On loading protocols and abutment use in implant dentistry

Clinical studies

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What is the difference between knowledge and wisdom? Knowledge is gained by gathering data, whereas, wisdom is earned by going through actual life experiences.

Kwon Jin-Soo

ABSTRACT

Research questions: The influence of immediate or delayed loading and the use of abutments in implant dentistry with regard to peri-implant tissues and the effect of risk parameters.

Methodology: Fifty partially edentulous patients each received three Brånemark TiUnite™ implants. The patients were randomly assigned to a test group (immediate loading) or a control group (delayed loading). The test patients received a temporary prosthesis within 48h. The prosthesis was attached directly at implant level (IL) or via abutments: a machine-milled surface (AM) or an oxidized surface (AOX, TiUnite™). Clinical examinations and intraoral radiographs were performed during a 5-year period. For a subgroup, crevicular fluid was analyzed with qPCR.

Results: Up to 1-year, six implants were lost. Thereafter, no implants were lost, resulting in 5-year cumulative survival rates of 93.9% and 97.0%, for test and control groups, respectively. After 5 years, significantly lower marginal bone loss (MBL) was found at superstructures connected to AM than at sites with superstructures attached to IL. Soft tissues retracted mostly during the first year and thereafter minor changes were seen. With time, proximal probing pocket depth, plaque and bleeding increased, whereas a minor decrease for bleeding was found between 3 and 5 years. Similar bleeding-on-probing levels were seen at 3 and 5 years for various connections. The prevalence of peri-implantitis was 4.0% and 9.1% at implant and patient level, respectively, after 5 years. Technical complications were scarce after the first year; the most common was porcelain chipping. In a multiple linear regression model, the independent variables – health change, medication for high blood pressure, periodontal disease experience, smoking (≤10 cigarettes per day), and proximal pocket depth – explained about 27% of MBL variations. The gene study demonstrated correlation between some genes and clinical findings, but there is need for more research.

Conclusions: The results demonstrated similar implant survival and marginal bone loss, irrespective of loading protocol. The use of a machined abutment should be preferred regarding marginal bone stability over time. There is still a lack of scientific support for placing superstructures directly on the implant. Factors related to systemic health and medications as well as periodontal disease experience and smoking, are associated with marginal bone loss. Peri-implantitis was found in 9.1% of the patients, indicating the need for supportive maintenance.

Keywords: abutment design; clinical studies; dental implants; dental prosthesis, implant-supported; gene expression; health; immediate implant loading; marginal bone loss; osseointegration; prosthodontics; risk factors; smoking; treatment outcome.

SAMMANFATTNING PÅ SVENSKA

Syfte: Att vid implantatbehandling studera betydelsen av direkt eller fördröjd belastning, användandet av distans och riskfaktorer avseende omgivande ben- och mjukvävnad.
Metod: Femtio patienter med partiell tandlöshet inkluderades. Patienterna randomiserades till en testgrupp (direkt belastning) eller en kontrollgrupp (fördröjd belastning) och varje patient erhöll tre Brånemark TiUnite™ implantat. På de tre implantaten byggdes implantatbron: direkt på implantatnivå (IL), med en maskinbearbetad, prefabricerad distans (AM) och med en distans med oxiderad titanyta (AOX, TiUnite™). Kliniska undersökningar och intraorala röntgenbilder utfördes under en 5-årsperiod. På ett urval av arton patienter togs exsudat från implantatfickan som sedan analyserades med molekylärbiologisk metodik.
Resultat: Under första året förlorades sex implantat och därefter inga flera, vilket ger en femårsöverlevnad på 93,9% och 97,0%, i test- respektive kontrollgrupp. Efter 5 år sågs signifikant mindre marginal benförlust kring implantat med maskinbearbetad distans jämfört med implantat som har bron byggd direkt på implantatnivå utan mellanliggande distans. Mjukvävnaden retraherade mest under det första året och därefter sågs mindre förändringar. Efter 1 år registrerades ökande periimplantära fickdjup approximalt. Plack- och blödnings-index ökade med tiden men en liten nedgång sågs för blödning mellan 3 och 5 år. Liknande nivåer för blödning vid sondering registrerades vid 3 och 5 år för IL, AM, AOX. Biologiska och tekniska komplikationer noterades. Förekomsten av periimplantit var 9,1% på patientnivå och 4,0% på implantatnivå efter 5 år. Tekniska komplikationer var få efter det första året, vanligast var porslins-”chipping”. I multipel linjär regressionsanalys med marginal bennivå som beroendevariabel sågs signifikanta samband med följande oberoende variabler: hälsoförsämring, medicinering för högt blodtryck, tandlossningserfarenhet, rökning (≤ 10 cigaretter per dag) och approximala fickdjup. De kan sammantaget förklara 27% av variationerna i marginal benförlust. En del gener korrelerade med kliniska fynd men fler studier behövs inom detta område.
Slutsatser: Användning av konventionell distans med maskinbearbetad titanyta bibehöll det marginala benet bättre över tid jämfört med att bygga bron direkt på implantatnivå. Ingen skillnad i marginal bennivå sågs vid direkt eller fördröjd belastning. Riskfaktorer att beakta kan vara hälsoförsämring, medicinering för högt blodtryck, tandlossningserfarenhet, rökning och djupa approximala fickor. Periimplantit sågs hos 9,1% av patienterna och stödbehandling över tid är viktig.

LIST OF PAPERS

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

I. **Göthberg C**, André U, Gröndahl K, Ljungquist B, Thomsen P, Slotte C. Immediately loaded implants with or without abutments supporting fixed partial dentures: 1-year results from a prospective, randomized, clinical trial. *Clin Implant Dent Relat Res*. 2014 Aug;16(4):487-500.

II. Slotte C, Lennerås M, **Göthberg C**, Suska F, Zoric N, Thomsen P, Nannmark U. Gene expression of inflammation and bone healing in peri-implant crevicular fluid after placement and loading of dental implants. A kinetic clinical pilot study using quantitative real-time PCR. *Clin Implant Dent Relat Res*. 2012 Oct;14(5):723-36.

III. **Göthberg C**, André U, Gröndahl K, Thomsen P, Slotte C. Bone response and soft tissue changes around implants with/without abutments supporting fixed partial dentures: Results from a 3-year, prospective, randomized, controlled study. *Clin Implant Dent Relat Res*. 2015 Mar 19. doi: 10.1111/cid.12315.

IV. **Göthberg C**, Gröndahl K, Omar O, Thomsen P, Slotte C. Complications and risks of implant-supported prostheses: 5-year RCT results. *Submitted for publication*.

CONTENT

ABBREVIATIONS

AM	Abutment machine-milled
ANOVA	Analysis of variance
AOX	Abutment oxidized
BoP	Bleeding on probing
CF	Crevicular fluid
CNC	Computer numeric controlled
CONSORT	Consolidated Standards of Reporting Trials
IL	Implant level
ICC	Intra-class correlation coefficient
ISQ	Implant stability quotient
MBL	Marginal bone loss
PPD	Probing pocket depth
qPCR	Quantitative polymerase chain reaction
RCT	Randomized controlled (clinical) trial
RFA	Resonance frequency analysis
SEM	Standard error of the mean

1 INTRODUCTION

1.1 Background and introductory remarks

The world population and the percentage of persons over age 65 are increasing. As per the literature, age is aligned with every tooth loss indicator.[1-6] Caries and periodontal disease (periodontitis) are the most common causes of tooth loss.

Right now, it's rare to be completely edentulous in Sweden. Among 70-year-olds in Jönköping, Sweden, the portion of edentulous people fell from 38% in 1973 to 1% in 2013.[5] In Swedish dentistry, focus has shifted to rehabilitating patients with partial edentulousness.[3,5]

Although the number of teeth missing per patient may decrease[7], ENREF 7 the overall number of missing teeth will probably continue to increase worldwide due to the aging population. So need for prosthetic treatment – especially in partially edentulous patients – will likely increase during coming decades.[8]

Teeth loss results in impaired oral function, diminished self-esteem and attractiveness, loss of social status, and an overall poorer quality of life.[9-11] Evidence also shows that implant-supported prostheses can restore some of these functions.[9,12-15] Oral prosthodontics restore normal function, esthetics, and comfort – regardless of number of teeth being replaced.

Nevertheless, in the clinical situation, it isn't always easy to select appropriate treatment, *e.g.*, when choosing between tooth-supported prosthetics or a more radical treatment including extractions and implant-supported prostheses placements.[16,17] For patients, dental implant treatments can be painful, tedious ordeals. Furthermore, treatment costs – as related to the individual and society – should be considered and more implant-supported prostheses-efficiency evaluations are needed.[18] A recently published study regarding single-tooth replacement demonstrates that a single implant is a cost-effective treatment option compared to a traditional three-unit fixed dental prosthesis.[12] Initial costs are higher for implant treatments – compared to fixed partial dental prostheses – and survival rate must be considered when determining cost-effectiveness.[19]

It's apparent that multiple host-related factors might be equally as important as actual technical solutions.[20] Moreover, patient expectations may vary and

can be an important factor to consider regarding treatment outcomes or patient satisfaction.[21] Women seem to have higher expectations than men.[22] To provide an accurate prognosis for a given treatment, it's evident that one must identify potential risk factors. Today, the known risk factors associated with implant treatment include smoking, previous periodontal disease experience, diabetes mellitus, poor oral hygiene, and poor general health.[23-29]

Brånemark and co-workers described the osseointegration concept in the 1960s.[30-34] They attempted to apply the osseointegration principle to anchor oral implants. But clinical results weren't very convincing in the first years, and it wasn't until the late 1970s that osseintegrated oral implants came into routine clinical use. At the 1982 Toronto conference[35], osseointegration was recognized internationally and accepted for clinical application. Now, rehabilitation of partially and fully edentulous arches with osseointegrated titanium implants is scientifically documented and considered highly predictable and safe.[28]

Since the advent of osseointegration, several alterations in the original treatment concept were introduced. Improvements of basic implant design functions and modifications of surgical and prosthetic approaches reflect the changes. Such technical changes include modifications of implant (anchored in bone) and abutment (transmucosal component) materials, designs, and surface properties.[36-40] Moreover, several innovative procedures were introduced, including development and inclusion of digital technologies to support planning, treatment, fabrication processes, and outcome assessments.[41-44] _ENREF_41 Although many publications on these topics are presented every year, it must be admitted that we often lack fundamental understanding of whether novel treatment methods actually provide better outcomes than conventional methods. Because commercial interests are strong in this treatment field, a need exists for randomized, prospective, independent, and comparative clinical studies.

Treatment times have been successively shortened, and in selected patients, it's possible to load implants immediately or early after their placement.[45-48] Due to this trend, many patients currently undergo treatment with immediate loading, *i.e.*, titanium-implant loading in an early biological process stage, which leads to osseointegration in the jaw bone. But well-designed, randomized controlled trials (RCT) for scientific documentation of immediate and early loading are still relatively limited – particularly regarding treatment of partially dentate jaws.[49-53]

Patient demands for good esthetic results in the soft tissues also increased in parallel with higher demands for shorter treatment times. These two requirements are not always easy to reconcile. Soft-tissue healing around implants after conventional implant placement (delayed loading) was systematically studied in animals[54-57] and to some extent, in humans.[58-61] But studies of soft-tissue reactions around implants in early loading (preclinical and clinical) are scarce.[62-65] Further evidence-based knowledge is needed to support clinical decisions–regardless of whether immediate or early loading protocols are applicable.

Implant survival shouldn't be the only parameter used to measure treatment success. Varying esthetic-result factors, long-term soft- and hard-tissue stability, and long-term restorative-component stability must also be investigated. Albrektsson et al. identified parameters that affect establishment and maintenance of osseointegration.[66] These parameters were reconsidered in relation to immediate loading to improve chances of fulfilling success criteria. Due to a new protocol introduction (*i.e.*, immediate loading) need arose for identifying factors most vital for successful osseointegration and long-term implant success in such cases. Among varying factors, bone status, implant site, and implant loading conditions were asserted to be decisive for implant success, while other parameters (*e.g.*, implant material characteristics and surgical approach) may help to compensate for suboptimal bone sites and loading conditions.[51,67,68]

To reduce complications, a well-thought-out treatment plan is necessary. When selecting appropriate prosthetic treatment, thorough documentation of clinical and radiographic parameters is crucial for evaluating total oral-cavity status.[69] Development and methodology applications that aid clinicians in appropriate decision-making are important factors for determining treatment success or failure during follow-up and monitoring.

