in the oral cavity. Indeed, recent studies have found wear particles in most investigated peri-implantitis lesions [77-79]—for instance, Wilson et al. [77] found Ti and/or cementum particles surrounded by inflammatory cells in 34 of 36 biopsies [77].

Eger et al. [80] found that ultrasonic scaling of modified Ti surfaces generates more particles than turned surfaces. When added to cell cultures containing bone marrow-derived macrophages, the sterile particles from a sandblasted surface induced the most substantial inflammatory upregulation of the pro-inflammatory cytokines IL1β, IL6, and TNFα, followed by sandblasted/acid-etched and turned surfaces. Additionally, when 0.01 μg/ml lipopolysaccharide was compared with particles from sandblasted/acid-etched surfaces, it triggered a 40–70-fold greater inflammatory response and increased osteoclastogenesis in the presence of RANKL. In vivo, particles from sandblasted surfaces induced severe bone resorption in a mouse calvarial model, with increased osteoclast and inflammatory cell activity [80]. Their findings may have clinical relevance for how implant surfaces are cleaned and possible inflammatory iatrogenic complications. For instance, one could speculate that closed debridement may generate more trapped particles than open flap debridement would do.

Kheder et al. [81] further explored the role of Ti particles in peri-implantitis. Their biopsy-based study compared mucosa retrieved from implant tops at abutment connections, implants with peri-implantitis, and healthy gingiva using whole-genome transcriptome analysis and immunohistochemistry. While Ti particles were found in both healthy and diseased implants, they were significantly more abundant in cases of peri-implantitis. Tissue from affected implant sites showed an overproduction of inflammatory cytokines and an osteoblast/osteoclast activity imbalance that favored bone resorption. In vitro validation confirmed a concentration-dependent activation of macrophages by

Ti particles, leading to elevated production of pro-inflammatory cytokines such as IL-18, IL-1β, IL-8, and CCL3. Interestingly, healthy implants also displayed FBRs, although a more balanced type, with moderate inflammatory cytokine levels regulated by abundant IL-10 production [81].

In her recent doctoral thesis, Olander [82] provided valuable insights into the effects of wear particles on peri-implant tissues. In a retrospective study involving 132 patients with 174 implants, zirconia abutments were associated with greater MBL than titanium (Ti) abutments annually and at the 5-year follow-up. Interestingly, MBL also varied depending on the restorative dentist, whereas factors such as BOP or general hygiene did not appear to impact [83].

In a subsequent study, biopsies were obtained from the peri-implant mucosa surrounding four zirconia abutments and five Ti abutments in nine patients at their 5-year follow-up. Wear particles were detected in all biopsies but were more abundant in the zirconia group. Further, qPCR analysis revealed (>2-fold) upregulation of several pro-inflammatory genes, including RANKL, in the zirconia group [84].

The above findings suggest an ever-ongoing immunological surveillance in the marginal tissues of both sick and healthy implants. The expression of this FBR appears to be associated with the choice of material and is prone to change over time in response to generated wear debris. Hence, the association between materials and particles with MBL should be further studied.

INTRAORAL CEMENTATION

Reviews on the effect of intraoral cementation of implant restorations on MBL have shown mixed results. One systematic review of 59 publications found MBL >2 mm in 2.8% of intraorally cemented crowns and 0% of screw-retained crowns. However, screw-retained single crowns were associated with more technical complications, whereas partial and full arch cases had similar outcomes [85]. In contrast, another review on 14 prospective multicenter studies using the same implant system found less MBL in cemented cases at 3 and 5 years [86].

In patients with confirmed cement excess, MBL and inflammation can be expected in the majority of cases. For example, Linkevicius et al. [87] observed an additional MBL of over 1.5 mm after the first year of loading and clinical signs of peri-implantitis in 42 of 73 investigated implants with cement excess.

Further, each of the 39 implants with excess in patients with a history of periodontitis was associated with peri-implantitis [87].

Cementum excess may occur in most intraorally cemented cases despite meticulous removal techniques—for example, Kim et al. [88] found residual cement on 73% of 109 cemented single restorations upon removal and a 3.66-fold higher risk for excess in cases with a submucosal restoration margin [88]. Upon removal, a recent in vitro study found cementum residues on each of 20 cemented crowns in a soft tissue imitation model. Only one of the twenty cases was detected on radiographs. Resin-modified glass-ionomer cement generated a 7.4% greater residue area than resin cement [89].

Removal of cement excess led to resolved mucosal inflammation and no further MBL in most reported cases. For example, Korsch et al. [90] found adverse peri-implant effects of a specific methacrylate cement and, therefore, removed and cleaned all superstructures with that particular cement, irrespective of the current peri-implant status. They then re-cemented all superstructures with zink-eugenol. The intervention significantly reduced BOP at 4 weeks and 1 year in patients with and without excess cement. Further, a complete remission of suppuration was observed at 4 weeks in all cases and 17 of 20 implants at 1 year [90].

The mixed outcome of existing data on cementum suggests that it is technique-sensitive. It also remains unknown if different types of cement may act as inflammatory agents to induce MBL. Based on the above studies, avoiding cementation in patients with a documented history of periodontitis is advisable.

1.5.2 MBL AND FAILURE FROM TREATMENT-RELATED FACTORS

THE OUTCOME IMPACT OF EXPERIENCE AND VOLUME

Higher treatment volumes and years of surgical experience are generally associated with better surgical outcomes across all surgical procedures [91-99], particularly in complex cases [92,100].

In joint arthroplasty, the rate of loosening and other complications is highly dependent on the experience and volume produced by the team, where “each

successively higher hospital volume category manifested a lower complication rate" in a big U.S. register study [99].

Clinician-dependent failure and MBL rates have also been found in implant dentistry, but the publications are sparse and often based on older implant types that are no longer in use [101]. One study found that the 5-year implant cumulative survival rate of turned implants improved from 75% to 99% with increased surgical experience [102]. A similar outcome was observed for HA-coated implants, with an increased survival rate from 86.5% to 97.6% at 4 to 8 years [103].

In a more recent study on 2,915 consecutive treatments, Jemt [65] reported that the highest risk for implant failure was related to the treating surgeon [65].

For MBL, Bryant [104] found that the difference between surgeons and prosthodontists at the same clinic varied significantly [104]. Further, Derks et al. [105] found more implants with MBL over 2 mm in implants restored by general dentists compared to specialists among Swedish patients treated at different clinics and followed for 9 years [105].

The effect of different isolated treatment steps on MBL, such as direct implant installation, different loading protocols, 1-stage/2-stage surgery, or mucosal grafts to thicken the mucosa, are often inconclusive when systematically reviewed. Perhaps many reports better reflect the overall skill and experience of the clinician who provided the treatment rather than some specific method, as indicated by the above studies and a previous review [106]. However, daily experience in a referral practice reveals that most cases sent for revision were already compromised from the outset of the original treatment, as demonstrated in the cases shown in Figures 4-7.

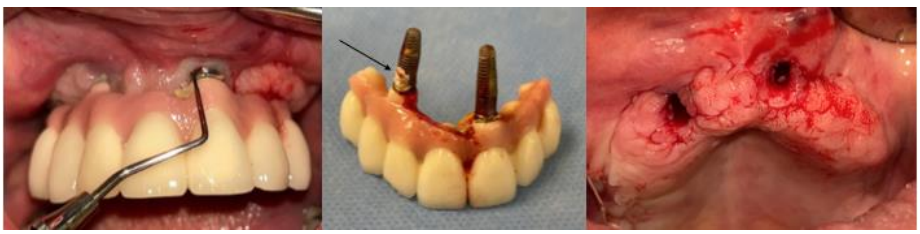

Figure 4. The case was referred for revision 10 years after the original treatment. The extension of the superstructure had been reduced in steps due to posterior implant failures. Cement (arrow) was likely pushed further into developing pockets during re-cementations. Consider the buccal position of implant platforms in the right picture.

Was the buccal bone ever sufficient? How did the posterior loose implants affect loading on the anterior ones? What was the primary reason for implant failures?

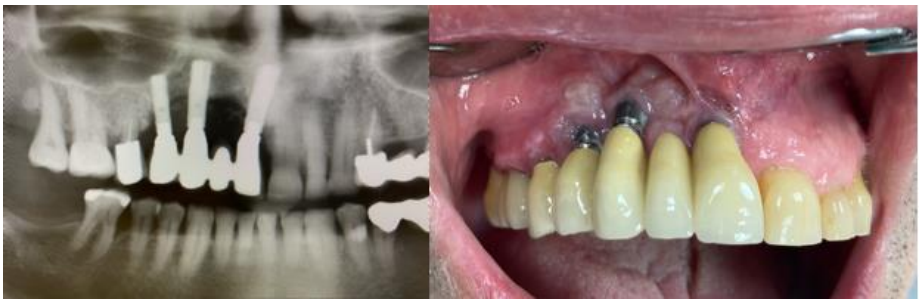

Figure 5. The case was referred for revision due to MBL. Consider the suboptimal implant placement with a buccal inclination and buccal/apical position of the implant platforms outside an original, imagined bony envelope. Again, was the buccal bone ever sufficient, and what was the primary reason for MBL?

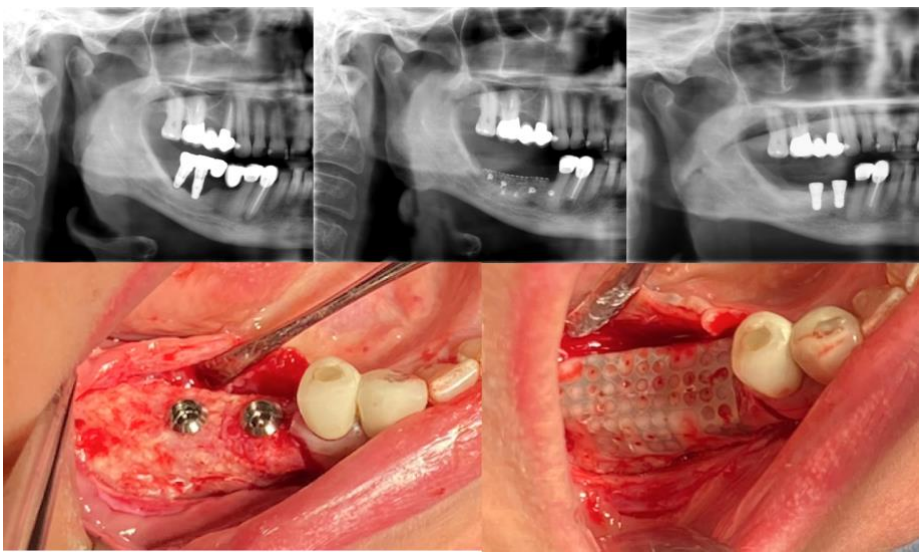

Figure 6. Revision case due to progressive MBL. Note the crestal bone resorption mesial to the implants. Were the implants originally placed supracrestally, or has the crest resorbed since insertion? Also, observe the loading conditions created by cantilever units on both implant- and tooth-supported restorations within the same quadrant and the narrow distance between the implants at the marginal level. The revision involved vertical and horizontal bone grafting, followed by the placement of tissue-level implants with their transmucosal portion partially submerged to account for expected minor resorption after vertical augmentation. The case was treated together with Dr. Henrik Nilsson.

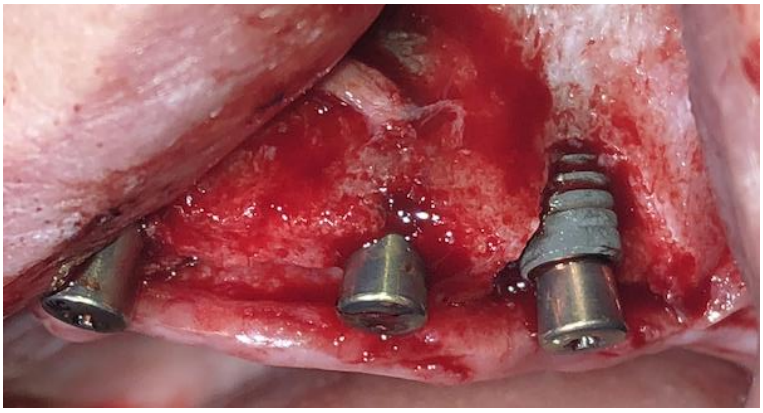

Figure 7. Marginal bone defect discovered during second stage surgery 4 months following implant insertion (abutments were connected right before the photo was taken). No bone regeneration was involved, the covering mucosa was intact, and no signs of infection were present. Dense fibrous tissue was found instead of bone upon discovery. How can we tell if bone resorption was arrested or progressive at the time of discovery? How many similar bone defects are left undetected following 1-stage surgery?