Such methods include evaluation of (i) clinical parameters and (ii) laboratory processing parameters such as biomarker.[70,71] In both cases, underlying biological processes must be deciphered. Rapid introduction of various new products, and the skyrocketing number of installed implants have revealed many complications related to oral implants placed in humans.[72,73] So more research on technical and biological complications is necessary for developing technologies that reveal causal and modifying factors in these processes.

In recent years, abutments usage has been challenged. Abutments (i) are considered redundant for prosthetic constructions, (ii) add unnecessary extra

cost for patients, (iii) increase leakage risk by creating double connections[74,75], and (iv) complicate superstructures' esthetic emergence profiles, with risk for visible metal. Yet abutments have been advocated for several reasons. Abutments are said to protect endosseous implants from excessive load and to reduce risk of bacterial leakage close to implants and bone crests.[71] Successful incorporation of an oral implant system relies on (i) osseointegration and (ii) adhesion of surrounding soft tissue to seal the tissues from bacterial penetration into the crestal bone.[54,76-80] ENREF_76 ENREF_76 ENREF_76

1.2 Implant material and surface topographies

Several organizations have provided guidelines for implant material standardization. The International Standards Organization, *e.g.*, provided the basis for such standards (International Standards Organization, standard references, Philadelphia 1996, ANSI-USA). The favorable long-term clinical survival rates reported for titanium and its biomedical alloys have made titanium the *gold standard* material for endosseous dental implants fabrication.[34,81] Titanium has high biocompatibility, high corrosion resistance, and low modulus of elasticity in comparison with other metals.[82] ENREF_82 Implant materials' physical and chemical properties are well documented and influence clinical outcomes from implant treatment.[83] These properties include the implant's surface roughness and chemistry as well as the design factors. ENREF_84[84-88] Standard grades of titanium (unalloyed) and titanium alloys maintain a very stable, insoluble oxide surface at normal temperatures.[82,89,90] The oxides can exhibit microscopically smooth or rough topographies at the micrometer level. Also important: various fabrication technologies provide specific and varying properties for implant surfaces. Technologies cover machining, particulate blasting, chemical (acid etching), or combinations of procedures[91,92] and new modification tools such as use of laser.[93,94] In a systematic review, Esposito et al. found no evidence that demonstrates that any particular type of dental implant had superior long-term clinical success.[95]

Whether – and the degree to which – implant surface characteristics influence adverse peri-implant biological responses and disease is a highly debated topic. As per Wennström[96] and Renvert[97], no clinical study evidence shows that implant surface characteristics affect either bone loss or peri-implantitis initiation, respectively. An opposing conclusion is that implant surface can affect biological response. Esposito et al. found that three years after loading, implants with turned (smoother) surfaces had a 20% reduction in risk of peri-

implantitis effects – compared to implants with rough surfaces.[95] But a tendency for early failures among implants with turned surfaces was reported – compared to implants with roughened surfaces.[95] An experimental study in dogs suggests that implant surface characteristics might influence outcome when treating peri-implantitis. Radiographic bone gain occurred at implants with turned, TiOblast and SLA surfaces, while at TiUnite implants, additional bone loss was found after treatment.[98]

1.3 Abutments and the peri-implant tissue

Many abutment materials (*e.g.*, titanium, stainless steel, gold, zirconia, and polyether ether ketone) and designs are available on the dental implant market. Traditional abutment material is commercially pure titanium (grade I-IV) due to its well-documented biocompatibility and mechanical properties. Esthetic awareness in implant dentistry drove development and use of alternative materials such as zirconia.[99,100]

In experimental animal studies, Abrahamsson et al. analyzed soft-tissue healing near abutments made of titanium, gold-alloy, dental porcelain, and Al_2O_3 ceramic. Results showed that gold-alloy and dental porcelain failed to establish soft-tissue attachment, while titanium or ceramic abutments (highly sintered 99.5% Al_2O_3) formed attachments with similar dimensions and tissue structures.[101] In a limited patient sample, Vigolo et al. assessed the marginal bone level and peri-implant mucosa around abutments made of gold-alloy or titanium on cemented single-tooth implant restorations, and they found no evidence of varying responses to the materials in a 4-year follow-up.[102]

In a recent review, Linkevicius et al. analyzed published research data regarding effect of zirconia or titanium as abutment materials on soft peri-implant tissues.[103] Overall, the research doesn't support any obvious advantage for titanium or zirconia abutments in comparison to each other. But zirconia abutments evoke better color response from the peri-implant mucosa and, consequently, a superior esthetic outcome.[104-108] This response is particularly evident in cases of thin peri-implant soft tissue and in regions in which implant placement is more superficial.[109] Others claim that the human eye could not distinguish change in color with a mucosa thickness exceeding 2–3 mm.[110,111]

Figure 1: External hex connection, Brånemark Implant System, Nobel Biocare AB. Reprinted by permission of © Nobel Biocare.

Brånemark's original implant was composed of an external hex with a butt joint (Figure 1). Initially, little interest in abutment-connection antirotational functions occurred because implants were used to treat fully edentulous patients and were connected with a one-piece metal superstructure. The implant's external hex portion wasn't added to the design for rotational stability but rather for enabling the implant's surgical placement.

A paradigm shift came with the internal-connection evolution. Each implant company developed its own internal connection design, which results in a confusing variation in terminology and connections. Reports in the literature claim that a morse tapered connection (*i.e.*, internal) seems to be more efficient in maintaining marginal bone level and minimizes bacterial leakage when compared to an external connection.[112,113] Moreover, loosening of abutment screws is a frequently occurring technical complication and the type of connection seems to have an influence on incidence of this complication. Loose screws were more often reported for externally connected implant prostheses.[114] As judged by the published literature, insufficient clinical evidence exists in randomized clinical trials for the superiority of a specific connection. Ultimately, this means that the clinical decision is a challenging one with no clear answer in scientific literature.

The soft-tissue connection to the implant's transmucosal component is critical because it relates to peri-implant tissue stability and prevention of peri-implant infection – with subsequent peri-implant structures destruction.

The primary function of a soft-tissue barrier at implants is to effectively protect the underlying bone and prevent access for microorganisms and their products. A mucosal seal surrounding dental implants with a true connective tissue attachment to the abutment may improve this protective function and prevent peri-implantitis.[115] The biologic width surrounding dental implants contains a coronal portion with junctional epithelium, followed apically by a connective tissue layer (Figure 2). Tomasi et al. reported a soft-tissue dimension of about 3.6 mm after 8–12 weeks of healing, including a barrier epithelium of 1.9 mm and a connective tissue portion of 1.7 mm.[116] Buser et al. described the peri-implant attachment as being rich in collagen fibers but sparse in cells and resembling scar tissue.[117] The natural dentition has dentogingival fibers running perpendicular to the tooth from the bone to the cementum. In contrast to the natural dentition, the connective tissue layer surrounding a dental implant abutment has fibers running in a parallel fashion – and thus need not have the same attachment quality – and may be more susceptible to apical migration of microorganisms.[61,118]

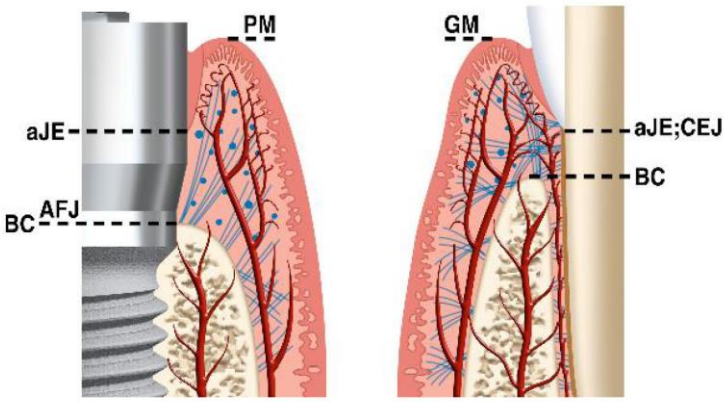

Figure 2: The tissue around an implant and a tooth. Reprinted by permission of © Nobel Biocare.

Covani et al. reported that soft tissues undergo minimal change at the buccal and proximal sites during the initial three months after surgery and immediate rehabilitation.[119] Varying study results are reported for immediate rehabilitation that favors[120] or penalizes[121] proximal soft-tissue height (papilla). Ideally, an esthetic gingival profile is established with gain in surrounding soft tissue and interdental papilla height; although it's still unclear which interventions are the most effective for maintaining or recovering the health of peri-implant soft tissues.[122] At multiple-implant restorations, peri-implant, soft-tissue topography reflects the underlying bone crest. Establishment of a *biological width* of the supracrestal soft-tissue barrier is similar to that described for the natural tooth.[123] Independent of implant geometry and insertion method (one- or two-stage procedure), experimental and clinical studies report that a soft-tissue seal of about 3–4 mm in height is established around the implant unit's transmucosal part.[124-128] If a minimum, peri-implant mucosa width is required, then marginal bone response (*i.e.*, bone resorption) may be regarded as an adaptative response to allow a stable soft-tissue attachment to form.[129] Although cellular and molecular mechanisms for such responses haven't been clarified, changes in the relationship between bone and overlying soft tissue may be one of the reasons for early marginal bone loss (MBL).[130] Linkevicius et al. claim that significantly less bone loss occurs around bone-level implants placed in naturally thick mucosal tissues, in comparison with thin biotypes.[131] A report by Puisys et al. recommend augmentation of thin soft tissues with allogenic membrane during implant placement to reduce crestal bone loss.[132] In contrast, others claimed that caution should be used in considering periodontal biotype at the patient level as a possible indicator of future peri-implant biotype.[133] Ross et al. suggest that implant diameter, gingival biotype, surgical technique, and/or the reason for tooth loss can influence the amount of gingival recession[134]; in this study, most recession occurred within the first 3 months between implant placement/provisionalization and definitive restoration. Use of a customized anatomic provisional abutment was found to reduce the amount and frequency of recession.[134]

Peri-implant soft-tissue dimensions around early or immediately loaded implants seem to be similar to those around conventionally loaded implants.[135,136] Non-removal of an abutment placed at the time of surgery results in a significant reduction of bone remodeling around the immediately restored, subcrestally placed, tapered implant – in cases of partial posterior mandibular edentulism.[137] A randomized controlled clinical trial assessed the effect of three abutment materials (titanium, gold-hue titanium, and zirconia) on peri-implant soft tissue and reported that abutment type did not influence

peri-implant variables after 2 years.[133] Gingival-margin, soft-tissue recession was observed only at 13% of implants irrespective of abutment type.

Contradicting results are reported in animal and human studies regarding influence of abutment surface roughness on composition and health of surrounding soft tissue. Whereas some studies reported that increased surface roughness increases the implant's biological seal[138-141], others failed to confirm this assertion.[142]

Previous studies also failed to show correlations between abutment surface roughness and inflammatory response in the surrounding soft tissue.[143,144] The aim of a recently published systematic review was to determine the peri-implant tissue response to different implant abutment materials and designs.[145] The authors concluded that the current literature provides insufficient evidence about effectiveness of various implant abutment designs and materials that favor stability of peri-implant tissues.[145]

A human histological study reported that an oxidized titanium surface provided an enhanced mucosal attachment by affecting collagen-fibers orientation. The researchers suggested that this may provide a strengthened mucosal attachment to the abutment and thereby prevent bacterial colonization and subsequent MBL.[138] But this was found after a short healing period (8 weeks), and it remains to be shown whether this attachment remains after longer follow-up. Piattelli et al. highlighted the importance of clarifying potential response of various types of cells to varying implant materials and topographies. In vitro studies using cell cultures and histological evaluation were performed in animals and humans to describe the physiological response to different surfaces.[146] Specific modifications were proposed in the surfaces to create an ideal surface that could "modulate" the cellular behavior (*e.g.*, by using laser).