MBL FROM DIFFERENT TREATMENT PROTOCOLS

The impact of different treatment protocols on survival and MBL is perhaps best demonstrated by the outcome of the discontinued Nobel Direct one-piece implant. In a 1-year study of 117 such implants, Sennerby et al. [107] found that 7.9% failed when installed with an aggressive protocol involving in situ grinding of the abutment part and direct loading. In contrast, 0% failed when a conservative protocol was used. Further, the aggressive protocol generated a 2.6 mm mean MBL, while the conservative protocol generated 1.6 mm [107]. A similar outcome was found in a multicenter study [108]. Tissue damage from generated heat and inflammation from wear particles are possible reasons for the adverse result of the aggressive protocol. Also, the implant design was likely suboptimal, as evidenced by the MBL in the control group.

In implant treatment, a more compromised situation often calls for a more conservative approach as a general rule. This allows for a stepwise evaluation of each treatment outcome before proceeding further. For instance, healing responses following tooth extraction or bone grafting can provide valuable insights into the patient's overall condition and the treated site. Additionally, a cautious approach can help prevent unnecessary complications, such as the failure of a simultaneous marginal bone graft adjacent to an otherwise successfully osseointegrated implant.

FUTURE PERSPECTIVES

Future studies on implant failures and biological complications could identify and categorize suboptimal factors from the original treatment. This approach may help pinpoint specific reasons for the widely recognized variations in success rates among clinicians. Additionally, a rigorous failure analysis could optimize revision treatments for affected patients.

1.5.3 MBL FROM PERI-IMPLANTITIS

ETIOLOGY

Similar to the progression of gingivitis into periodontitis, it is believed that peri-mucositis serves as a precursor to peri-implantitis [51]. However, in the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, researchers admit that "the histopathologic and clinical conditions leading to the conversion from peri-mucositis to peri-implantitis are not completely understood" [49]. Prospective studies on the initiation and progression of peri-implantitis have not been conducted for ethical reasons; thus, current knowledge is primarily based on cross-sectional clinical studies and animal experiments. Both conditions are classified as infectious diseases [49].

PREVALENCE

In a cross-sectional study, Derks et al. [109] used only radiograms to assess the onset and progression of peri-implantitis, assuming an infectious cause across all identified cases [109]. Other authors used various levels of radiographic MBL with BOP^+ to define peri-implantitis, resulting in a prevalence of up to 43% of implants in single studies [110,111] and 22% in a systematic review [112]. Peri-mucositis, considered a precursor to peri-implantitis [113], has been shown to increase over time, resulting in peri-mucositis diagnoses in 75-90% of implants and 100% of patients at 10 years in individual studies [110,111,114].

Such high rates of "progressive disease" are inconsistent with the long-term implant survival and MBL data discussed above. Instead, these studies indicate a reduced risk of implant failure over time, pointing to false positives. As previously discussed, an overestimation of cases with progressive MBL may be partly due to the low predictive power of periodontal indices for MBL

[52,57]. Indeed, the consistently high rates of BOP^+ around implants suggest that it is the expected biological response rather than a clear sign of disease [115].

The preferred cross-sectional study design may also contribute to overestimating actual disease [110]. In these studies, "progressive MBL" is often discerned from some degree of MBL observed in a radiograph taken several years after baseline, coupled with BOP^+ [49]. Considering that the majority of implant sites may bleed when probed and that most MBL occurs within the first years of loading, many cases of arrested MBL in a steady state are likely to be falsely regarded as progressive with that study design.

Clinical protocols that produce false-positive diagnoses can have significant negative consequences for patients. Affected individuals may endure the emotional distress of being incorrectly labeled with a disease. More critically, they risk undergoing diagnostic and therapeutic procedures that can be painful [116] or non-beneficial [52,68,80,117].

In a multicenter study involving 1,162 consecutive recall patients, MBL was measured since the last annual radiogram instead of the baseline level. The study observed a peri-implantitis prevalence of 1.2% at the implant level. Pathological MBL was defined as a yearly rate exceeding 0.2 mm since the last radiograph, following the criteria established by Albrektsson et al. [3]. Also, all 14 affected implants had cemented restorations, the preferred fixation method in 71% of the patients in that study [118].

FUTURE IMPLICATIONS

Future studies may benefit from reporting patients with pain and/or profuse bleeding or suppuration at inspection or from palpation since these conditions require treatment to relieve symptoms. As previously suggested, these parameters may also be valuable for predicting future MBL [52,58].

Further, true endpoints such as the loss of prostheses, implant failure, or having undergone surgical intervention should be included to measure the actual patient suffering and healthcare burden associated with peri-implantitis.

ANIMAL MODELS

Without prospective studies on the onset and progression of peri-implantitis, our understanding of its etiology relies heavily on animal studies. When

studying biofilm-related peri-implant conditions, undisturbed plaque formation by cessation of oral hygiene measures is arguably the most honest method [119]. While such studies have consistently shown increased peri-mucositis and a microbial shift toward a more anaerobic flora, they have failed to produce clinically relevant MBL [120,121]. For example, Ericsson et al. noticed no ongoing bone resorption around Brånemark implants after 3 months of plaque accumulation in 5 beagle dogs despite "marked signs of inflammatory changes" in the peri-implant mucosa [122]. Saito et al. noticed no MBL around Brånemark implants and adjacent teeth after 6 months of plaque accumulation in four mongrel dogs [123]. Watzak et al. [121] looked at three types of implants in 9 baboons, each with 12 implants. They found that all implants survived and maintained high marginal bone levels within the expected range of physiological remodeling despite severe peri-mucositis after 1.5 years of plaque buildup and loading. Additionally, the study lacked a control group with plaque management [121].

THE LIGATURE MODEL

To accelerate MBL around implants, most preclinical studies utilize submarginal ligatures to induce bone resorption [119,124]. This approach, known as the "ligature-induced experimental peri-implantitis model" or simply the "ligature model," was adapted from the well-established "ligature-induced periodontitis model" used to stimulate bone resorption around teeth [125,126].

Cotton retraction cords and silk sutures are commonly used as ligature materials. These are inserted into peri-implant pockets to promote plaque accumulation. However, both materials are known to trigger significant FBRs.

Research outside the field of dentistry has repeatedly demonstrated sterile FBRs to silk and cotton. For example, silk sutures have been associated with persistent granulomatous inflammation [127], while cotton has been linked to osteolytic, tumor-like lesions [128]. For instance, one study reported "a remarkable higher number of foreign body giant cells that characteristically spread from the periphery into (silk) implants" compared to polypropylene implants in vivo [127]

The first ligature-induced peri-implantitis experiment identified on PubMed was published in 1991. Hickey et al. [129] found increasing CAL loss during 45 days with subgingival 4-0 silk ligatures and plaque accumulation in two micropigs [129].

A subsequent study by Lindhe et al. [130] found that the mean MBL around implants in five beagle dogs was 3.2 mm after six weeks, with submarginal cotton ligatures replaced at three weeks with new ligatures pushed into the bottom of the growing marginal bone defects [130]. The ensuing MBL was described as a tissue response to submucosal infection rather than the ligature itself [130]. Since the initial studies, the artificial use of ligatures has heavily dominated experimental animal research on MBL and, in contrast to undisturbed plaque accumulation, usually causes big bone craters around implants in just a few months [119,124].

Despite their demonstrated effectiveness for inducing MBL around implants, the researchers generally did not consider an isolated effect of different ligature materials. For example, Lindhe et al. [130] acknowledged that ligature removal "converted an "active", progressive periodontitis into a "resting" non-progressing lesion" in experiments on teeth [131]. At the same time, they dismissed the ligature itself as a potential artificial cause for MBL without looking into its possible one-off effect [130]. Instead, the authors referred to an experiment from 1966 that used ligatures to induce periodontitis in germ-free and normal rats [132]. None of the teeth in either group developed MBL in that study, but it was still used as proof that ligatures cannot induce MBL without plaque build-up [130,132].

Subsequent preclinical studies make similar claims without investigating a potential ligature effect. For example, Carcuac et al. [133] claimed that "while a ligature made of cotton or silk may not induce bone loss by itself, the developing inflammatory process in the connective tissue that results from the biofilm formation mediates tissue destruction during the experiment" [133].

In a year 2000 review of the model, Baron et al. [124] suggested that MBL could potentially result from aseptic mechanisms and argued that "bone resorption might occur in the immediate vicinity of the ligature by the ligature itself." [124] Martins et al. [119] made a similar argument in a 2014 review, stating that the traumatic action of ligatures may independently contribute to MBL. This is particularly true when ligatures are frequently replaced or added to, pushing new ligatures to the bottom of existing pockets [119].

Admitting to some differences between ligature- and undisturbed plaque-associated tissue breakdown, more recent experiments have included a period of spontaneous progression after ligature removal, which permits undisturbed plaque accumulation to occur between ligature removal and analysis. These

experiments have shown a quicker progression of bone resorption for modified surfaces than for turned surfaces in some experiments, along with worse outcomes after surgical intervention [134,135].

Over the years, the ligature model has been used to study a wide range of variables related to MBL, including but not limited to pathophysiology [129,130,133,136-140], different implant types and surfaces [69,135,136,141-146], peri-implant microbial composition [147-151] , and various treatment strategies, such as antimicrobial [135,152-154] and tissue-regenerative interventions [155-158].

Even preclinical conclusions have proven challenging to draw from published ligature experiments. For instance, Solderer et al. [159] concluded that "it is still impossible to answer the question of which implant types are more preservative and which are more prone to cause an infection in preclinical settings" in a systematic review of 36 experiments in dogs [159].

For clinical relevance, Stavropoulos et al. [67] recently published a systematic review. While moderately rough implant surfaces demonstrated more spontaneous MBL than turned surfaces after ligature removal in preclinical studies, this pattern could not be confirmed in clinical studies [67]. Instead, clinical studies with follow-ups ranging from 5 to 30 years showed similar MBL outcomes [67].

When considering the results of preclinical studies on plaque and ligature-induced peri-implantitis, along with the known biological and immunological effects of common ligature materials, one might wonder whether plaque can induce MBL without a ligature rather than the other way around. Validation of this method is long overdue.

2 AIMS

The general aim of this thesis was to explore potential iatrogenic causes for MBL around oral implants in experimental and clinical situations.

Specific aims were:

- To review existing animal models used to induce experimental peri-implantitis around oral implants and investigate the amount of MBL they produce and to what extent they have been validated to represent relevant clinical conditions. (Study I)

- To investigate the eventual immunological response to ligatures used in peri-implantitis experiments and their potential to induce MBL in aseptic environments. (Study II)

- To investigate if MBL and its associated inflammatory/immunologic response progresses or attenuates after prolonged exposure to aseptic ligatures. (Study III)

- To compare intraorally cemented and screw-fixed dental implant restorations by investigating their clinical, radiographic, and immunological effects. (Study IV)

3 MATERIALS AND METHODS

The first part of the thesis consists of a systematic review of animal models used to induce MBL. (Study I)

The second part consists of two in vivo studies that explore and validate the ligature model and investigate potential aseptic, artificial causes for MBL and peri-implant inflammation in response to the ligatures per-se, rather than the plaque traditionally accumulated on them. (Study II-III)

The third part is a prospective clinical crossover study comparing radiographic MBL and soft tissue response to chair-side cemented (CR) and screw-retained (SR) single implant restorations. (Study IV)

3.1 SYSTEMATIC REVIEW (STUDY I)

3.1.1 SEARCH STRATEGIES

Searches were performed in the databases PubMed/Medline, Web of Science, and ScienceDirect, with the following terms:

(((dental implant) OR oral implant)) AND (experimentally induced periimplantitis) OR experimentally induced peri-implantitis) OR experimental periimplantitis) OR experimental peri-implantitis) OR ligature induced periimplantitis) OR ligature induced peri-implantitis) OR ligature) OR plaque induced periimplantitis) OR plaque induced peri-implantitis) OR plaque accumulation) OR mechanical overload) OR bacterial inoculation)

In addition, a manual search of related journals was conducted, and the reference lists of identified studies and relevant reviews on the subject were scanned for possible additional studies.