Long-term effects should be studied clinically regarding various material usages, surface topographies, and designs of the transmucosal portion of the implant unit.[145] More studies are needed to clarify mechanisms involved in soft-tissue maintenance and to evaluate the function of abutments as a transmucosal component in the implant-superstructure complex.

1.4 Loading protocols for dental implant treatment

A healing period of 3–6 months before loading was originally considered as a standard procedure using dental implants for treatment of patients. Later on, the conventional treatment protocol was questioned, and immediate loading was introduced to eliminate waiting time for healing. Many clinical-based studies show positive outcomes with reduced cost and time – and high success rates.[147-152] A recently published systematic review found evidence for similar implant survival rates for immediate loading – compared to early and conventional loading in partially edentulous patients with extended edentulous sites in the posterior zone – provided that strict inclusion/exclusion criteria are followed.[50]

Unfortunately, the literature isn't always consistent regarding loading protocol definitions. As per a Schrott et al. review, the definition of terms is as follows: immediate loading within one week, early loading between 1 week and 2 months, and conventional loading after 2 months.[50]

When studying alternative loading protocols, many authors claimed the need for treatment modifiers for a successful outcome. These modifiers include bone quality, primary stability, insertion torque, implant stability quotients (ISQ) values, implant length, need for substantial bone augmentation, timing of implant placement, surface characteristics, and presence of parafunctional and smoking habits. [49,51,153,154]

In non-functionally loaded conditions, a moderately rough implant surface (*e.g.*, an oxidized surface) has been shown to promote initial bone healing, remodeling, and mechanical linking between the implant and bone.[155-157] Furthermore, such a surface has been associated with a high clinical long-term success[83,158-161], although some studies report that no difference exists compared to machined surface.[49,162,163] Another study claimed that surface roughness may not be the key factor for successful osseointegration of immediately or early loaded implants.[164] One study, which used a mini-pig model and implants with a hydrophilic sandblasted, large-grit, and acid-etched surface, compared immediate loading and delayed loading after direct installation and found that the two different methods resulted in similar levels of bone-to-implant contact (BIC).[165]

Interestingly, the initial healing of soft tissues was promoted by the application of a fixed prosthesis immediately after implant placement, possibly due to the guidance of soft tissue during initial healing and

ultimately resulting in increased soft-tissue stability.[166] But opinions vary in the literature regarding need for an immediate, temporary, or definitive prosthesis to obtain optimal results in surrounding soft tissue. So far, few studies have investigated soft-tissue reactions around implants after immediate or early loading.[167-170] So it's difficult to draw clear conclusions due to measurement heterogeneity and contradictory findings in these studies. Long-term, prospective, controlled clinical trials are necessary to identify the relationship between loading protocols and esthetic outcomes.[171]

1.5 Marginal bone loss (MBL)

Marginal bone loss around dental implants can potentially lead to implant failure. Clinical studies have reported MBL of 0.9 to 1.8 mm during the first year of loading and 0.05 to 0.13 mm annually thereafter.[32,172] Regarding MBL, the original success criteria for an implant was defined as less than 2 mm of MBL during the first year after prosthesis insertion and less than 0.2 mm of annual bone loss thereafter.[173,174] Different reports have later revised these criteria. For example, Albrektsson et al.[175] only accepted an average bone loss < 1.5 mm during the first year of function and thereafter of < 0.2 mm annually. The ICOI Pisa Consensus Conference[176] has simplified and updated a Health Scale specific for endosteal implants and claimed success as <2 mm radiographic bone loss from implant insertion surgery (including the first year).

So it's crucial to minimize MBL in the early treatment and loading stages. Most studies use the time at prosthesis insertion as baseline, but loss also occurs between implant placement and prosthesis insertion. Åstrand et al. found the bone loss between implant placement and prosthesis insertion to be several times higher than between prosthesis insertion and a 5-year follow-up.[177]

There is no clearly known single cause for MBL around dental implants and many reasons have been suggested, *e.g.*, surgical techniques, implant positioning, tissue thickness, presence of micro-gap in the prosthesis connection, and implant design.[178,179]

Effect of repeated abutment changes on MBL has been addressed. Preliminary, short-term data (4-month post-loading) in a human study showed that repeated abutment changes don't significantly alter bone levels.[180] The same conclusion was drawn in another clinical study[181], while a previous study showed that non-removal of abutments placed at the time of

surgery resulted in a statistically significant reduction of crestal bone resorption.[182]

Experiments have shown that plaque accumulation in the peri-implant area leads to inflammatory reactions and subsequent tissue breakdown.[183-185] This may also be the result of bacterial colonization in the implant-abutment interface (micro-gap).[186-188] Consequently, the implant abutment connection's vertical location may influence peri-implant bone reaction.[189]

Besides microbiological explanations, Zarone et al.[190] proposed that biomechanical factors may influence bone remodeling around implants. Occlusal forces – or lack of passive, prosthetic-framework fit – can exert stress in the system. A specific passivity level has not yet been established.[191] Finite element analysis has suggested that loading forces affecting the implant-bone interface may ultimately lead to MBL.[192,193] But animal experiments have revealed conflicting results.[194-197] In an animal experimental study, Isidor et al. demonstrated that implants could fail due to excessive occlusal load.[197] In another study, Naert showed that overload in an uninflamed peri-implant environment did not negatively affect osseointegration but supra-occlusal contacts in the presence of inflammation significantly increased plaque-induced bone resorption.[198] Taken together, the role of biomechanical factors as evaluated in animal studies is yet unclear because studies report conflicting results. It's unclear whether occlusal overload alone has the ability to create bone loss around osseointegrated dental implants. Chang et al. observed higher remodeling peri-implant bone activity around implants subjected to high loading forces.[199] Unfortunately, scientific evidence is scarce when it comes to the role of overload (*e.g.* bruxing habits) on MBL and osseointegration loss.[200]

Extremely compact bone in the mandible's posterior region was discussed as a risk factor for long-term marginal bone stability that surrounds implants.[201] Other risk factors that correlate with MBL were identified, *e.g.*, smoking[202-205], oral hygien[205], and periodontitis experience.[206,207] _ENREF_205

1.6 Methods for evaluating implant status

Various parameters may be adopted in clinical evaluations of implants, *e.g.*, plaque assessment, mucosal conditions, peri-implant probing depth, width of peri-implant keratinized mucosa, peri-implant sulcus fluid analysis, suppuration, implant mobility and discomfort, resonance frequency analysis, and radiographic evaluation.[208]

1.6.1 Clinical parameters

Oral hygiene assessment: Formation and development of microbial biofilms at oral implants are important factors to the pathogenesis of peri-implant disease, and the presence of clinically detectable plaque has been correlated with pathology development.[208] Mombelli et al. first proposed monitoring presence and development of plaque around implants[209]; they used an index – modified from the Silness Löe index[210] that was developed to monitor dental plaque formation: Score 0: No detection of plaque. Score 1: Plaque only recognized by running a probe across the implant's smooth marginal surface. Score 2: Plaque can be seen by the naked eye (visible plaque). Score 3: Abundance of soft matter.

Mucosal bleeding: As a result from peri-implant infection redness and swelling along with bleeding of the peri-implant mucosa may develop (peri-implant mucositis). Similar to monitoring plaque formation, Mombelli et al.[209] proposed a Sulcular bleeding index, modified from Löe[211], which represents peri-implant mucositis like this: Score 0: No bleeding when a periodontal probe is passed along the mucosal margin near the implant. Score 1: Isolated bleeding spots visible. Score 2: Blood forming a confluent red line on margin. Score 3: Heavy or profuse bleeding. Nevertheless, the mucosal conditions have a weak correlation with changes in implant crestal bone levels.[212]

Probing pocket depth: Under healthy conditions, peri-implant, soft-tissue crevice depth is around 3–4 mm, although higher values can be found in areas in which the implant is intentionally placed deeper. Superstructure design influences opportunities for peri-implant probing. So superstructure removal is strongly recommended before probing in implant studies (Figure 3). Probing force and angulation, probe-tip diameter, inflammatory status, and soft-tissue firmness influence the extent of probe penetration.[208] Probing-depth measurements at implants and teeth may not be fully comparable due to structural tissue differences.[118] Animal studies revealed that a probe extends closer to the marginal bone at an implant site than at the tooth.[208,213] In an animal study, no difference between teeth and implants was shown under normal healthy conditions.[214] In contrast, under pathologic conditions, probing at implants was significantly deeper than at teeth.[215,216] In a clinical study, Mombelli et al. showed that peri-implant pocket probing is more sensitive to force variation than periodontal pocket probing.[217]

Bleeding on probing (BoP): BoP in the peri-implant sulcus is used to assess inflammatory changes in the peri-implant tissues and has been recommended

when monitoring peri-implant soft-tissue conditions.[218] Animal studies have shown a clear correlation between absence of BoP and healthy conditions – and the reverse, *i.e.*, BoP when peri-implant mucositis or peri-implantitis were present.[216] But some clinical studies could not find such correlations.[219] This may be attributed to different probing forces used in different studies. Other investigations reported high negative predictive values of absence of BoP, which indicates stable peri-implant conditions.[220] Moreover, one study reported higher accuracy of BoP assessment at implant sites than at tooth sites.[221] Consequently, concomitant histology and biomolecular analyses should be carried out to validate such approaches.[221] Besides BoP, suppuration that reflects many PMN cells has been shown to be associated with severe peri-implant inflammation and tissue breakdown.[222-224]

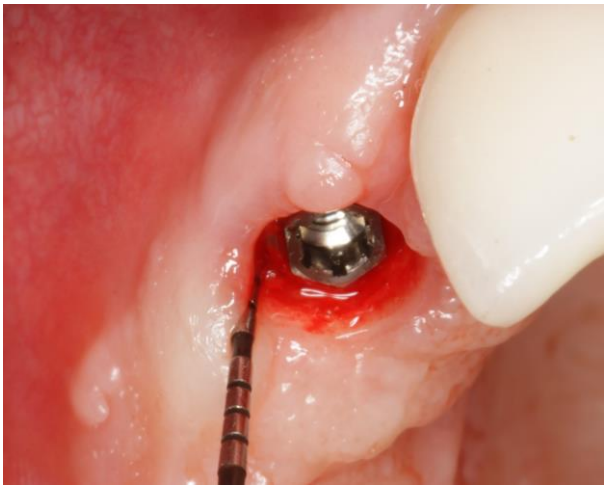

Figure 3: Clinical probing of peri-implant tissues after removing the superstructure.