3.1.2 INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria covered in vivo studies on experimentally induced peri-implantitis in animal species using any induction method. Studies that focused

only on periodontitis were excluded unless they also examined peri-implantitis. Research on mucositis around implants was not included.

3.1.3 STUDY SELECTION

Two reviewers independently read the titles and abstracts from the electronic searches. Full reports were obtained for studies that met the inclusion criteria or had insufficient data for a clear decision. Disagreements were resolved by consensus through discussion.

From the final included studies, the extracted data (when available) included publication year, animal species, number of animals, implant site, number of extracted teeth, implant healing period, study design, implant characteristics (shape, size, material, surface), number of implants per animal, surgical stages, time from abutment connection to peri-implantitis induction, use of randomization and loading, pre- and post-operative care, peri-implantitis induction method, ligature details, diagnostic markers, and peri-implant bone defect parameters. The authors were contacted for any missing data.

In addition, the reviewers also searched all included studies for experiments that investigated the isolated effect of different ligature types used to induce peri-implantitis.

3.1.4 ANALYSES AND META-REGRESSION

Descriptive statistics were used to report the data, standardizing vertical MBL across the finally included studies. The continuous outcome was vertical MBL, with the implant as the statistical unit. The untransformed proportion for vertical MBL was calculated using the random-effects DerSimonian-Laird method, accounting for variations in implant surfaces, animal species, and peri-implantitis induction methods, including different ligature regimens. Meta-regression analyzed vertical MBL using the induction period as a covariate, with significance set at $p<0.05$. Analysis was conducted using OpenMeta (Analyst) software.

3.2 IN VIVO STUDIES (STUDY II, III)

The in vivo studies used a modified version of the "ligature induced peri-implantitis model" to test the aseptic marginal bone and soft tissue reactions to commonly used ligature materials. A total of 16 New Zealand white rabbits were used for the experiments. In short, implants were installed in the femur and/or tibia of each rabbit under strict aseptic conditions. A single loop ligature of sterile silk or cotton chord was tied around the neck of each selected test implant, while contralateral pristine implants were used as control. The wounds were then closed in layers, followed by a healing time of 8 (study II) and 12 (study III) weeks, respectively. After sacrifice, clinical, histological, and immunological (with qPCR) analyses were performed on retrieved tissues.

3.2.1 MATERIALS

All implants (Ospol Regular, Malmö, Sweden) were made of commercially pure grade 4 Ti and measured 8 mm long and 4 mm in diameter. A minimally rough surface was selected to limit the surface area of titanium exposed to the tissues and, hence, the immunological reaction to the implant itself [39]. Silk (3-0 braided silk suture material, Ethicon, Cincinnati, OH, USA) and cotton (non-impregnated cotton gingival retraction cord GingiKNIT non-impregnated; Kerr Dental, Bioggio, Switzerland) chords were used as ligatures due to their common use in previous ligature studies.

3.2.2 STUDY DESIGN

Ten (n=10) rabbits were used for study II and 6 (n=6) for study III. In study II, six implants per rabbit were installed in the femur and tibia, according to Figure 8. The right and left legs were randomly alternated. Histological tissue reactions against silk versus cotton ligatures were compared in the femur. The same histological comparison was performed with silk ligature in the proximal tibia versus a non-ligated contralateral control implant. In the distal tibia, bone and marginal soft tissues from silk-ligated versus contralateral control implants were investigated with selected qPCR markers.

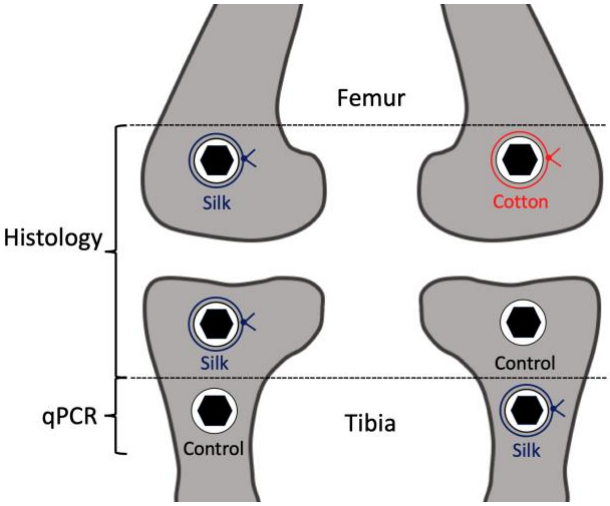

Figure 8. Overview of implant placement in study II. A total of ten rabbits (n=10) were included in the study.

In Study III, one implant was installed in each femoral leg of all rabbits. One randomly selected implant in each rabbit was ligated with silk, and the contralateral implant was used as a non-ligated control. All implants were investigated with both qPCR and histology (Figure 9).

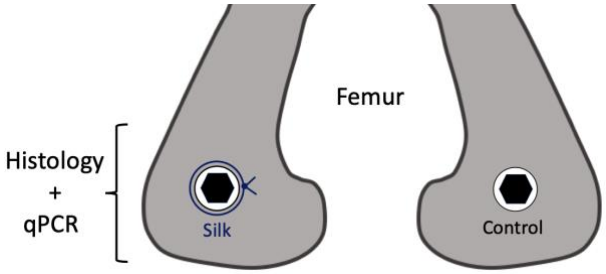

Figure 9. Overview of implant placement in study III. A total of 6 rabbits (n=6) were included in the study.

3.2.3 EXPERIMENTAL PROCEDURE

The surgical procedure is detailed in studies II and III of this thesis. In short, general anesthesia was induced, and analgesics were administered both pre- and postoperatively. Each implant site was shaved, disinfected, and isolated by a sterile surgical drape. After injection of local anesthesia, the implant sites

were exposed by a sharp incision through the skin and then periosteum, followed by periosteal elevation. Implant osteotomies were prepared with burs of increasing diameter under constant irrigation with sterile saline solution. After installing the implants halfway, single-loop ligatures were tied around the neck of selected implants with a surgical knot. The implant insertion was then finished, leaving the ligature compressed against the cortical bone plate. All wounds were closed in layers with interrupted, resorbable sutures. Rabbits were monitored for signs of infection, sickness, or poor condition throughout the healing period. The healing period was uneventful for all animals.

All animals were euthanized via intravenous injection of pentobarbital at 8 (study II) and 12 weeks (study III). The implant sites were surgically exposed and inspected for signs of infection, such as pus. In study II, the soft tissue (i.e., the periosteum) covering the implant tops was left untouched at the femoral and proximal tibial sites. These sites were then resected *en block* and processed for histology with cut-and-ground resin-embedded sections, as described in study II of this thesis.

The distal tibia sites were used for qPCR. The soft tissue in contact with implant margins was removed with a punch, and the marginal bone was then trephined out. Soft and hard tissue specimens were then processed with qPCR as described in detail in study II of this thesis.

In study III, implant sites were exposed similarly, but only a small piece of marginal soft tissue and ligature was removed for qPCR. The implant, surrounding bone, and covering soft tissue were then resected en bloc and histologically processed. Only soft tissue was processed for qPCR in this study.

3.2.4 OUTCOME MEASURES

- Clinical investigation - All implant sites were investigated for signs of infection at sacrifice, such as suppuration, fistulas, and edema.
- Quantitative histological investigation – All ground sections were investigated in a light microscope. The marginal bone level was measured from implant top to first bone contact on both sides (anterior and posterior). The first bone contact was ascertained with 40x magnification.
- Qualitative histological investigation - Marginal bone and soft tissue adjacent to implants and ligatures were examined.

- For studies II and III, marginal soft tissue was analyzed using qPCR, while marginal bone was also examined in study II. Table 1 lists the selected qPCR markers and their associated main biological entity.

Table 1. Selected qPCR markers and their main biological entity (Studies II and III).

Main biological entity	**Assay**
Bone mineralization	ALPL, OC
Bone resorption	CTSK, TRAP
Bone formation	ALPL, OC
Fibroblast	FGF2
M1 macrophage	CD11β, IL1β, IL6, TNFα, IL8
M2 macrophage	ARG1
Macrophage	MCP1
Neutrophils	NCF1
B Lymphocytes	CD19
T lymphocytes	CD4, CD8
Endothelial cells	VEGFα
The complement system	C5aR1
Reference gene	ACTβ, GAPDH, LDHA

3.2.5 STATISTICAL ANALYSIS

The difference between the distances from implant top to first marginal bone contact for test versus control implants was analyzed using a non-parametric Wilcoxon Signed Rank with the pair considered as the control and test samples from the same rabbit.

The distance between the ligatures and bone was used as a second measure of bone loss, considering that all ligatures were compressed against the bone at the study's outset.

Gene expression results were reported as calibrated normalized relative quantities (CNRQ). Mean and 95% confidence intervals were reported for each assay for each group. The difference in mean between the test and control groups in the soft-tissue and bone samples was analyzed using a non-parametric Wilcoxon Signed Rank Test, with the test and control samples from the same rabbit considered paired.

3.3 PROSPECTIVE CLINICAL CROSSOVER STUDY (STUDY IV)

The prospective clinical study compared chairside cemented (CR) versus screw-retained (SR) implant superstructures in the posterior upper or lower jaw during the first year of loading.

3.3.1 STUDY DESIGN

Twenty-four adult patients received a single Ti implant (Astra EV, Dentsply Sirona Implants, Mölndal, Sweden) in the premolar or first molar region, without bone augmentation, by one specialist in oral surgery. Patients were then randomized into two groups to receive either an SR or identical CR crown (Ti abutment, Atlantis® Abutment, Dentsply Sirona Implants, Mölndal, Sweden, and full-contour zirconia crowns) for 16 weeks (P1), then switched to the other type for another 16 weeks (P2). Finally, all patients received an SR crown for the last 20-week period (P3). One specialist in oral prosthodontics provided the prosthetic treatment.

3.3.2 OUTCOME PARAMETERS

Plaque index, BOP, PPD, CAL, and intra-oral radiographs for MBL measurement were taken at baseline, 16, 32, and 52 weeks. MBL was measured from the implant top to the first marginal bone contact point at each implant's mesial and distal side, and the mean value was considered. At week 32, peri-implant soft tissue biopsies were harvested and processed for qPCR. The selected qPCR markers are presented in Table 2.

Table 2. Selected qPCR assays and their main biological entity for study IV.

Main biological entity	Assay
Bone mineralization	ALPL, OC
Bone resorption	CTSK, TRAP, RANKL
Fibroblast	FGF2, TGFβ
Pro-inflammatory markers	CD11β, IL1β, IL6, TNFα, IL8, IFN-γ
Anti-inflammatory markers	ARG1, IL10
Macrophage	MCP1
Neutrophils	NCF1
T lymphocytes	CD4
Bacteria	16s rRNA
Reference gene	ACTβ, GAPDH, LDHA

3.3.3 STATISTICAL ANALYSES

Descriptive statistics included the mean, standard deviation, and percentages. Tests used were Kolmogorov–Smirnov (normal distribution), Levene's test (homoscedasticity), Student's t-test or Mann-Whitney (two independent groups, continuous variables), Pearson's chi-squared or Fisher's exact test (categorical variables), and Wilcoxon signed ranks test (longitudinal comparisons).

A two-way mixed ANOVA was performed with follow-up time as a within-subject variable and group as a between-subject variable to assess changes in CAL, PD, and BOP over time between groups, with Tukey post-hoc tests for differences. Statistical significance was set at $p < 0.05$.