1.6.2 Radiographic examination

Follow-up evaluations with intra-oral radiographs are used in most studies to determine marginal bone changes at implants. Intraoral radiography is regarded as a standard procedure in the evaluation of oral implants and has been shown to correlate with clinical parameters.[225-227] Despite the relatively good diagnostic accuracy, the probability of predicting clinical implant instability from radiographic examination can be low in populations with a low prevalence of such a condition.[228]

When comparing bone levels in serial radiographs it is essential that a standardized, reproducible technique is used. A modification of the parallel technique has been evaluated in a study by Fernández-Formoso and co-workers who found the gold standard technique preferable to the modified technique. However, the precision was high for both methods and high enough for clinical use.[229]

1.6.3 Resonance frequency analysis (RFA)

Measuring implant stability is an important implant-success evaluation method, and several functional osseointegration assessment methods are available.[230] Implant stability is achieved at two stages: primary and secondary. An implant's primary stability comes from mechanical engagement with cortical bone; secondary stability develops from bone regeneration and remodeling around the implant after insertion.[231]

Meredith et al. introduced RFA as a non-invasive tool to measure implant stability.[232] A transducer that's (i) attached to an implant and (ii) excited over a frequency range – to measure the transducer's resonance frequency (RF). Basic RF measurements (in Hz) are translated to implant stability quotients, ISQ.[233,234] RFA has been thoroughly studied and validated in vitro and in animal models.[232,235,236] It's a helpful diagnostic device for measuring implant stability and useful in detecting circular bone loss[237], and it demonstrates a high degree of inter-operator reliability and repeatability.[238] Even so, the clinical reliability for detecting partial vertical bone loss is low.[237] In addition, clinical reports demonstrated the benefits of this technology – particularly in compromised implant cases or when immediate or early implant loading is performed.[239,240] Atieh et al. claimed that RFA measurement at the time of implant placement isn't sufficiently accurate to determine implant stability and osseointegration during immediate loading protocols.[241] It is apparent that single readings using RFA are of limited clinical value. The prognostic value of RFA technology in predicting loss of implant stability has yet to be established in prospective clinical studies.[233,242,243]

1.6.4 Crevicular fluid analysis using quantitative polymerase chain reaction (qPCR)

The crevicular fluid (CF) around teeth and implants represents an inflammatory exudate that contains a mixture of serum proteins, inflammatory cells, surrounding tissue cells, and oral bacteria.[244-246] CF volume and content were analyzed in relation to orthodontic tooth movement[247,248], periodontal diseases[249-252], and implants.[249,253,254] Using

cellulose paper strips, designed for CF sampling, several studies have found associations between the increased CF volume and presence of inflammation in the gingival/periodontal tissue[255] and around implants.[256] Nevertheless, the impact of several parameters – including the sampling method, time, evaporation, and other factors – has been indicated to affect reliability of CF volume measurements.[257,258] Moreover, it's evident that the change in the CF volume, per se, doesn't provide clear information about biological mediators that are potentially involved in local processes around the implant and/or abutment. That said, molecular analyses of the content of CF mainly focused on detection of specific proteins in the CF – including inflammatory cytokines[259-261], tissue degrading enzymes[262,263], and tissue degradation products.[264,265] Up to now, protein-targeting procedures implemented technologies such as ELISA[265-267], western blotting[262], and spectrophotometry.[264]

CF proteomic analyses limitations are mainly attributed to (i) limited CF volume and (ii) sensitivity of most available technologies for simultaneously measuring several factors. These limitations inhibit simultaneous analysis of a wide range of biological mediators (*i.e.*, inflammation and tissue destruction factors plus factors that govern tissue regeneration and remodeling). Such analysis would allow for direct correlations between clinical parameters and biological factors that might mediate and/or reflect underlying processes of osseointegration around an implant.

Quantitative polymerase chain reaction (qPCR) is a highly sensitive tool that provides opportunities for analyzing panels of selected factors in limited biological materials. For instance, in a series of experimental studies on osseointegration mechanisms, qPCR was used with a sampling method to analyze gene expression that denotes several biological activities – including inflammation, cell migration, bone regeneration, and remodeling.[155,156,268] Many of these studies were done on very limited biological material, *i.e.*, implant-adherent cells after implant unscrewing. Interestingly, molecular activities in those cells strongly correlated with the degree of bone formation and biomechanical stability at the bone-implant interface.[155,269]

In the present thesis, a suggested supposition is that the combination of CF sampling and subsequent qPCR analysis of selected markers for inflammation, bone formation, and remodeling will allow for comparative and correlative analyses between the clinical parameters around implants and the underlying biological processes. Furthermore, such combination may provide a sensitive tool for early detection of biological complications around

implants – besides opportunities for implant screening and monitoring in clinical care setting.

1.7 Risks and complications

Although implant treatment is regarded as safe and reliable, complications do occur[270] – namely, biological and technical complications that sometimes lead to implant loss and even loss of the prosthetic superstructure.[72,271,272] In a systematic review, Pjetursson et al. reported a positive learning curve in implant dentistry; higher survival rates and lower complication rates occur in newer studies compared with older studies. Still, complications incidence is high, so it's important to identify problems and their etiology for better treatment outcomes.[273]

Many researchers discuss various factors that may influence treatment outcome, and a multifactorial background is likely. Porter et al. reported main predictors for implant success, namely: bone quantity and quality, patient's age, dentist's experience, plus implant placement location, implant length, axial loading, and oral hygiene maintenance.[274] They, among others, claimed that primary predictors of implant failure are poor bone quality, chronic periodontitis, systemic diseases, smoking, unresolved caries or infection, advanced age, implant location, short implants, acentric loading, an inadequate number of implants, parafunctional habits, and absence/loss of implant integration with hard and soft tissues.[274,275] Inappropriate prosthesis design might also contribute to implant failure.[274] Esposito et al. ENREF_277 divided implant failures into four groups: biological failures (related to the biological process), components' mechanical failures (implant fractures, connecting screws, coatings, and prostheses), iatrogenic failures (nerve damage and incorrect implant alignment), and functional failures (phonetical, aesthetical, and psychological problems).[276]

1.7.1 Biological complications

Careful treatment planning is essential for prevention of biological implant complications related to soft and hard tissue surrounding the implant.[277,278] These factors are important: (i) improved clinical research reporting (based on collaboration among clinicians, epidemiologists, and clinical trials specialists); (ii) applying consistent case definitions; and (iii) assessing random patient samples of adequate size and function time.[279,280]

Total implant loss is, of course, the most dramatic complication, and this is an easy outcome to study.[281] When an implant doesn't osseointegrate, it can

be regarded as an early failure – in contrast to a late failure that results from loss of an achieved osseointegration under functional conditions. A recent study reported that early implant loss occurred in 4.4% of patients (1.4% of implants), while 4.2% of the patients who were examined 9 years after treatment presented with late implant loss (2% of implants). Overall, 7.6% of the patients had lost at least 1 implant.[282] More failures are reported for implants placed in the maxilla than for those placed in the mandible.[283] Further, higher failure rates are present for treatment with overdentures and less for single tooth restorations.[278]

The definition and prevalence of peri-implant infections are controversial. Mucositis is a soft-tissue inflammation around the implant, with no bone loss. In contrast, peri-implantitis is characterized by crestal bone loss in conjunction with BoP and/or pus formation with concomitant deepening of peri-implant pockets.[284] So the diagnosis of peri-implantitis is based on clinical findings in combination with MBL detected in radiographs.

A Zitzmann et al. review reported 80% mucositis prevalence at patient level and 50% at implant level; corresponding figures for peri-implantitis were 28–56% and 12–43%, respectively.[285] The included studies in this review had ≥50 implant-treated subjects who exhibited a function time of ≥5 years.

In a recent meta-analysis, Atieh et al.[26] found slightly lower prevalence rates of mucositis and peri-implantitis: 63.4% of participants and 30.7% of the implants had peri-implant mucositis, whereas peri-implantitis occurred in 18.8% of participants and 9.6% of the implants. Higher frequency of peri-implant diseases was recorded among smokers (summary estimate of 36.3%).

A recent review reported large variation in prevalence of peri-implant mucositis (ranged from 19 to 65%) and peri-implantitis (ranged from 1 to 47%).[280] Since bone loss around implants is considered a time-dependent event, the inclusion of subjects in studies and the time of follow-up are mandatory for correct reporting of peri-implantitis prevalence.[280] Peri-implantitis rates are often higher if expressed on the patient level rather than implant level. For instance, Dvorak et al. reported that 24% of the patients and 13% of the implants were affected.[286]

Early diagnosis is important for preventing extensive problems. Insufficient evidence exists regarding ways in which infections should be treated, and the treatment prognosis is uncertain.[287] Surgical treatment is often necessary for creating space to clean around implants, and extensive plaque control is mandatory for successful long-term outcomes.[288,289]

Smokers as well as periodontitis patients are more likely to develop peri-implant lesions.[290-292] A recently published systematic review reports a low level of evidence for risk associated with implant treatment in patients with systemic conditions and underscores need for future studies.[293]

1.7.2 Technical complications

Technical complications are more frequent at implant-supported restorations compared with fixed tooth-supported prostheses. Sometimes the complications lead to implant loss and even prosthetic superstructure loss.[272,294,295] Periodontal receptors efficiently encode tooth load when subjects contact and gently manipulate food using the teeth, especially for the fine motor control. Consequently, important sensory-motor functions are lost or impaired when these receptors are removed in conjunction with teeth extractions.[296-299]

Technical complications for single crowns on implants reached a cumulative incidence of 8.8% for screw loosening, 4.1% for loss of retention, and 3.5% for veneering material fracture after 5 years.[300]

Pjetursson et al. reported that the survival rate was significantly better for metal-ceramic fixed dental prostheses compared to gold-acrylic fixed dental prostheses. The survival rate of metal-ceramic implant-supported fixed dental prostheses (FDPs) was 96.4% after 5 years and 93.9% after 10 years. Only 66.4% of the patients were free of any complications after 5 years. The most frequent complications over the 5-year observation period were veneering material fractures (13.5%), peri-implantitis and soft-tissue complications (8.5%), loss of access hole restoration (5.4%), abutment or screw loosening (5.3%), and loss of retention of cemented FDPs (4.7%).[72]

Romeo et al. reported that no increase occurs in complication rate due to cantilever presence.[301] Similarly, Aglietta et al. found no detrimental effects on bone levels due to presence of a cantilever extension *per se*.[302]

To minimize incidence of complications, dental professionals should make great effort when selecting reliable components and materials for implant-supported FDPs, and patients should be placed in a well-structured maintenance system after treatment.[72]

2 AIM

The four clinical studies in this thesis aimed to:

- Evaluate implant failures and marginal bone loss (MBL) in patients subjected to immediate or delayed (conventional) loading.
- Evaluate influence of abutment use and abutment surface design on MBL and soft tissue stability.
- Study biological and technical complications associated with implant treatment.
- Assess potential impact of risk factors on MBL.
- Explore a sampling technique and qPCR to determine gene expression as a non-invasive tool for monitoring implant healing.

3 PATIENTS AND METHODS

The thesis is based on 50 patients selected to participate in a prospective, randomized, double-blinded, parallel-arm, longitudinal, clinical trial. Results were reported after 1 year (study I), 3 years (study III), and 5 years (study IV). Eighteen of the 50 patients participated in study II, which explored a non-invasive diagnostic tool as a complement to clinical evaluations for monitoring healing and identifying peri-implant disease-specific genes.

3.1 Ethical considerations

The regional ethical review board for research at Linköping University (doc. no. M102–05), Linköping, Sweden approved studies I-IV, which were run as per Good clinical practice requirements [303], the International Conference on Harmonization guidelines, and the Declaration of Helsinki for patients participating in clinical studies. CONSORT guidelines for clinical studies were adopted.[304]

Each patient was thoroughly informed of overall requirements and procedures after explaining the study purpose, planned treatment, potential risks, and possible complications. Alternative treatment was also discussed. All information was given in verbal and written forms. Then participant signed the informed consent document.

No financial supporters influenced the studies and their results.

3.2 Patient selection and study design

The studies were conducted on partially edentulous patients who had been referred for prosthetic rehabilitation to the Institute for Postgraduate Dental Education in Jönköping, Sweden. All clinical examinations and interventions occurred in the Periodontology and Prosthetic dentistry departments.

From 2005 to 2008, 200 patients were screened for eligibility. One patient declined to participate and 149 did not meet inclusion criteria. So 50 patients (32 women, 18 men; average age 67; range 35–87) were included.

Inclusion criteria were: healthy adults, necessary dental pretreatment performed, tooth extractions, and eventual bone augmentation performed at least 3 and 6 months, respectively, before implant placement, and sufficient bone volume for 3 implants to be placed with good primary stability. These

exclusion criteria were used: smoking >10 cigarettes/day, severe malocclusion, and known bruxism.

The included patients were randomly assigned to a test group (immediate loading) or a control group (delayed loading), and each patient was assigned a code that was not revealed to the surgeon until implants were placed. Due to a logistics error, one patient was erroneously assigned to the test group. So 26 patients were assigned to the test group and 24 to the control group.

Table 1. Patients' age, sex, medical status, smoking and periodontal disease.

		Test (n=26)	Control (n=24)
Patient's information	Age [Mean (SEM)]	68.0 (1.3)	66.1 (1.1)
	Gender [Female (n)/male (n)]	16/10	16/8
Concurrent diseases	Cardiovascular disease (n)	12	9
	Diabetes mellitus, type II (n)	2	1
	Rheumatoid arthritis (n)	0	1
	Tumor disease (n)	3	3
	Osteoporosis (n)	1	0
	Respiratory disease (n)	1	0
Medications	Blood pressure medication (n)	11	11
	Statins (n)	2	7
	Low-dose antiplatelet drugs (n)	7	8
	Corticosteroids (n)	0	1
	Hypothyroid medication (n)	2	0
	Other hormone medication	1	0
Smoking	Smokers (≤10 cig/day) (n)	8	7
	Non-smokers (n)	18	17
Periodontal disease	No loss of marginal bone (n)	11	9
	Horizontal loss ≤1/3 of marginal bone (n)	9	7
	Horizontal loss >1/3 of marginal bone ± angular defects and/or furcation involvements (n)	6	8

3.3 Implants and abutments

Brånemark Mark III implants (Nobel Biocare AB) with a TiUnite™ oxidized surface were used and titanium abutments (Multiunit abutment™, Nobel Biocare AB) that had two surface designs: one with a commercially available machine-milled (AM) surface and one with a TiUnite™ surface (AOX) that was especially manufactured for this study. The most commonly used implant length was 13 mm (65%), followed by 10 mm (28%). Similarly, regular platform implants (Ø 3.75 mm) were most frequently used (80%), while narrow platform implants (Ø 3.3 mm) were used in the other sites. Both implant lengths and dimensions were evenly distributed between the two groups. In total, one hundred fifty implants were installed. Within each patient, the implants were randomly assigned to attach the superstructure directly at implant level (IL) or via abutments: one with a machine-milled surface (AM), and one with an oxidized surface, TiUnite™ (AOX) (Figure 4).

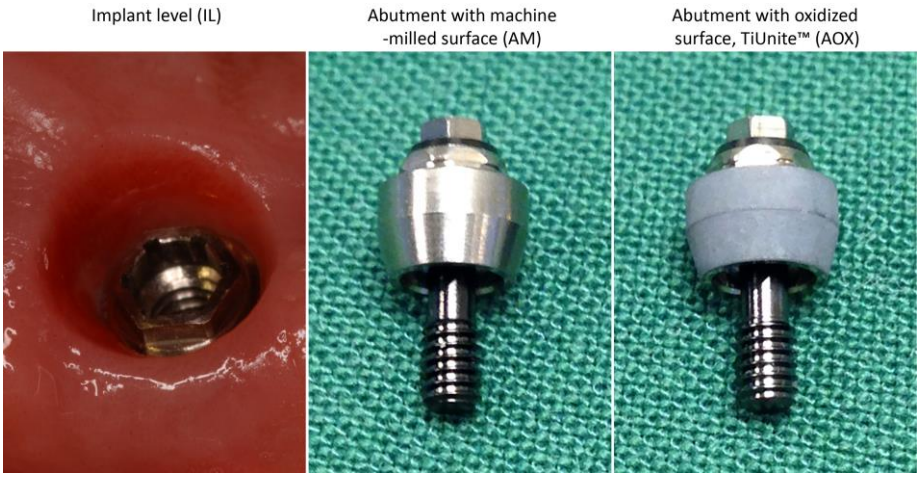

Figure 4: Different connection types.

3.4 Clinical procedures

A periodontology specialist performed all surgeries. Alveolar bonecrest width was measured 3 mm below the top of the crest using a calibrated caliper. Three implants were placed at a center-to-center distance of at least 7 mm and abutments were placed on 2 of the 3 implants. The third implant received a healing abutment. Per oral antibiotics were prescribed postoperatively, either Kåvepenin (fenoximetylpenicillin) [AstraZeneca AB, Södertälje, Sweden], 2 g twice daily for 5 days or Dalacin (clindamycin) [Pfizer AB

Täby, Sweden], 300 mg twice daily for 5 days. Patients were instructed to refrain from mechanical brushing in the operated area and instead rinse with chlorhexidine 0.1% (Hexident, Ipex Medical AB, Solna, Sweden) for 4–6 weeks. At 10 test implant sites and 16 control implant sites, previous bone augmentation was done using sinus lifting with placement of a bone substitute (no significant difference between the groups). The osteotome technique was used at 15 implant sites (4 tests and 11 controls, p .05). Particulate autogenous bone, with a guided tissue regeneration barrier, was applied at two sites in the test group and particulate autogenous bone alone was placed at nine sites (five tests and four controls).

A prosthetic dentistry specialist implemented the prosthetic treatment. The test group (immediate loading) received an implant-supported temporary bridge within 2 days. The final bridge was manufactured after 6 months. The control group had 1-stage implant surgery with implants loaded with the permanent bridge after 3–4 months. Temporary acrylic bridges were manufactured with bridge cylinders in metal and built with slight occlusal contacts in centric occlusion and group contacts in functional movements. No cantilever units were built on the temporary prostheses to avoid excessive functional loading during the early follow-up period. The final prosthesis comprised three units in 28 patients (14 tests, 14 controls) and 4 units in 22 patients (12 tests, 10 controls). Six patients received a bridge with a cantilever unit (4 tests, 2 controls). The permanent bridges consisted of titanium frameworks (ProceraTM, Nobel Biocare AB) covered with porcelain; they were designed with freedom-in-centric and with no steep cuspal inclinations or extreme lateral contacts. Temporary and permanent bridges were screw-retained. After temporary and final fixed partial prosthesis placement, a dental hygienist instructed patients in oral hygiene. If needed, repeated instructions were given at all scheduled follow-up visits.

Most patients received treatment in the posterior maxilla (40 cases) followed by posterior mandible (6 cases), frontal maxilla (2 cases) and frontal mandible (2 cases). Figure 5 shows clinical and radiographic images from a test patient.

The most common bone quality was type 3 (73%). Distribution of bone resorption was A (42%), followed by B (33%) and C (23%).[305] The average bone crest width was 6.65 (0.18) mm in the test group and 7.19 (0.17) mm in the control group, hence, significantly wider in the control group (p<0.05).

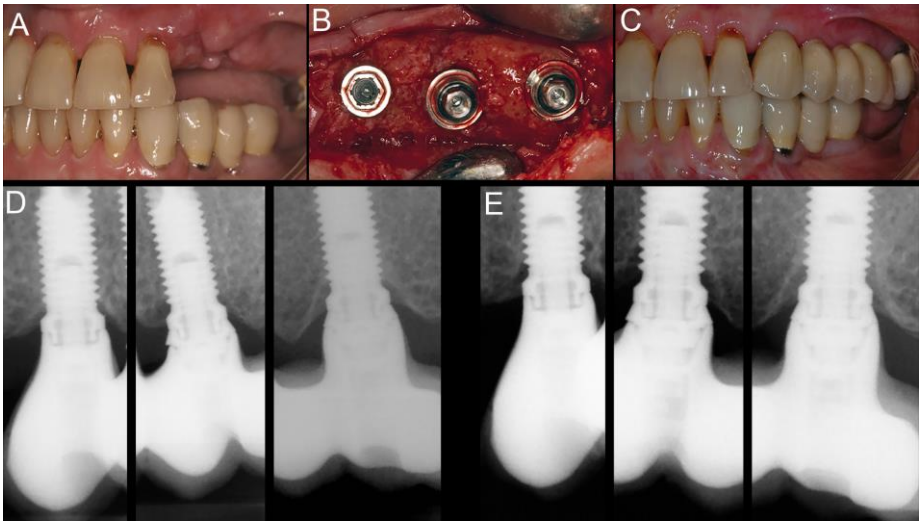

Figure 5: Clinical and radiographic images from a test patient. A) Pre-operative view. B) Three implants placed in the left maxilla. C) Permanent fixed prosthesis placed 6 months after surgery. D) Intra-oral radiographs at 1-year follow-up. E) Intra-oral radiographs at 5-year follow-up.

3.5 Clinical examinations and data collection

All clinical assessments (study I, III, IV) were made after superstructure removal with measurements taken on the day of surgery; after 2 days; 2 and 4 weeks; 3 and 6 months; and 1, 2, 3, 4, and 5 years (Figure 6).

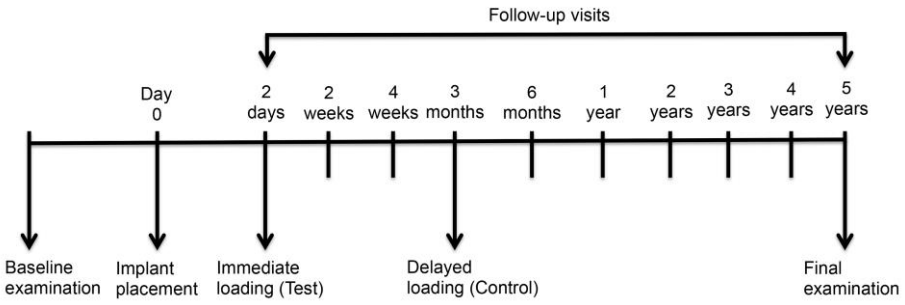

Figure 6: Outline of treatment and follow-up.

A dental hygienist, unaware of the given treatment, performed most examinations while a periodontist and a prosthodontist took measurements at surgery and after 2 days, 2 weeks, and 4 weeks. For practical reasons, these latter measurements weren't blinded.

The success of each implant was evaluated as per Smith & Zarb[306] criteria and modified as follows: the implant was considered a failure when (i) peri-implant radiolucency was noted on radiographs and/or (ii) clinical mobility was present.

Definitions and prevalence of peri-implant infections are controversial. The studies applied a peri-implantitis definition that includes crestal bone loss in conjunction with bleeding on probing (BoP) and/or pus formation with or without concomitant deepening of peri-implant pockets.[284] So the diagnosis of peri-implantitis was based on clinical findings in combination with MBL detected in radiographs.

RFA (Ostell mentor device; Ostell AB, Göteborg, Sweden) was used to determine the ISQ during and after surgery.

Plaque and sulcus bleeding scores, as per Mombelli et al.[217], were measured at mesial, distal, buccal, and lingual sites.

Peri-implant probing pocket depth (PPD) and BoP were measured at six sites of each implant (mesiobuccal, mesiolingual, distobuccal, distolingual, buccal, and lingual sites). BoP was assessed as 0 = no bleeding, 1 = minute bleeding and 2 = abundant bleeding from the pocket.

Soft-tissue coronal height – from the implant or abutment platform to the mucosal margin (Figure 7) – was measured to the nearest 0.5 mm with a periodontal probe (Hu-Friedy PCP UNC-15, Hu Friedy Inc, Leimen, Germany) at six sites.

Biological and technical complications, such as dehiscence, mucositis, hyperplasia, screw loosening, and porcelain fractures, were recorded at each follow-up appointment. Further, occlusion and jaw function, and changes in oral and general health status were registered.

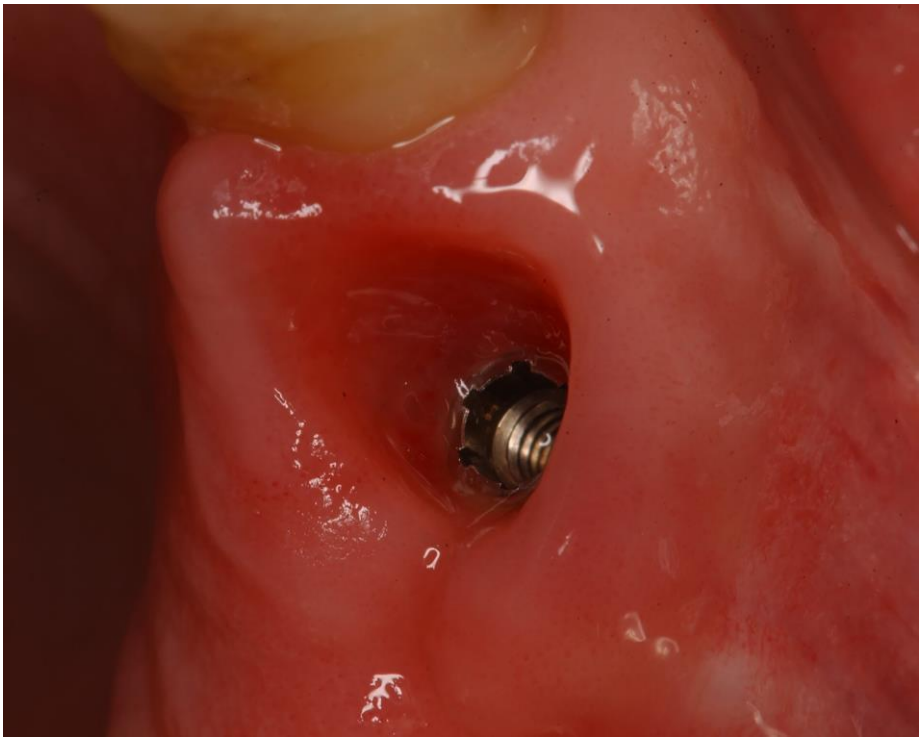

Figure 7: Soft tissue around a dental implant.