The qPCR Gene expression was reported as calibrated normalized relative quantities (CNRQ) with means and 95% confidence intervals. The mean difference between groups was assessed using the DeltaDeltaCT method, normalized against three reference genes following the modified GeNorm algorithm.

4 RESULTS

4.1 SYSTEMATIC REVIEW (STUDY I)

The systematic search yielded the inclusion of 133 studies in the qualitative synthesis and 35 studies in the statistical analysis (Figure 10)

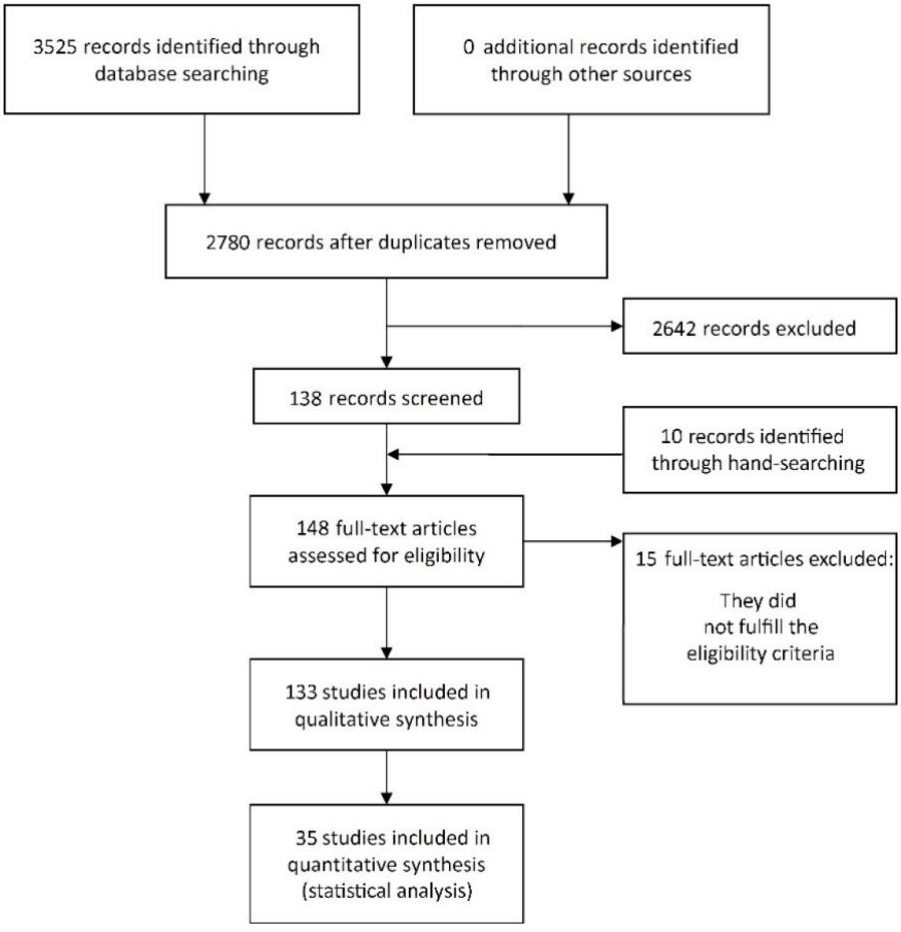

Figure 10. Reinedahl et al 2018. Overview of the study selection process for the systematic review's qualitative synthesis and statistical analysis.

4.1.1 QUALITATIVE SYNTHESIS (*N*=133 STUDIES)

EXPERIMENTAL ANIMAL SPECIES

The animals used included dogs (94 studies), monkeys (n=26), mice (n=8), micro- or minipigs (n=3), and rats (n=2).

METHODS USED TO INDUCE MBL

Eighty-nine percent (n=119) of the 133 studies used marginal ligatures to accumulate plaque and induce experimental peri-implantitis, sometimes in conjunction with other provocations such as occlusal overload or inoculation with porphyromonas gingivalis. Seven studies used a period of plaque accumulation alone, four occlusal overload, two bacterial inoculations, and one immunization and injection with lipopolysaccharide.

LIGATURE APPLICATION AND VALIDATION OF LIGATURE EFFECT

Ligatures were used for periods ranging from 7 to 660 days. Cotton was the most commonly used material, followed by silk, unspecified materials, combinations of different materials, metal wire, resorbable suture material, and dental floss. The ligatures were managed in one of three ways: (1) left in place for the entire duration of the experiment, (2) regularly replaced with new ligatures pushed to the bottom developing peri-implant pockets, or (3) incrementally added by placing new ligatures on top of the existing ones.

While some studies stated that ligatures—artificial, non-clinical elements—acted merely as carriers of plaque and could not induce MBL independently, the present authors found no experiment designed to verify these claims. Hence, the isolated ligature effect was not evaluated in the included studies.

4.1.2 STATISTICAL ANALYSES (*N*=35 STUDIES)

Estimated vertical MBL varied significantly for different ligature materials and ligature management. Cotton material and incrementally added ligatures generated the most MBL. The MBL also varied between animal species and implant surface types (Table 3).

Table 3. Reinedahl et al. 2018. Estimated vertical MBL in relation to various implant surfaces, animal species, method of induction, and ligature regimen.

Factor	MBR (in mm) (95% CI), *p*-Value, SE	Heterogeneity	Number of Measurements * Included for the Meta Regression (Number of Studies)
Surface			
Turned	2.265 (1.786, 2.745), $p < 0.001$, 0.245	$I^2 = 99.5\%$, $p < 0.001$	29 (21)
Acid-etched	2.864 (2.491, 3.237), $p < 0.001$, 0.190	$I^2 = 94.72\%$, $p < 0.001$	12 (6)
SB	2.509 (1.555, 3.463), $p < 0.001$, 0.487	$I^2 = 98.03\%$, $p < 0.001$	6 (5)
SB+F	1.697 (-0.640, 4.034), $p = 0.155$, 1.193	$I^2 = 98.94\%$, $p < 0.001$	3 (2)
SBAE	2.175 (1.658, 2.693), $p < 0.001$, 0.264	$I^2 = 98.44\%$, $p < 0.001$	21 (10)
SBAE/HA-coated	2.700 (2.174, 3.226), NA, 0.268	NA	1 (1)
HA-coated	2.349 (1.254, 3.444), $p < 0.001$, 0.559	$I^2 = 98.58\%$, $p < 0.001$	12 (7)
HA-plasma	1.650 (1.410, 1.890), $p < 0.001$, 0.122	$I^2 = 0\%$, $p = 0.683$	2 (1)
CaP-plasma sprayed	0.469 (-0.010, 0.949), $p = 0.055$, 0.245	$I^2 = 75.67\%$, $p < 0.043$	2 (1)
TPS	2.184 (1.523, 2.844), $p < 0.001$, 0.337	$I^2 = 98.16\%$, $p < 0.001$	13 (9)
Cancellous	1.932 (1.432, 2.431), $p < 0.001$, 0.255	$I^2 = 0\%$, $p = 0.990$	4 (1)
Anodized	3.462 (3.273, 3.651), $p < 0.001$, 0.097	$I^2 = 16.1\%$, $p = 0.311$	4 (2)
Overall	**2.295 (2.042, 2.548), $p < 0.001$, 0.129**	**$I^2 = 99.18\%$, $p < 0.001$**	**109 (35 **)**
Species			
Dog	2.389 (2.152, 2.626), $p < 0.001$, 0.121	$I^2 = 98.3\%$, $p < 0.001$	98 (30)
Monkey	1.649 (1.254, 2.044), $p < 0.001$, 0.202	$I^2 = 96.75\%$, $p < 0.001$	9 (4)
Rat	0.800 (0.393, 1.207), NA, 0.208	NA	1 (1)
Mouse	0.579 (0.549, 0.609), NA, 0.015	NA	1 (1)
Overall	**2.295 (2.042, 2.548), $p < 0.001$, 0.129**	**$I^2 = 99.18\%$, $p < 0.001$**	**109 (35 **)**
Method			
Plaque	0.689 (0.507, 0.871), $p < 0.001$, 0.093	$I^2 = 66.17\%$, $p = 0.007$	7 (2)
Inoculation	0.800 (0.393, 1.207), NA, 0.208	NA	1 (1)
Ligature			
Cotton	2.730 (2.478, 2.982), $p < 0.001$, 0.129	$I^2 = 97.88\%$, $p < 0.001$	66 (22)
Silk	1.683 (1.296, 2.070), $p < 0.001$, 0.197	$I^2 = 98.98\%$, $p < 0.001$	28 (8)
"Dental floss"	2.361 (1.675, 3.046), $p < 0.001$, 0.350	$I^2 = 86.05\%$, $p = 0.007$	2 (1)
Steel	2.607 (1.359, 3.856), $p < 0.001$, 0.637	$I^2 = 98.08\%$, $p < 0.001$	5 (2)
Overall	**2.295 (2.042, 2.548), $p < 0.001$, 0.129**	**$I^2 = 99.18\%$, $p < 0.001$**	**109 (35 **)**
Ligature regimen			
Only one	2.000 (1.647, 2.352), $p < 0.001$, 0.180	$I^2 = 98.91\%$, $p < 0.001$	37 (13)
Exchange	2.303 (2.030, 2.576), $p < 0.001$, 0.139	$I^2 = 97.89\%$, $p < 0.001$	48 (14)
New on top	3.123 (2.409, 3.838), $p < 0.001$, 0.365	$I^2 = 98.66\%$, $p < 0.001$	20 (6)
Overall	**2.395 (2.098, 2.620), $p < 0.001$, 0.133**	**$I^2 = 99.2\%$, $p < 0.001$**	**105 (33) *****

CI—confidence interval, SE—standard error, MBL—marginal bone loss, SB—sandblasted, SB+F—sandblasted + fluoride modified, SBAE—Sandblasted/acid-etched, HA—hydroxyapatite, NA—not applicable (there is only one study for this category), TPS—Titanium plasma-sprayed. * Some studies performed measurements of MBL in different implant surfaces, and/or different time points, and/or different methods. ** The total number of studies is always 35 for the factors "surface", "species", and "method", even though the sum of the studies for all categories under the same factor is higher than 35. The reason for this is that some studies performed measurements of MBL in different conditions, as explained in the footnote "*" above. An exception is made for "ligature regimen" (see footnote "***" below). *** The ligature regimen was not clearly informed in four measurements performed in two studies.

4.2 EXPERIMENTAL STUDIES (II, III)

4.2.1 CLINICAL RESULTS

All animals in both studies healed without complication and showed no signs of ailment throughout the healing time. Further, the tissue inspection during sacrifice revealed no signs of infection. While carefully removing the marginal soft tissue and ligatures from Study II implants selected for qPCR, small saucerization like bone defects were detected around the test implants but not on the control implants. An example can be observed in Figure 11.

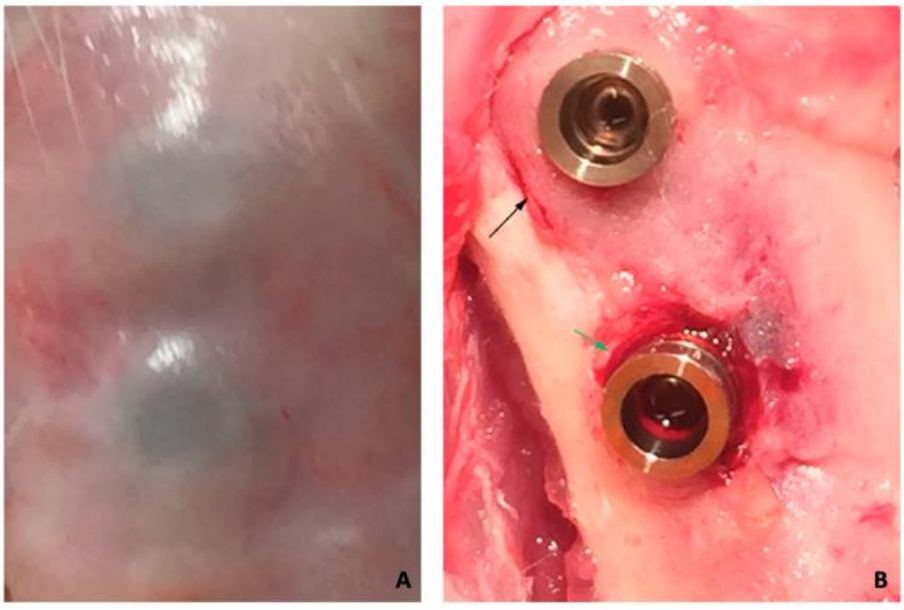

Figure 11. Reinedahl et al. (2019) Clinical photo from sacrifice at 8 weeks (Study II). A. Soft tissue covering two tibial implants, with no signs of infection. B. A small saucerization-like defect was observed around the inferior implant after soft tissue and silk ligature removal, involving the first thread of the implant. Some callus formation was visible around both implants, likely due to a tenting effect of the implants on the overlying periosteum.

4.2.2 HISTOMORPHOMETRIC RESULTS

In study II, the distance from the implant top to the first bone contact was significantly longer for silk-ligated implants compared to non-ligated controls ($p=0.007$) (Figure 12). The corresponding difference between silk and cotton-ligated implants was not significant ($p=0.37$).

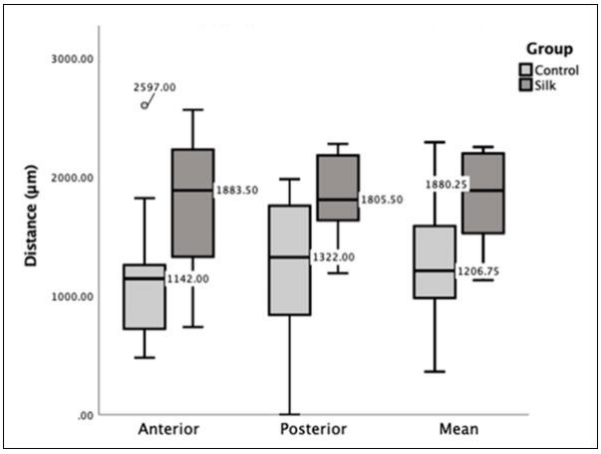

Figure 12. Reinedahl et al.(2019). Distance from implant top to first bone contact for 10 test (silk ligature) and 10 control implants (non-ligated) in the proximal tibia (Study II).

In study III, the distance from the implant top to the first bone contact was longer for test implants (silk ligature) than for controls (non-ligated). The difference was not statistically significant, likely due to the small number of included implants (Figure 13).

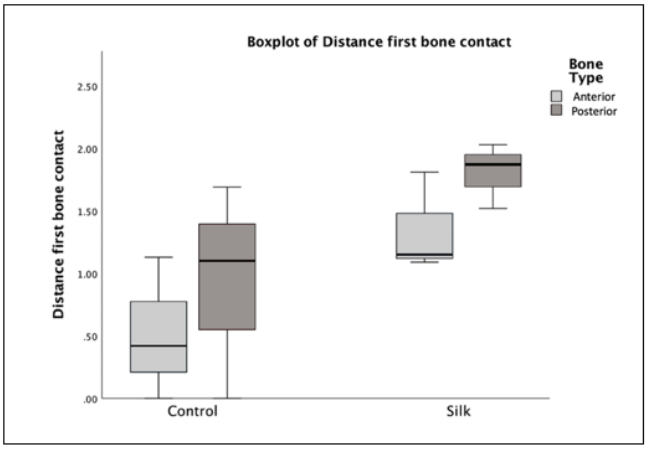

Figure 13. Reinedahl et al.(2024). Distance from implant top to first bone contact for 10 test (silk ligature) and 10 control implants (non-ligated) in the proximal tibia (Study III).

4.2.3 QUALITATIVE HISTOLOGICAL RESULTS

At 8 weeks (Study II), the silk and cotton ligatures (cotton only in Study II) were surrounded by resorbed bone surfaces at a distance from the ligatures. However, no osteoclasts were detected, indicating no ongoing bone resorption. In all cases, a fibrous tissue capsule separated the ligatures from the bone, and often, small marginal bone defects were observed around the implants, with cell infiltrates extending along the marginal implant surfaces. The soft tissue capsules contained numerous macrophages. Further, macrophages fused to MNGCs frequently formed elongated rims around the outer borders of the silk ligatures.

At 12 weeks (Study III), similar soft tissue capsules persisted, characterized by dense cell infiltrates outlining the ligatures and extending downward along the implant surfaces into small marginal bone defects. Further, elongated MNGCs frequently outlined the ligatures even at this time point. However, at this stage, thin layers of newly formed bone occasionally appear on the concave bone surfaces beneath the ligatures. Notably, a small neoformed bone knob was observed in contact with one of the ligatures, the only such occurrence in Studies II and III. These findings suggest a potential partial recovery of resorbed bone by 12 weeks.

By contrast, the test implants were fully osseointegrated at the marginal portion at both time points, with neo-formed bone occasionally extending over the implant tops.

Histological images from studies II and III can be observed in Figures 14 to 17.

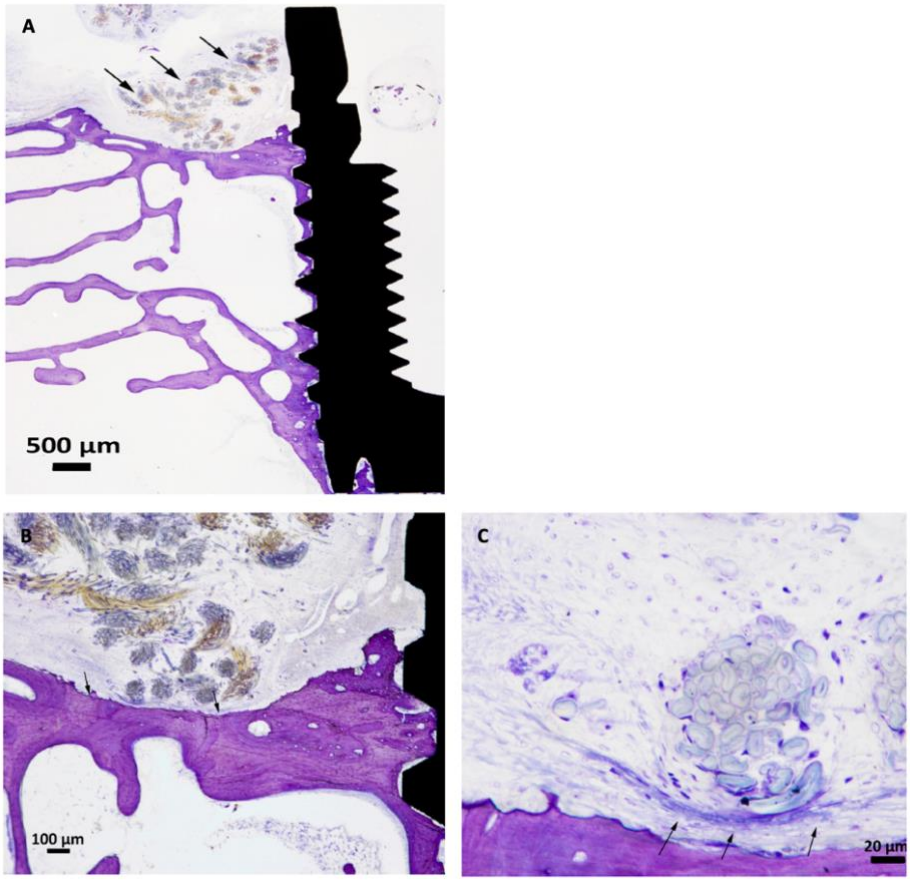

Figure 14. Cotton ligature at 8 weeks. A. A typical section shows the cotton ligature (arrows) positioned above the bone. B. MBL can be observed in the first two implant threads and poor osseointegration apical to the first marginal bone-implant contact. A soft tissue layer separates the periosteal bone surface from the cotton ligature, showing bone resorption (arrows), though no osteoclasts can be detected. C. The macrophage presence in the soft tissue between the bone and cotton appeared lower than in the silk sections. Reinedahl et al. (2019)

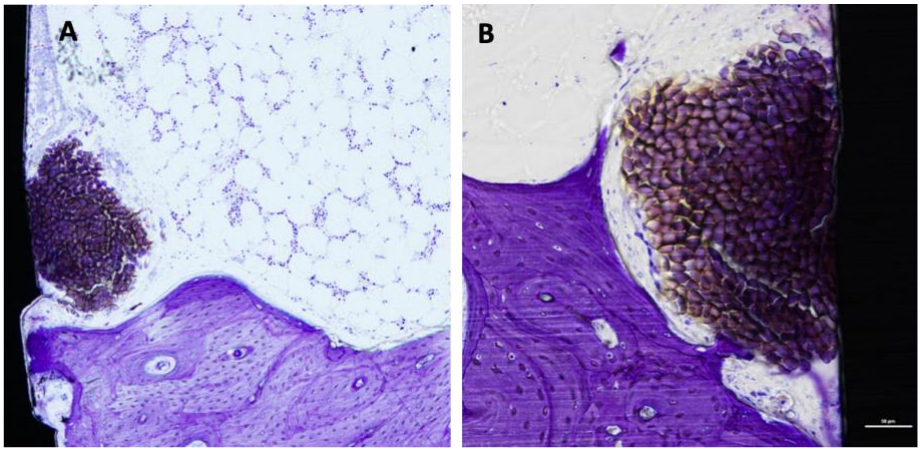

Figure 15. Two silk ligatures at 12 weeks A. A typical silk ligature specimen has distance osteogenesis between the implant surface and the bone inferior to the ligature, and cell infiltrates reach down along the implant surface along the first groove. Also, a thin neo-formed bone layer is present on the concave surface beneath the ligature. (B) A small, neo-formed "bone-nob" appears to be in contact with the ligature, the only case in studies II and III.

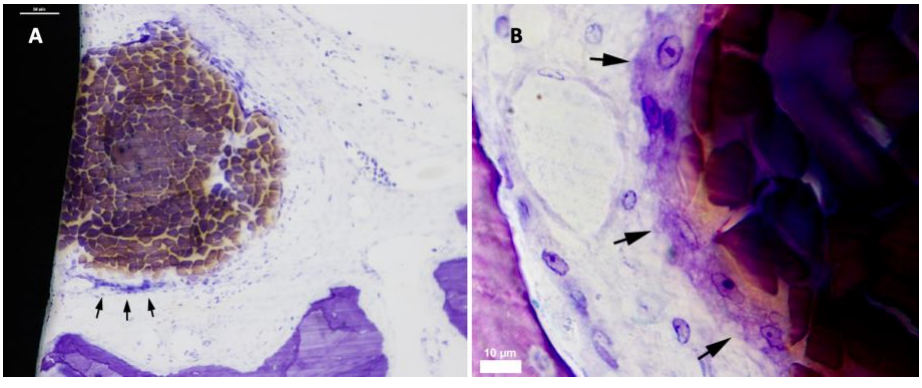

Figure 16. Large, elongated FBGCs (Arrows) partly outline the silk ligatures at (A) 12 weeks and (B) 8 weeks (Figure B from Reinedahl et al. (2019).

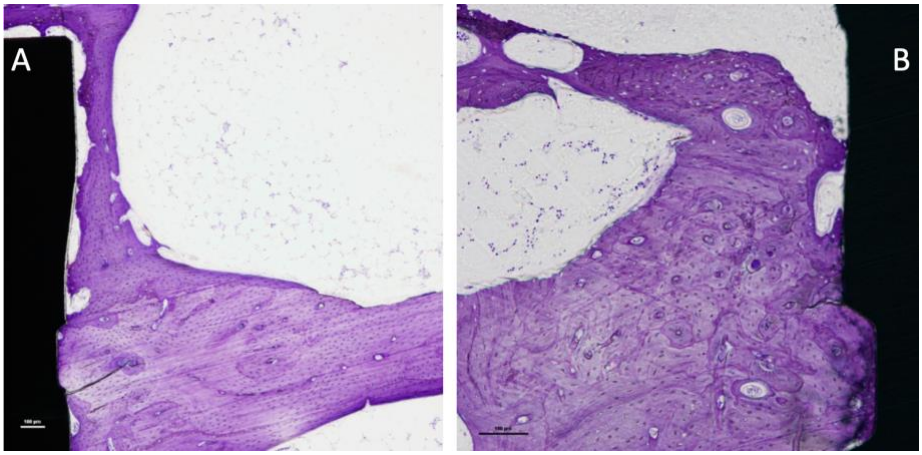

Figure 17. In both cases, control implants at 12 weeks (Reinedahl et al. 2023) and (B) 8 weeks show good osseointegration. Additionally, neo-formed bone is visible, extending over the top of the left implant.