3.6 Radiographic examinations

Using a parallel method, intra-oral radiographs were captured immediately after implant placement and then after 1, 3, and 5 years. In the control group (delayed loading), radiographs were also obtained at time of loading (*i.e.*, after 3–4 months). Distance was measured between a reference point (the implant-abutment junction or the implant or prosthetic reconstruction) and the marginal bone level at mesial and distal sides of each implant. Further, presence of peri-implant radiolucency was registered. After 3 years, MBL was examined with regard to whether the neighbor was an implant, a tooth, or an edentulous area.

An oral and maxillofacial radiology specialist performed all measurements and interpretations without knowing treatment allocation.

3.7 Gene expression analyses and microscopic analyses (study II)

3.7.1 Sampling procedure

Eighteen patients were selected (9 tests, 9 controls) for this exploratory study. CF was collected using standardized paper strips (PeriopaperTM, Proflow, Amityville, USA) at the implants provided with abutments from each patient at 2, 14, 28, and 90 days after surgery (Figure 8).

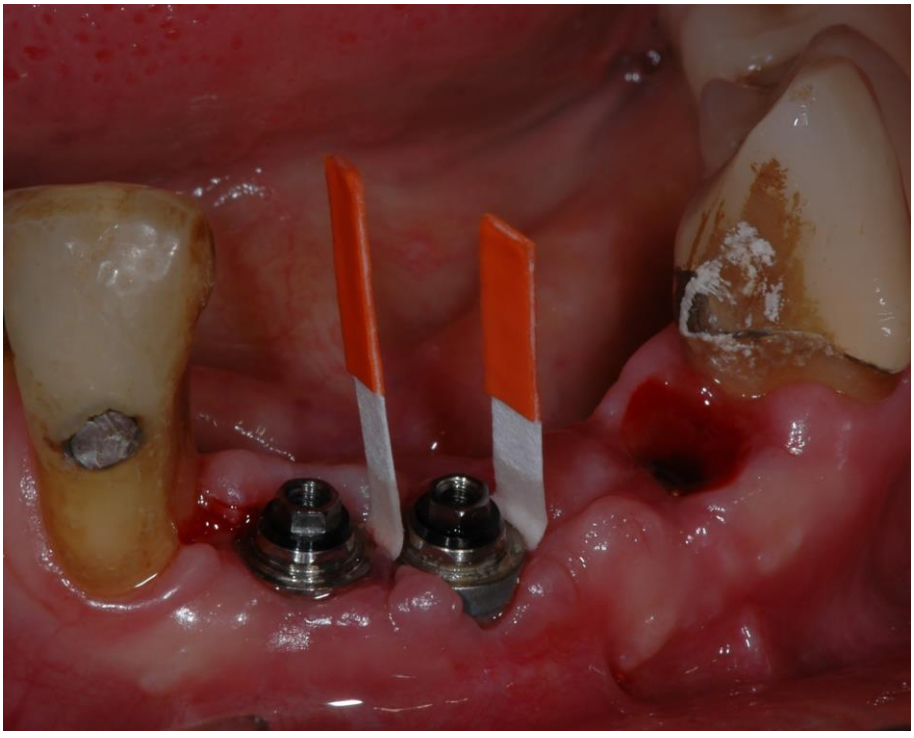

Figure 8: Postoperative crevicular fluid sampling after 2 weeks

Healing caps and fixed dental prostheses were removed and cotton rolls were applied to avoid saliva contamination in the sampling areas. One strip was inserted in the crevice at the abutment's mesial side for 1 minute and thereafter placed in RNAlater® (Ambion, Applied Biosystems, Austin, TX, USA). After a wash-out period of 2 minutes, the sampling procedure was repeated.

3.7.2 Quantitative polymerase chain reaction (qPCR)

RNA from cells attached to the strips was extracted and purified and converted to cDNA. Quantitative PCR assays for interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), osteocalcin (OC), alkaline phosphatase (ALP), cathepsin K (CK), tartrate resistant acid phosphatase (TRAP), and 18S ribosomal RNA were designed and validated (Figure 9). Relative gene expression levels were calculated by normalizing gene expression of each gene using 18S ribosomal RNA and the delta-delta Ct method using 90% efficiency for each assay.[307,308]

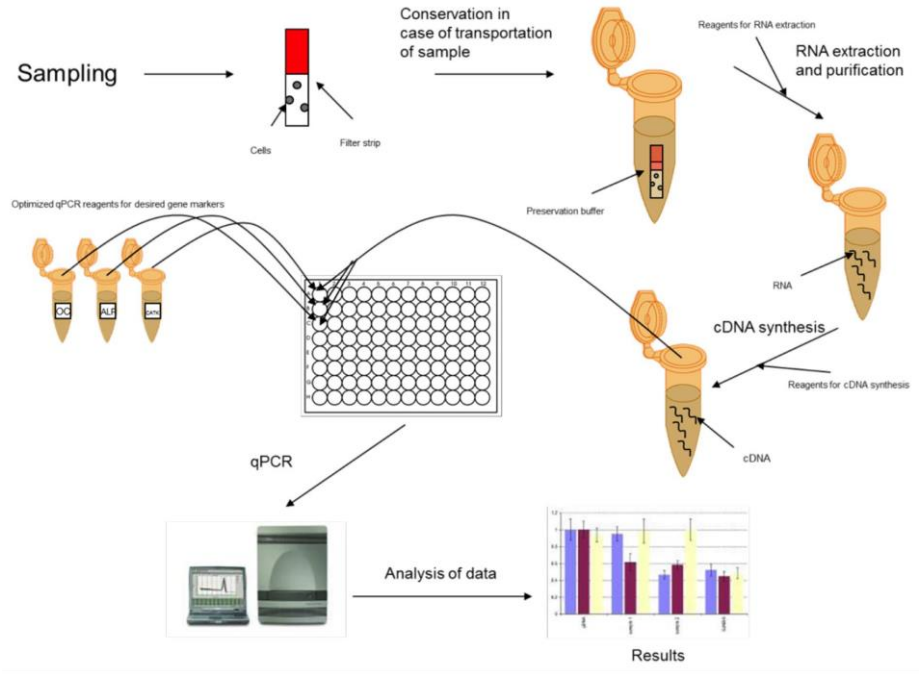

Figure 9: Schematic representation of the sampling procedure and qPCR analysis.

3.8 Power analysis

All data were transferred to IBM SPSS. The primary outcome was peri-implant MBL after 1 year. Based on the literature, expected bone loss 1 year after conventional loading is 1.2 mm. A difference of 0.4 mm between the groups, with a standard deviation of 0.8 mm and 80% power with $\alpha < 0.05$, gave a sample size of 63 implants in each group.

3.9 Calibration and blind examination

The clinical examiners were calibrated before the study's start. Duplicate measurements of soft-tissue height and pocket depth were made to assess intra- and inter-examiner reproducibility. Intra-examiner intra-class correlation coefficients (ICCs) were 0.94 (95% confidence interval [CI]: 0.90–0.96) for the prosthodontist; 0.94 (CI: 0.91–0.97) for the periodontist; and 0.96 (CI: 0.94-0.98) for the dental hygienist. The inter-examiner ICC was 0.91 (CI: 0.85–0.95).

3.10 Statistics

All data were transferred to IBM SPSS. Descriptive and analytical statistics were generated. Mean and SEM or medians (min-max) were calculated for each parameter. Student's t-test, Mann-Whitney U test, one-way ANOVA, and the Kruskal-Wallis test were used for group comparisons. Spearman rank correlation was run to study correlations between gene expressions and clinical parameters (study II).

Secondary outcomes were implant survival, peri-implant soft-tissue level, PPD– plus values for plaque prevalence, sulcus bleeding, and BoP.

MBL from surgery to 3 and 5 years was the dependent variable in univariate and stepwise multiple regression analyses. Independent variables were general health, medication, periodontal disease experience, smoking (≤10 cigarettes/day), allocation to intervention (test and control), type of connection (IL, AM, AOX), ISQ, plaque, mucosal bleeding, PPD, and abundant BoP.

In the analyses, BoP occurrence was coded into two groups – depending on if none or if at least one of the two proximal registrations showed positive findings. Only the registrations for abundant bleeding were included as positive findings in the regression analyses.

When analyzing 5-year results, the test and control groups were merged, and results were adjusted for effect from age and type of connection.

All reported p values were two-sided and considered statistically significant when $p<0.05$.

4 RESULTS

After one year, all 50 patients were eligible for examination. After three years, 46 patients were eligible (1 patient died of natural causes, 2 patients could not attend due to deteriorated general health, 1 was excluded because of conversion to full-arch prosthesis after implant loss). After five years, 44 patients were eligible (4 patients for reasons as mentioned after 3 years, and 2 additional patients, *i.e.*, one moved to another location and one could not attend due to deteriorated general health).

4.1 Studies I, III, and IV

4.1.1 Implant survival

Up to 1 year, four implants were lost in patients subjected to immediate loading (test group) and two in the delayed loading (control group). Lost implants were replaced after appropriate healing time but excluded from the study. No additional loss of implants occurred thereafter, resulting in 5-year, cumulative, survival rates of 93.9% and 97.0%, test and control, respectively (no significant intergroup difference); see Figure 10.

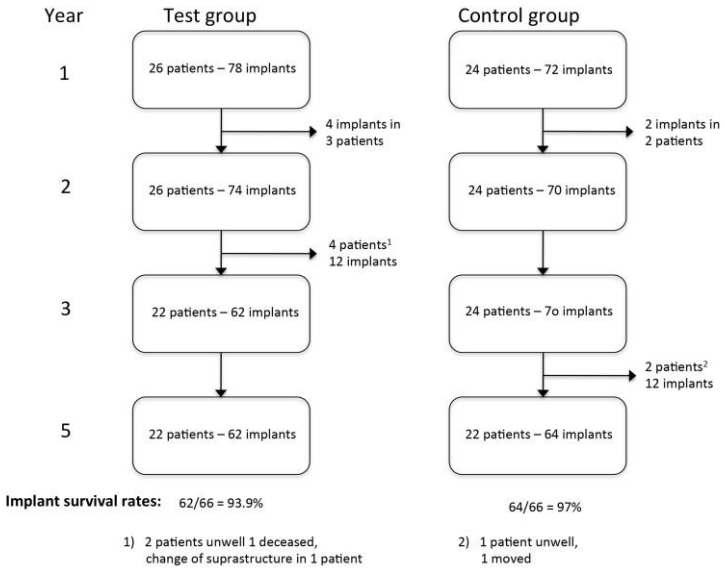

Figure 10: Flow-chart during the follow-up.

Some sites (n=58) were unaccounted for due to missing clinical assessments or unreadable radiographs.

4.1.2 Marginal bone loss (MBL)

After 1 year, the MBL around the implants was, on average (SEM), 1.33 mm (0.08) in the test (immediately loaded) group, and 1.25 mm (0.08) in the control group with no significant intergroup difference (Table 2). Analyses of merged groups revealed significantly larger mean bone loss at implants without abutment – compared with implants with machine-milled (AM) oxidized (AOX) abutments (Table 3).

Between 1 and 3 years, non-significant MBL occurred: 0.36 mm (0.08) and 0.33 mm (0.06) in test and control groups, respectively. When merging the two groups and comparing bone loss regarding type of connection, a non-significant difference was found from surgery to 3 years between IL (1.81 mm [0.93]) and AOX (1.77 mm [0.14]), while significantly lower bone loss was found at AM (1.42 mm [0.17]) compared with IL ($p<0.05$). Between 1 and 3 years, AOX displayed significantly more bone loss than IL, 0.51 mm (0.11) and 0.22 mm (0.62), respectively.

Between 3 and 5 years, a similar MBL occurred in the test and control groups: 0.22 mm (0.09) and 0.22 mm (0.07), respectively (NS). After 5 years, the total MBL was 1.88 mm (0.09) (test and control group merged; Table 3).