4.2.4 QPCR RESULTS

The qPCR 8-week results are presented in Figure 18 (study II) and 12-week results (study III) in Figure 19.

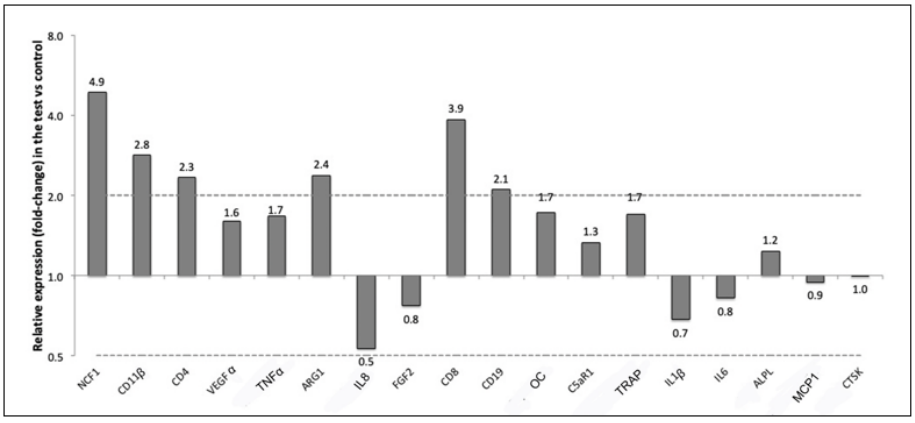

Figure 18. Reinedahl et al.(2019). Up- and down-regulation of selected genes in soft tissue at 8-weeks for test (silk ligature) versus control (non-ligated) implants (Study II).

The qPCR results from study II revealed a more than twofold up-regulation of NCF1, CD8, CD11β, ARG1, CD4, and CD19, alongside a twofold down-regulation of IL8.

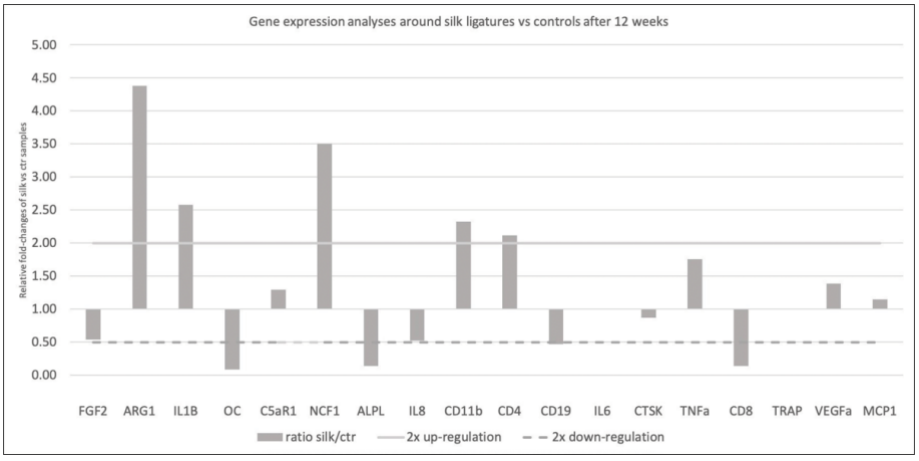

Figure 19. Reinedahl et al.(2023). Up- and down-regulation of selected genes at 12 weeks (Study III).

The qPCR results from study III revealed a more than twofold up-regulation of ARG1, NCF1, IL1β, CD11β, and CD4 and a more than twofold down-regulation of OC, ALPL, and CD8.

4.3 CLINICAL PROSPECTIVE CROSSOVER STUDY (IV)

4.3.1 IMPLANT SURVIVAL AND EXCESS CEMENT

The implant survival rate was 100% at 1 year. Excess cement was found on 18 out of 24 (75%) CR restorations: 100% in group A and 50% in group B.

4.3.2 RADIOGRAPHIC MBL

Both groups experienced MBL with CR crowns and bone gain during all periods with SR crowns (Table 4). During P1 (BL-W16), group A (SR) showed

slight bone gain (+0.007 ± 0.152 mm), while group B (CR) had a bone loss (-0.512 ± 0.703 mm), a statistically significant difference (p = 0.006). In P2 (W16-32), group A (CR) had a bone loss (-0.121 ± 0.263 mm), and group B (SR) had a bone gain (+0.255 ± 0.243 mm) (p < 0.001). Both groups showed bone gain in P3 (W32-52), with no significant difference (p = 0.843). The overall change from BL to W52 was not significantly different between the groups (p = 0.932). Group B showed significant MBL differences only between P1 and P2 (p = 0.005). In two group B cases, bone loss reached the implant's rough surface during P1 but partially recovered with SR crowns (Table 4).

Table 4. Radiographic marginal bone level changes within and between groups A and B (Study IV).

Follow-up period	MBL (mm)				
	Group A		**Group B**		*p* value*
	Mean ± SD (min, max)	*p* value**	Mean ± SD (min, max)	*p* value**	
BL-W16	0.007 ± 0.152 (-0.293, 0.328)	0.248 [a] (BL-W16_ BL16-W32)	-0.512 ± 0.703 (-2.234, 0.473)	0.005 [a] (BL-W16_ BL16-W32)	0.006 [b]
W16-W32	-0.121 ± 0.263 (-0.494, 0.316)	0.131 [a] (BL16-W32_ BL32-W52)	0.255 ± 0.243 (-0.174, 0.784)	0.136 [a] (BL16-W32_ BL32-W52)	< 0.001 [b]
W32-W52	0.106 ± 0.228 (-0.246, 0.585)		0.122 ± 0.337 (-0.537, 0.933)		0.843 [b]
BL-W32	-0.114 ± 0.246 (-0.502, 0.363)		-0.257 ± 0.611 (-1.450, 0.799)		0.799 [b]
BL-W52	-0.008 ± 0.244 (-0.415, 0.474)		-0.135 ± 0.560 (-1.317, 0.760)		0.932 [b]

* Comparison between Group A and B
** Longitudinal comparisons
BL - baseline
MBL - marginal bone loss
SD – standard deviation
W – week
[a] Wilcoxon signed ranks test
[b] Mann-Whitney test

4.3.3 CLINICAL PARAMETERS AND INDICES

PPD and CAL tended to follow the trend of the MBL measurement as expected. During P1, mean PPD increased in both groups, more in Group B (CR) (+1.1 mm) than in Group A (SR) (+0.2 mm), with a significant difference (p = 0.002). PPD decreased in both groups from W16-52, notably between W32 and W52. Changes differed significantly between groups (p < 0.001). CAL followed a similar trend but without statistically significant differences (p = 0.258; Two-way ANOVA).

BOP followed a somewhat different pattern than MBL, PPD, and CAL. In group A, BOP increased during P1-2 (BL 12.1%, W16 33.3%, W32 32%) and decreased in P3 (W52 18.1%). In group B, BOP increased during P1, decreased in P2, and rose again in P3 (BL 16.7%, W16 45.8%, W32 30.6%, W52 43.1%). The difference was significant at W52 (p < 0.001).

Plaque control was excellent in both groups throughout the study, ranging from 0-2.8% in group A and 0-4.2% in group B, with no significant differences.

4.3.4 QPCR RESULTS

Figure 20 presents the qPCR results from peri-implant gingival tissue from Group A and Group B at week 32. In group A with cemented restorations, the pro-inflammatory marker IL6 was 7.09-fold upregulated, and the bone mineralization marker ALPL was 2.3-fold downregulated. No other markers showed >2-fold up- or down-regulation. The bacterial marker 16S rRNA was < 2-fold upregulated with a minor difference between the groups, demonstrating similar bioburden for both groups.

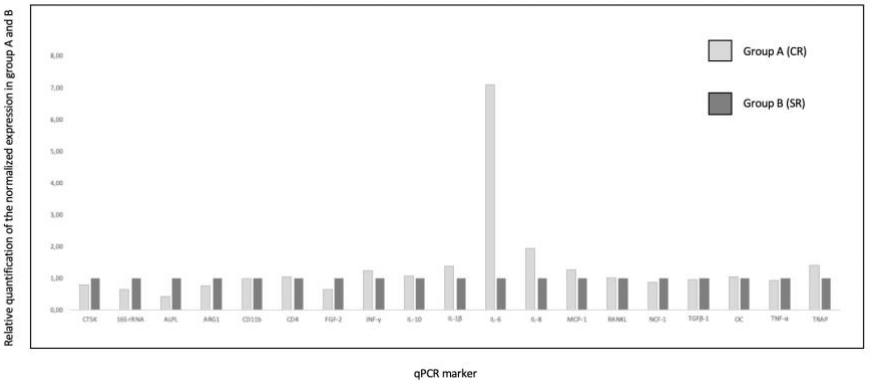

Figure 20. qPCR results from peri-implant soft tissue biopsies at week 32, following 16 weeks with CR in Group A and SR in Group B.

5 DISCUSSION

5.1 THE IMMUNE SYSTEM AND ITS IMPORTANCE FOR OSSEOINTEGRATION AND ITS PERTURBATION

Donath was the first investigator to suggest that osseointegration was merely a foreign body reaction [29]. As a trained histopathologist, Donath challenged the then-popular idea that titanium was a bioinert material [160]. However, at that time, no methods were available to conclusively prove that Ti implants could be recognized as foreign bodies and, indeed, activate the immune system.

With the new millennium came a new scientific field, osteoimmunology, which enabled researchers to explore immune responses to implants using advanced methods such as ELISA and qPCR. In orthopedics, the dominant reason for failure of hip and knee arthroplasties was aseptic loosening, a condition less studied in dental implants. Over the years, various hypotheses were proposed to explain aseptic loosening, including "metal disease" and "plastic disease" (gliding surfaces made of high-density polyethylene) (for an overview, see Campbell 1995) [161]. Using ELISA, orthopedic researchers identified elevated immune responses associated with aseptic loosening, leading Harris [162] to conclude that a massive immune reaction caused aseptic loosening [162].

Understanding immune reactions to implants and their role in success and failure is complex. In dentistry, Trindade et al. [42] were the first to demonstrate that titanium implants caused an elevation in immune-related factors, albeit a milder reaction compared to materials like copper and PEEK [43,163]. Inspired by Donath's findings, Albrektsson et al. [164] introduced a new definition of osseointegration. This definition marked a significant departure from earlier ones, suggesting that the immune system immediately recognizes titanium as a foreign material and forms bone around the implant to safeguard surrounding tissues. This shielding reaction, composed of bone tissue, relies on proper control of surgical trauma and other factors, and it is what we call osseointegration. In orthopedics, where conventional hip replacements involve significant trauma, the result is distance osteogenesis instead of osseointegration. While distance osteogenesis

functions well in orthopedic implants, it seems unlikely that oral implants would succeed under the same mechanism.

5.2 MAIN FINDINGS

This thesis explored marginal bone loss (MBL) around oral implants due to immunological reactions to materials. The ligature model, utilized in 119 of 133 reviewed animal studies on peri-implantitis, was found to lack validation. The immunological effects of artificially added ligatures, commonly used to accelerate bone resorption in these experiments, had neither been considered nor tested (Study I).

Two in vivo studies (Studies II and III) were conducted using a novel rabbit model, where sterile ligatures made of cotton retraction cords and 3-0 silk sutures were placed around implants under aseptic conditions and protected from contamination through layered wound closure. The experiments revealed aseptic immunological reactions toward the ligatures, resulting in MBL. Rich inflammatory cell infiltrates, dominated by macrophages and MNGCs, outlined the ligatures, which were also encapsulated by fibrous tissue adjacent to resorbed bone surfaces—indicative of a chronic FBR of the fibrous encapsulation type, with ongoing frustrated phagocytosis.