After 5 years, a significantly lower MBL was found at implants connected to AM (1.60 mm [0.18]) than at sites with IL (2.09 mm [0.13]).

To conclude, a significant difference of MBL between IL and AOX and between IL and AM for the total group was found after 1 year but the difference between IL and AOX disappeared after 3 and 5 years (Table 3).

No influence on bone loss was found when the implant faced a tooth, an implant, or an edentulous area up to 3 year (Figure 11); consequently, additional analyses weren't performed after 5 year.

Table 2. Mean (SEM) marginal bone loss (MBL) at implant sides, in millimeter, from surgery to 1 year, between 1 and 3 years and between 3 and 5 years with regard to superstructure connection.

	Test (immediate loading)			**Control (delayed loading)**		
	Surgery-1 year	1-3 years	3-5 years	Surgery-1 years	1-3 years	3-5 years
Implant-level (IL)	1.65 (0.12)$^{\alpha}$	0.23 (0.08)	0.14 (0.15)	1.54 (0.12)	0.21 (0.09)	0.42 (0.10)
n	48	43	43	43	48	44
Machine-milled (AM)	0.97 (0.17)$^{\alpha}$	0.43 (0.14)	0.21 (0.16)	1.10 (0.14)	0.21 (0.10)	0.13 (0.11)
n	43	38	40	41	45	42
Oxidized (AOX)	1.32 (0.13)	0.44 (0.19)	0.31 (0.17)	1.10 (0.13)	0.56 (0.12)	0.11 (0.13)
n	44	38	40	41	46	42
Total (all connection types)	1.33 (0.08)	0.36 (0.08)	0.22 (0.09)	1.25 (0.08)	0.33 (0.06)	0.22 (0.07)
n	135	119	123	125	139	128

α = p<0.01, Implant level vs. Machine-milled abutment (in test group)

Table 3. Mean (SEM) marginal bone loss at implant sides, in millimeter, at different times with regard to superstructure connection. Data pooled for immediately (test) and delayed (control) loaded groups.

	Merged groups				
	Surgery- 1 year	1-3 years	Surgery-3 years	3-5 years	Surgery- 5 years
Implant-level (IL)	1.60 (0.08)$^{\alpha\beta}$	0.22 (0.62)$^{\gamma}$	1.81 (0.93)$^{\delta}$	0.28 (0.09)	2.09 (0.13)$^{\varepsilon}$
n	91	91	85	87	84
Machine-milled (AM)	1.04 (0.11)$^{\alpha}$	0.31 (0.08)	1.42 (0.17)$^{\delta}$	0.17 (0.10)	1.60 (0.18)$^{\varepsilon}$
n	84	83	81	82	79
Oxidized (AOX)	1.21 (0.09)$^{\beta}$	0.51 (0.11)$^{\gamma}$	1.77 (0.14)	0.2 (0.11)	1.95 (0.15)
n	85	84	81	82	79
Total (all connection types)	1.29 (0.56)	0.34 (0.49)	1.67 (0.79)	0.22 (0.06)	1.88 (0.09)
n	260	258	247	251	242

α = p<0.001, Implant level vs. machine-milled abutment
β = p<0.05, Implant level vs. oxidized abutment
γ = p<0.05, Implant level vs. oxidized abutment
δ = p<0.05, Implant level vs. machine-milled abutment
ε = p<0.05, Implant level vs. machine-milled abutment

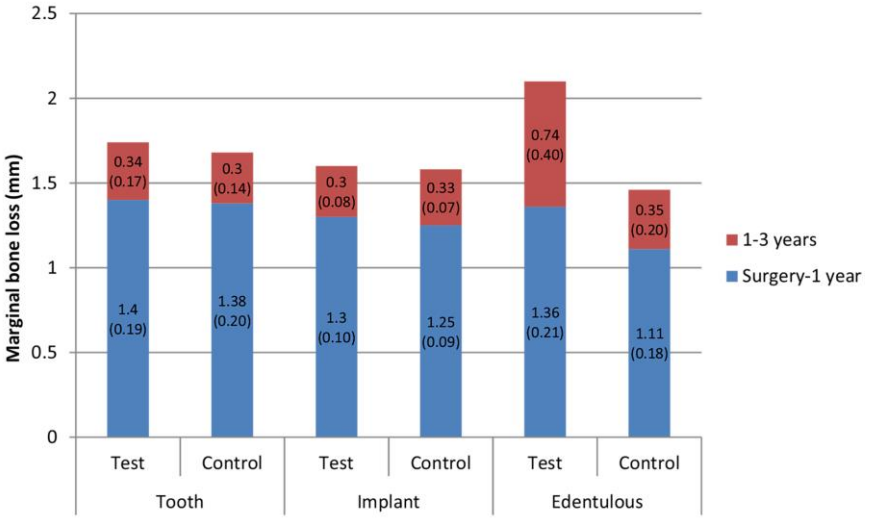

Figure 11: Mean (SEM) MBL, in millimeter, around implant sites facing a tooth, an implant, or edentulous area. No significant within- and inter-group differences were found.

4.1.2.1 Multiple linear regression analyses, marginal bone

MBL was the dependent variable (Table 4) during stepwise, multiple-linear regression analyses. Significant independent variables from univariate analyses were entered in the multiple regression analysis.

For the merged group (test and control) after 3 years, the independent variables smoking (≤10 cigarettes/day), abundant BoP, and ISQ after 2 days could significantly explain MBL. These independent variables explained about 9.7% of the variation in MBL after 3 years.

After 5 years, these independent variables could explain MBL: health deterioration, high blood pressure medication, periodontal disease experience, smoking (≤10 cigarettes/day), and PPD. These independent variables explained about 27% of the variation in MBL after 5 years.

Table 4. Multivariate linear regression analysis with the marginal bone loss (MBL) as a dependent variable (surgery to 5 years).

Independent variable		**Adjusted R^2**	**β-coefficient**	**95% confidence interval (c.i.)**	***p-value***
Adjusted for	Age	0.267	0.015	-0.002 – 0.032	0.085
	Abutment/no abutment		-0.031	-0.228 – 0.166	0.758
Periodontal disease experience			-0.243	-0.341 – -0.145	0.000
Deteriorating health			-1.085	-1.472 – -0.699	0.000
Smoking ≤10 cigarettes/day			-0.458	-0.821 – -0.095	0.014
Medication for high blood pressure			-3.117	-5.514 – -0.721	0.011
Proximal pocket depth			-0.183	-0.335 – -0.032	0.018

4.1.3 Resonance frequency analysis (RFA)

The ISQ at surgery showed a highly significant correlation to assessments of bone quality and degree of resorption. The most common bone quality was type 3 (73%), followed by type 4 (15%), and type 2 (12%). Distribution of bone resorption was A (42%), followed by B (33%) and C (23%), D (1%), and E (1%). The average bone crest width was 6.65 mm (0.18) in the test group and 7.19 mm (0.17) in the control group, hence, significantly wider in the control group ($p<0.05$).

Regression analyses on test and control data (pooled), which used implant failure and MBL as dependent variables, could not demonstrate any correlation to bone quality, resorption, or crest width. Accordingly, the ISQ as measured during surgery and during the 1-year follow-up wasn't found to correlate to the dependent variables (study I).
In general, the delayed loading (control) group demonstrated higher ISQ values throughout the 5-year period, and a significantly higher ISQ was found in the control group compared to the test group at 2 and 4 weeks

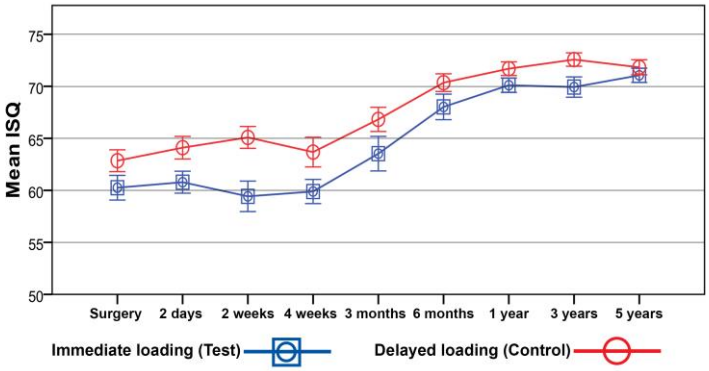

Figure 12: Mean implant stability measurements (ISQ) during follow-up. Error bars show SEM.

(Figure 12). Both groups revealed essentially similar unchanged ISQ levels from surgery to 4 weeks. After 4 weeks, a similar increase of RFA values was found in both groups.

4.1.4 Soft-tissue variables

4.1.4.1 Plaque and mucosal bleeding

Regarding plaque and mucosal bleeding, the types of superstructure-connection (IL, AM, and AOX) and loading groups (test and control) are merged. Initially, plaque and inflammation scores were low irrespective of treatment. With time, plaque and mucosal bleeding increased, whereas a minor decrease for bleeding was found between 3 and 5 years (Figure 13).

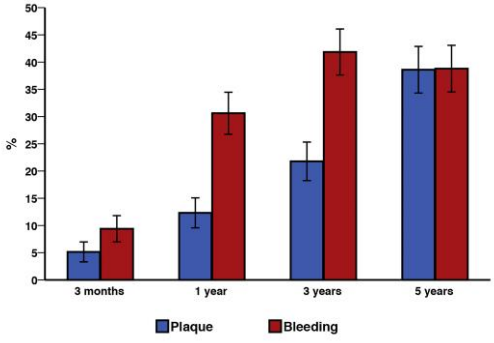

Figure 13: Plaque and mucosal bleeding percentages (%) during the follow-up period. Data pooled for test and control groups. The column graph shows the mean values and the SEM of the pooled percentages for immediate (test) and delayed (control) loading groups. Statistically significant differences ($p<0.05$) are reported as following: For plaque, between 3 months and 1 year; between 1 year and 3 years; between 3 years and 5 years. For bleeding between 3 months and 1 year and between 1 year and 3 years. No significant difference was reported for the bleeding between 3 years and 5 years.

Plaque prevalence and mucosal bleeding after 5 years were 38.6% and 38.8%, respectively. Figure 13 shows the statistically significant differences.

4.1.4.2 Pocket probing depth (PPD) and bleeding on probing (BoP)

PPD for the merged groups showed only minor alterations at all implant sites up to 3 year, and thereafter, a marked increase occurs, especially for proximal sites (Figure 14). The increase between 3 and 5 years for proximal sites was not statistically significant (for any connection type). PPD values were similar in test and control groups up to the 3-year examination for all superstructure connections. At 3 and 5 years, a nonsignificant difference was found when comparing test and control groups at proximal sites and buccal sites. At buccal sites, the PPD underwent the smallest changes. A significantly lower PPD was found at buccal sites compared with proximal and lingual sites ($p < 0.05$). Significantly higher proximal PPD was found around IL compared with AM and AOX after 3 years ($p < 0.05$) and 5 years ($p<0.001$). At buccal sites, PPD decreased at IL and AM, while PPD at AOX showed a small increase up to 1 year. From 3 years, a small increase in PPD was found for AM and AOX sites, whereas at IL sites, the PPD remained largely unchanged up to 5 years. The lingual PPD underwent only minimal changes up to 3 years and then a small increase was observed.

The frequency of BoP increased over time (Figure 15). At 3 and 5 years, minute BoP was found in 30–40% of the sites, while abundant BoP occurred at about 20% of the sites. No statistical differences with respect to BoP and type of superstructure connection were found.

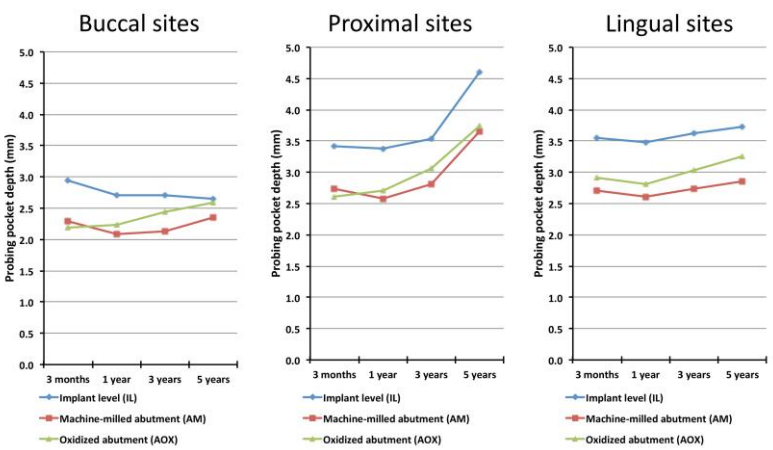

Figure 14: Mean PPD, in millimeter, at surgery, 1 year, 3 years, and 5 years with regard to superstructure connection.