To assess clinical relevance, a crossover RCT evaluated biological responses to cemented and screw-fixed implant restorations (Study IV). Excess cement was found in 75% of cemented restorations upon removal of the crowns and was associated with inflammation and radiographic MBL. These included radiographic MBL, a sevenfold upregulation of pro-inflammatory IL-6, and a 2.3-fold downregulation of the bone formation marker ALPL, as shown by qPCR analysis. Replacing cemented restorations with screw-fixed ones and carefully removing visible cement debris from the peri-implant mucosa resulted in gradual MBL recovery by the 1-year endpoint.

The following sections will discuss the main findings from the systematic review, experimental studies, and clinical trial related to what is known today about the immune regulation involved in response to any implanted material. Relevant clinical implications will also be discussed.

5.3 ASEPTIC MECHANISMS BEHIND MBL IN EXPERIMENTAL PERI-IMPLANTITIS STUDIES

5.3.1 THE LIGATURE EFFECT WAS NOT VALIDATED

The systematic review found that the ligature model had not been validated. The immunological effect of artificially added ligatures, used to accelerate bone resorption in 119 of 133 reviewed experimental peri-implantitis studies, had not been considered or validated. In many publications, ligatures were simply regarded as tools to transport bacteria deeper into peri-implant pockets without adequately evaluating their independent effects.

A single reference was cited as validation in one of the earliest experiments: a 1966 study by Rovin et al. [132] that investigated the effect of silk ligatures around the teeth of germ-free and normal rats. Unlike normal rats, the study observed that germ-free rats did not mount a detectable inflammatory response and was cited as sufficient evidence that ligatures do not trigger bone resorption. However, this conclusion overlooked several critical factors. First, whether germ-free or normal, MBL was not observed in any test animals in Rovin et al.'s study. Further, germ-free rats have an underdeveloped immune system with impaired cytokine production—notably a diminished production of pro-inflammatory cytokines, crucial for triggering bone resorption [165,166]. Additionally, Rovin et al.'s [132] study did not include other ligature types, such as cotton, dental floss, or stainless-steel wire fixed with cyanoacrylate, which have been used in subsequent research (Study I).

Numerous publications have demonstrated potent pro-inflammatory reactions to silk and cotton in sterile environments, including foreign body granulomas, bone resorption, and, in severe cases, tumor-like osteolytic lesions known as gossypiboma [127,128,167-169]. In biomaterials research, cotton was sometimes used as a negative control "to induce intense FBR" [170]. Moreover, several trials involving plaque accumulation have failed to provoke clinically relevant bone resorption, even after observation periods of up to 1.5 years [121-123]. MBL generally occurred most rapidly following the most often repeated ligature placements. This highlights the need for further investigation into the direct role of ligatures in triggering immune responses and their potential contribution to bone resorption.

The poorly supported claim regarding the passive role of ligatures has been repeatedly cited and perpetuated in subsequent publications [133,171]. Notably, the critical reflections of the method's frequent users focused on researchers' control over MBL progression, such as the frequency of ligature exchange or adding more ligatures [134]. However, any potential immunological reaction to the ligatures was entirely disregarded.

As a result, the ligature model has been rightly criticized over the years, and its relevance must be questioned [67,115,119,124,159]. This includes studies using a so-called spontaneous progression period following ligature removal, which reported more rapid MBL progression on modified surfaces compared to turned surfaces—a claim that was recently refuted in a comprehensive systematic review comparing ligature-based studies with clinical realities derived from long-term outcome studies [67].

5.3.2 ASEPTIC LIGATURES INDUCE MBL

The fibrous encapsulation, persistent immune cell infiltrates along ligature surfaces, and peripheral bone resorption observed around aseptic ligatures at 8 and 12 weeks in Studies II and III demonstrate a prolonged fibrous encapsulation-type FBR, with ongoing frustrated phagocytosis by macrophages and MNGCs. The histological and immunological findings suggest a relatively stable condition, with arrested bone resorption and a regulated inflammatory response. This is likely influenced by the extended healing times, as 8 and 12 weeks in rabbits correspond to approximately 7 and 11 months in humans. In contrast to successful osseointegration, fibrous encapsulation represents implant rejection if present around an entire implant. Therefore, the histological and molecular differences between these material-dependent responses may provide valuable insights into the mechanisms underlying osseointegration and its perturbation.

FIBROUS ENCAPSULATION TYPE FBR – HISTOLOGICAL ASPECTS

In their studies on bone-fixed implants of different materials, Trindade et al. [43,163] observed osteolysis and fibrous encapsulation in response to copper implants, a FBR similar to that observed against ligatures in this thesis. However, their analysis focused on earlier time points. By day 10, bone resorption around copper was so extensive that no bone-implant contact remained. Additionally, fibrous proliferation was evident between the copper

implants and the resorbed bone surfaces, with macrophages forming MNGCs within dense inflammatory infiltrates. These findings highlight bone tissue's rapid ability to resorb and isolate perceived threats. Notably, a slight down-regulation of RANKL was detected at 10 days compared to sham sites, suggesting that the required bone resorption had been completed by then [163].

In their subsequent 4-week study [43], a more structured tissue response was observed. The inflammatory infiltrates had partially subsided, and fibrous encapsulation formed, creating a barrier between the bone and the implants. Additionally, distance osteogenesis occurred at the periphery of the fibrous capsules, further isolating the rejected copper implants from the bone marrow. This reaction contrasted starkly with the osseointegration seen around titanium implants, where contact osteogenesis began within 10 days, progressing to corticalized osseointegration by 28 days. Their findings emphasize the material-dependent nature of immune-mediated responses to bone implants and that inflammatory reactions attenuate to some extent once separation from bone is established and no further provocation occurs [43].

Compared to Trindade et al.'s studies, the present aseptic ligature experiments examined immune responses at later time points equivalent to ~7 and 11 months in humans, considering rabbits' faster bone metabolism. It was noteworthy that immune cell infiltrates persisted around the ligatures even after a prolonged period, while fibrous capsules remained well-formed, isolating the ligatures and their associated inflammatory cell infiltrates from the adjacent resorbed bone surfaces.

At 12 weeks, discrete neo-bone formation was observed on some previously resorbed bone surfaces around the ligatures, a feature absent at 8 weeks. Notably, one specimen exhibited a small neo-formed bone knob in direct contact with the ligature (Figure 15). These findings suggest that new bone formation may partially close or narrow the fibrously encapsulated osteolytic zone adjacent to a hostile material over time. Further, it highlights the dynamic nature of the immunological response to materials over time and its potential influence on MBL.

IMMUNOLOGICAL ASPECTS

The qPCR results in Studies II and III should be interpreted with caution, as only a few markers (NCF1, CD11β, and CD4 at 8 weeks) with at least a twofold difference in up- or down-regulation for test and control samples

reached statistical significance ($p < 0.05$). Gene expression varied quite widely across biopsies for some genes, and study III included a limited number of samples. The following section will discuss markers that were consistently up- or down-regulated by more than twofold in silk-ligated compared to pristine implants in both studies.

The up-regulation of NCF1 at both 8 and 12 weeks suggests ongoing reactive oxygen species (ROS) production in response to the ligatures. NCF1, a key subunit of the NOX2 complex, facilitates the conversion of oxygen into superoxide—a ROS and precursor to more potent nitric oxide-dependent reactive species (RNS). Persistent ROS/RNS release is a hallmark of frustrated phagocytosis, a process where macrophages and neutrophils encounter particles too large to engulf. When unable to degrade an object internally, these phagocytes release ROS/RNS extracellularly for degradation, leading to tissue damage and chronic inflammation, as observed histologically around silk and cotton ligatures in Studies II and III [172,173].

The up-regulation of the M2 macrophage marker Arg1 at 8 and 12 weeks suggests a regulatory response to persistent inflammation induced by the ligatures. Arg1 creates an immunosuppressive environment by reducing nitric oxide availability, thereby limiting ROS production and promoting M2 macrophage polarization [174]. Generally, Arg1 up-regulation is associated with pathological conditions related to immune regulation, tumor growth, and chronic inflammation [175,176].

The up-regulation of CD11β further supports a sustained but regulated inflammatory response to the silk ligatures. As a key component of the Mac-1 integrin, CD11β facilitates leukocyte migration and adhesion, particularly in neutrophils, making it more prominent in M1 macrophages [177]. However, more recent studies have also demonstrated a regulatory role; for example, Yao et al. [178] found that leukadherin-1, a CD11β agonist, attenuates LPS-induced pro-inflammatory responses in macrophages [178].

The up-regulation of CD8 and CD4 in both experimental studies suggests the involvement of cytotoxic T cells (CD8) and T-helper cells (CD4) in the immune response against silk ligatures. T-lymphocytes likely regulate macrophage activity during FBR, promoting adhesion and fusion into FBGCs [179]. However, pristine Ti implants were used as controls, and previous findings indicate that Ti surfaces attenuate T-cell activity at 12 weeks [44].

Additionally, Rodriguez et al. [179] reported that macrophages can mount a normal FBR without T-cells [179].

The regulated and controlled inflammation observed in response to the ligatures in studies II and III may explain why traditional ligature studies often require repeated interventions, such as replacing or increasing the number of ligatures, to sustain an ongoing bone resorptive process. Introducing new ligatures into the depths of existing pockets likely triggers new bone resorption to create safety distance and space for immune defense against the ligature/plaque agents again.

5.3.3 CLINICAL IMPLICATIONS OF LIGATURE STUDIES

Although silk and cotton ligatures are artificial research tools and not clinically relevant, their resulting FBRs—osseointegration versus fibrous encapsulation with frustrated phagocytosis—are highly relevant, as the former signifies success and the latter failure in implant integration.

Future studies on the dynamic and persistent immunological responses to implant-related materials, similar to those observed for ligatures, may provide insights into why marginal bone levels around implants sometimes fluctuate over time. For instance, Chrcanovic et al. [66] reported a radiographic marginal bone gain in 11.7% of 1,045 implants across 227 patients followed for over 20 years [66].

Similarly, Jemt et al. [68] found radiographic marginal bone gain in 36% of 752 implants in 145 patients previously diagnosed with peri-implantitis on average 9.1 years earlier. In their study, marginal bone levels were not influenced by dental hygiene treatments or peri-implantitis surgery performed during these years, suggesting that immune-material interactions beyond the presence or absence of infection regulate MBL [68].

The sterile, modified ligature model introduced in Studies II and III could be applied to investigate immunological responses to clinically relevant materials, such as the effects of different cements or wear debris from implants and superstructure materials.

5.4 CEMENT-RELATED MBL

Study IV investigated MBL in relation to material and treatment variables in a clinical setting. The comparison between screw-retained and chairside cement-retained restorations was motivated by the frequent use of both methods in clinical practice and the mixed outcomes reported in the literature. While some studies have associated cement retention with greater MBL [85], others have noted the opposite [86]. The crossover RCT design was chosen for its ability to facilitate both within- and between-group analyses.

The proportion of cases with excess cement (75%) in this study was consistent with previous findings by Kim et al. [88] (72.5%) and somewhat higher than Korsch et al. [90] (60%). However, it is essential to note that Korsch et al. examined restorations with 3.5 or more years in function, during which routine maintenance may have removed some of the previously present excess cement. Factors such as submucosal restoration margins and broad emergence profiles may contribute to excess cement retention [88], although this study did not investigate these factors.

The radiographic MBL observed during periods with cemented restorations correlated with the high prevalence of cement excess and was likely driven by increased inflammation, as indicated by IL-6 upregulation. Excellent oral hygiene in both groups and a similar bacterial bioburden based on 16S rRNA expression suggests a cement-induced rather than infection-driven cause [180]. However, bacterial species were not compared [181].

It is important to note that the expression of pro-inflammatory cytokines—whether IL-6, TNF-α, IL-1β, IL-17, or others—cannot reliably distinguish between infection, particle-induced inflammation, autoimmune conditions, or other causes of local bone resorption [23]. Instead, multiple factors must be considered. Notably, inhibition of IL-6 in a recent mouse study arrested plaque/silk-induced MBL around implants, underscoring IL-6´s role in triggering local bone resorption [182].

The absence of changes in RANKL expression in this study suggests that active bone resorption was not ongoing at the analysis time. Instead, the downregulation of ALPL indicates reduced osteoblast activity, potentially impairing new bone formation near the cemented restorations [183]. As previously demonstrated, local bone resorption occurs rapidly in response to foreign body agents [163]. Therefore, a fibrous tissue barrier likely had already

formed, isolating the bone from the cement-induced inflammatory response and containing the process. Similar to the observation of Olander et al. [83], BOP did not correlate with bone resorption [83].

The recovery of MBL observed during all periods with screw-fixed restorations, facilitated by the cross-over design, was particularly noteworthy. The intervention was intentionally minimal, avoiding debridement of implant or abutment surfaces and leaving granulation tissue untouched. It consisted solely of removing the cemented crown, replacing it with a screw-fixed restoration, and carefully removing visible cement debris from the peri-implant mucosa without disturbing the granulation tissue.

Figures 21 to 23 show the most severe case of the study. At 16 weeks, the patient presented with substantial cement excess (Figure 21) and associated radiographic MBL. After the restoration was exchanged, partial MBL recovery was observed at 1 year (Figure 22). A radiograph taken during a recall visit two years after the study's conclusion revealed near-complete spontaneous regeneration of the bone defect (Figure 23).

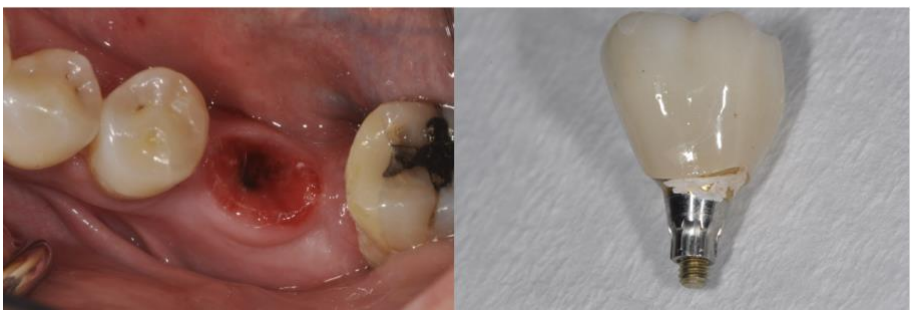

Figure 21. The bone defect correlated with the study's most significant detected cement excess. Note the granulation tissue and dislodged cementum particles. Note also the wide emergence profile of the abutment that may have contributed to the excess.

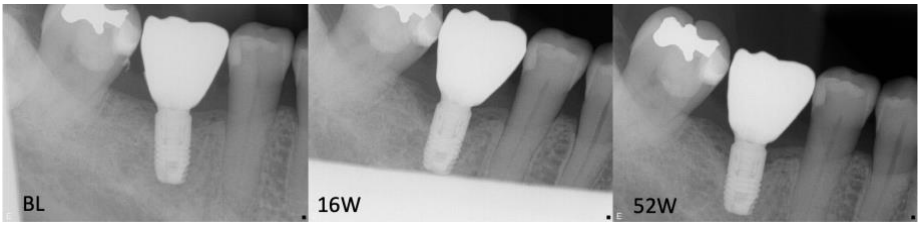

Figure 22. One of the included study patients presented with a buccal bone defect at week 16 of the experiment, which was by far the most severe case in the study. After exchange to a screw-retained crown, MBL improved slightly until 1 year. The cementum excess visible on the distal side of the crown and mesial side of the tooth was removed after the radiograph was taken.

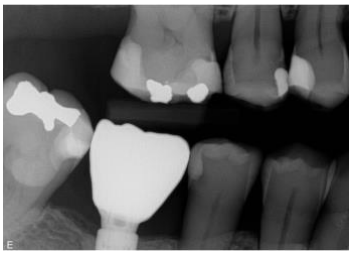

Figure 23. A radiograph taken during a routine follow-up visit 2 years after the study's conclusion revealed nearly complete spontaneous marginal bone regeneration.

Given the high prevalence of cement excess detected upon removal and its associated biological complications, we conclude that cemented fixation should be avoided whenever possible.

The study design facilitated the minimally invasive intervention applied in this study, as the cemented restorations were intentionally designed to be removable for exchange purposes. However, this raises the question of whether a similar approach—removal and ex situ cleaning of restorations and abutments—could offer comparable benefits for screw-retained restorations in cases of newly detected MBL, profuse bleeding, or suppuration. Such a method could help preserve delicate marginal tissues and minimize the risk of dislodging material particles into the peri-implant mucosa—an established iatrogenic concern in orthopedics that remains insufficiently understood in the peri-implant context.

It is crucial to recognize that we still lack a complete understanding of the mechanisms regulating marginal bone levels around osseointegrated implants or what defines a "normal" biological response across the diverse range of

implant and superstructure types, clinical protocols, and practitioner skill levels. Future research will provide greater clarity on these ongoing debates.

5.5 LIMITATIONS

SYSTEMATIC REVIEW

A broad range of studies was deliberately included to explore research in the field comprehensively. The qualitative analysis was prioritized to determine if the ligature model had been validated.

The meta-analysis included a heterogeneous group of studies encompassing different animal species, ligature types, and ligature application methods. MBL was evaluated using varied techniques, including radiography, histology, and clinical assessment. Consequently, significant heterogeneity was observed across most parameters, necessitating a cautious interpretation of the results. For example, a recent systematic review by Solderer et al. [159] found no significant effect of ligature type or application method on MBL at 12 weeks in dog experiments. However, a similar trend was noted, with cotton causing more MBL than silk [159].

SAMPLE SIZE

The sample size across all studies was relatively small, particularly in experimental Study III, which was further affected by the loss of one animal due to implant displacement. This small study format was a deliberate decision, balancing ethical considerations (reduction) with the anticipated learning curve associated with the pioneering study design. Generalizability was deemed less critical, as marginal ligatures are artificial constructs and do not represent clinical reality.

The clinical study (Study IV) also had a small sample size but demonstrated significant differences between groups. Given the cross-over design requiring multiple restoration swaps and biopsies, a more extensive study would have been difficult to perform.

IMMUNOLOGICAL ANALYSIS

The qPCR markers were selected to focus on innate immune reactions to biomaterials, particularly macrophages, central to FBR in bone and soft tissue [15,184]. Bone markers were included to assess the immune response's impact on bone resorption/formation, along with markers of the adaptive immune system that were suspected to be involved in aseptic loosening in some previous studies [185].

Although qPCR is inherently biased toward predefined targets, it offers high specificity and sensitivity. Future studies could benefit from high-throughput methods like RNA sequencing to explore the transcriptome and uncover unexpected regulatory networks relevant to FBRs. RNA sequencing could complement qPCR by providing broader insights, followed by qPCR validation to ensure specificity.

A limitation of these studies was the lack of protein-level analyses, as upregulation of mRNA does not always translate into increased protein expression. ELISA-based approaches, such as those used by Wong et al. [186] in rabbit cytokine studies, could provide additional insights, especially given the frequent use of rabbits in dental implant research [186].

TRANSLATIONAL LIMITATIONS

Rabbit models have a long history in osseointegration research, dating back to Professor Brånemark's seminal work. They have also been instrumental in developing moderately rough implant surfaces [187]. Our use of long rabbit bones allowed for the placement of human-sized implants under aseptic conditions.

However, rabbits exhibit a 3–4 times higher bone turnover than humans [188], necessitating careful consideration in experimental design and result interpretation. While the turnover rate in dogs, commonly used for ligature studies, is closer to that of humans [189].

Further, while rabbits' femoral and tibial bones can house natural-size implants, their cortical bone plates are thinner, and their marrow is fattier than humans [188]. This sometimes poses a challenge in reaching primary stability for implants, along with the risk of displacing the implants into the marrow, as was the case with one of the implants of the studies in this thesis. Considering that the bone resorption seen from ligature studies in dogs is often rapid, with an attenuating trend already at 8 weeks [159], shorter experiments in rabbits

would likely better capture the resorptive phase, as compared to the arrested bone resorption and M2-regulated inflammatory response observed in studies II and III in the present thesis.

TIMING OF LIGATURE PLACEMENT

This thesis placed implants and ligatures simultaneously, deviating from customary protocols that allow osseointegration before ligature placement. This approach reduced the risk of contamination and infection from repeated procedures. Also, a previous traditional-type ligature experiment showed bone defects similar to those from immediate ligature placement compared to delayed ligation [156]. We deliberately chose a relatively smooth implant type, considering that some modified surfaces trigger strong M2 macrophage-associated reactions in vitro and even spontaneous healing of marginal bone defects in vivo [39,40].

HISTOLOGICAL LIMITATIONS

The resin-embedded cut and ground sections used in this study did not allow for immunohistochemical staining. This method was chosen to preserve and observe the calcified bone. Recently, techniques enabling immunohistochemical staining for resin-embedded sections have become more readily available [190]. These may be a good option for future studies.

6 CONCLUSIONS

- Ligatures made of silk, cotton, and other materials have been used in most pre-clinical studies on the onset, progression, and treatment of peri-implantitis, being the method of choice in 119 out of 133 identified experiments in the systematic review. The extent of MBL induced by ligatures varies depending on the material and application method; however, the high heterogeneity among the included studies must be considered. Notably, the inflammation or MBL caused directly by the ligatures—artificial additions that do not reflect clinical conditions—has not been investigated. Plaque accumulation without ligature generates zero to negligible MBL (Study I)

- Cotton and silk ligatures induce aseptic and persistent Peri-implant inflammatory responses in the long bones of rabbits, marked by rich cell infiltrates dominated by macrophages, multinucleated giant cells (MNGCs), and fibrous encapsulation. These ligatures also cause significant MBL around titanium implants after 8 weeks. (Study II)

- The inflammatory response to silk ligatures persists over a more extended observation period of 12 weeks but does not progress. Over this time, signs of neo-bone formation appear, partially closing the gap between the ligature and the bone surface. (Study III)

- Chairside cemented implant restorations are associated with a high prevalence of cement excess associated with radiographic MBL, increased probing pocket depth, and clinical attachment loss. Bleeding on probing does not reliably predict ongoing MBL. Switching to screw-fixed restorations can facilitate MBL recovery. (Study IV)

7 FUTURE PERSPECTIVES

The discovery of immunological reactions to osseointegrated implants and prosthetic materials represents a paradigm shift in dental implant research, providing new opportunities to investigate the molecular mechanisms underlying osseointegration and bone resorption. Collaboration across various research fields could be valuable for further exploring these mechanisms. While interdisciplinary collaboration within dentistry is vital, partnerships with orthopedics, osteoimmunology, and rheumatology are equally important. Moreover, oncology research on manipulating macrophage polarization to halt tumor progression may offer insights into foreign body responses to implants. Identifying specific molecular and genetic mechanisms could lead to targeted interventions that improve implant outcomes—potentially even tailoring them to individual patients.

At the same time, this new understanding of immune regulation in osseointegration raises important questions. What defines a normal, healthy foreign body response at the marginal complex where bone interfaces with soft tissue and restorative materials engage with implants? Can fluctuations in inflammation and marginal bone levels—both upward and downward—be anticipated biological responses to foreign bodies? The findings of this thesis suggest so, but additional research is necessary to guide the next generation of implant materials.

For clinical research on progressive MBL, big data on true endpoints—such as prosthesis loss, implant failure, and surgical interventions—should be integrated alongside patient-reported complications, including pain, profuse bleeding, or suppuration, that necessitate intervention regardless of their predicted impact on future MBL. Given the considerable variability in success rates among clinicians, future studies should also aim to identify and categorize suboptimal elements from the outset of failing implant treatments. Quality registries, widely utilized in orthopedics, could offer valuable frameworks for such investigations.

In conclusion, progressive MBL around osseointegrated implants is a multifactorial condition that requires a multidisciplinary research approach. Hopefully, future discoveries will emerge from such collaborative efforts.

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