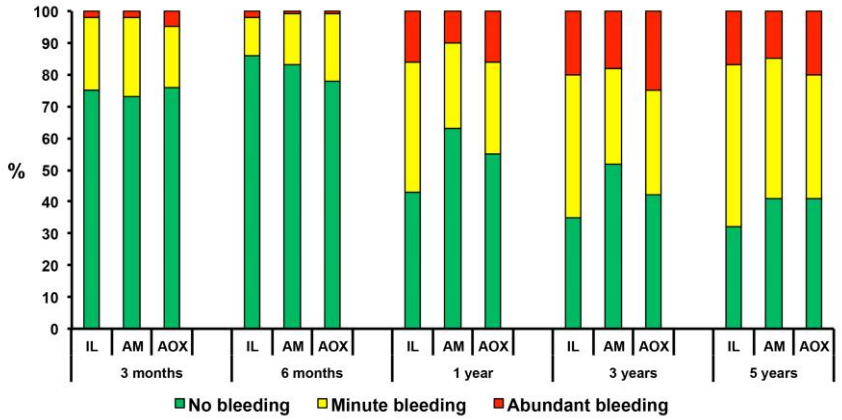

Figure 15: BoP percentage (%) regarding superstructure connection. The data is pooled for immediate (test) and delayed (control) loading groups.

4.1.4.3 Soft tissue height

The most pronounced retractions in the soft tissue occurred during the first year, and thereafter only minor changes at proximal and buccal sites were found (Figure 16). Significantly higher proximal soft-tissue height was found in the control group versus test group at surgery (p<0.005), after 2 days (p<0.001), and at 2 and 4 weeks (p<0.005). Thereafter, no differences were found. At proximal sites, the retraction was most pronounced in the control group, and this difference was statistically significant up to 1 year (p<0.001). Accordingly, the buccal sites displayed the same baseline height difference and retraction at AOX and AM between test and control groups. After implant surgery, the mean (SEM) buccal soft-tissue height varied between 1.04 mm (0.31) (AOX) and 2.85 mm (0.21) (IL). Retractions during the first year were 1.17 mm (0.14) in the test group and 1.79 mm (0.16) in the control group. When merging test and control groups, over the first year, a larger retraction was found buccally (1.49 mm [0.11]) than proximally (0.94 mm [0.08]) (p<0 .05). Between 1 and 3 years, minor retractions of the buccal soft-tissue height were found (on average 0.08 mm [0.06] in the test group and 0.07 mm [0.07] in the control group). At the proximal sites, a minor height increase was found (0.13 mm [0.06] and 0.14 mm [0.07], test and control groups, respectively). At the 5-year examination, the soft-tissue height shows no significant differences between test and control groups. When analyzing the proximal and buccal sites, no differences exist between AOX and AM, but a significant difference (p<0.001) exist when comparing IL with AM and AOX. Lingual soft-tissue height remained stable throughout the study period with no intergroup difference.

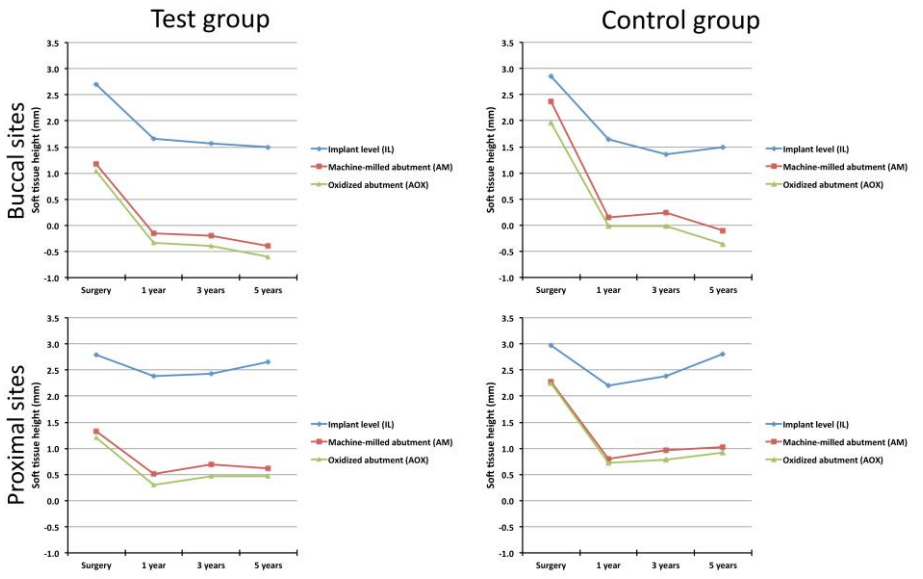

Figure 16: Mean soft-tissue height at implant sites in millimeter at surgery, 1 year, 3 years, and 5 years for superstructure connection.

4.1.5 Complications

Figures 17 (A and B) display biological and technical complications. After 3 years, peri-implantitis (5 patients) affected 3 test and 3 control implants. After 5 years, 5 implants (3 tests and 2 controls) showed peri-implantitis (4 patients). One control patient, who exhibited 2 implants with peri-implantitis after 3 years, wasn't possible to re-examine because she moved to another location. So after 5 years, the four implants with peri-implantitis were the same as were recorded at the 3-year examination and only one implant (control) was added to the group. Hence, after 5 years, peri-implantitis prevalence was 9.1% at patient level and 4.0% at implant level.

Few technical complications occurred after the first year; the most common was porcelain chipping (Figure 17 B).

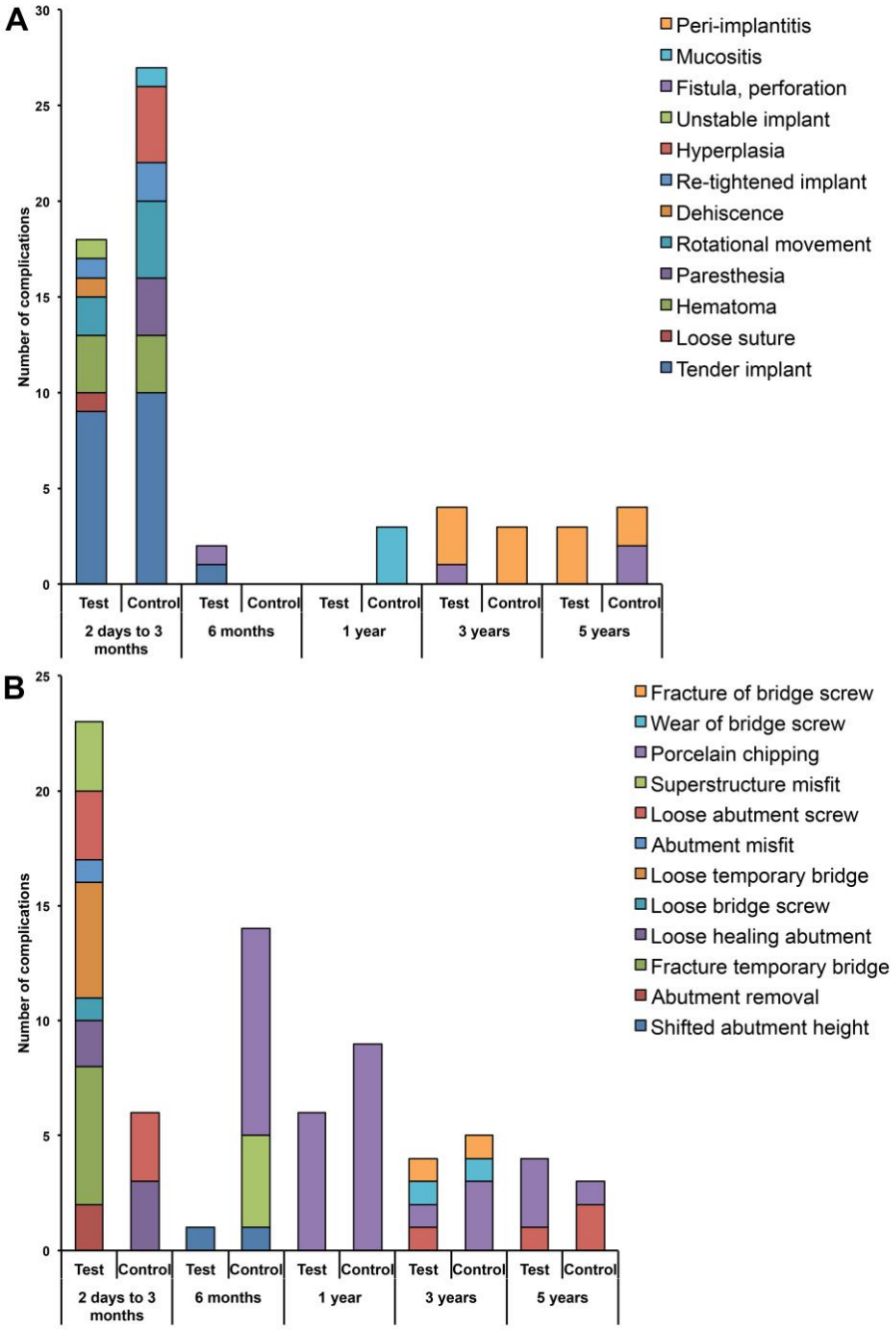

Figure 17: Biological (A) and technical (B) complications from day-2 to 5-year examinations. Data pooled for type of connection.

4.2 Study II

4.2.1 Analyses of peri-implant crevicular fluid (CF) after placement and loading of dental implants

At the two-day sampling (postoperatively), a considerable flow of peri-implant CF was observed at most sites. The flow gradually diminished during subsequent sampling. At day 90, hardly any fluid could be clinically detected.

4.2.1.1 Microscopic findings

Light-microscopic (LM) analyses showed relatively large quantities of cells at the strips' rims (Figure 18A), but some cells had also penetrated into the cellulose mesh. Most of these cells were granulocytes and monocytes, but occasional cells with mesenchymal morphology were also recorded (Figure 18B). Scanning electron microscopy analysis showed the same appearance with cells entrapped in the strips' cellulose fiber network. The most common cells were erythrocytes followed by platelets. But granulocytes and larger cells were also observed and surrounded by fibrin (Figure 18C). No attempts were made to determine the number of cells at various time points.

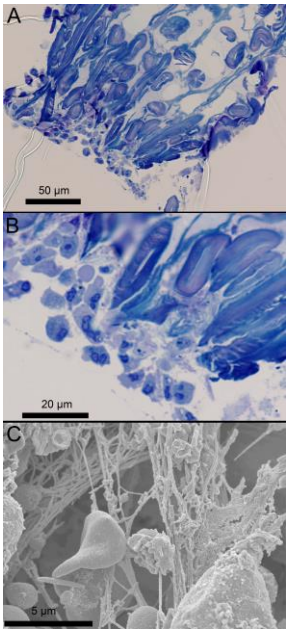

Figure 18: (A) LM image of filter strips after insertion in a peri-implant crevice for 60 seconds. Cells penetrating the rim of the cellulose filter strip. (B) Higher magnification of Figure 18A. Most of the cells are granulocytes/monocytes but occasional cells with mesenchymal morphology are also found. (C) SEM images of filter strips after insertion in a peri-implant crevice for 60 seconds. An erythrocyte and a thrombocyte are shown attached to the fibrin mesh. Magnification × 7250.

4.2.1.2 qPCR analysis

Table 5 shows the relative expression levels of various genes in the selected panel. Higher expression of TNF-α was detected during the early time points and gradually decreased thereafter, while OC and ALP revealed a relative increase over time. No major changes were found for IL-1β, CK, and TRAP. The following is a short summary of findings for the particular genes:

IL-1β Two days postoperatively, significantly higher expression was found at machine-milled abutments in the conventional loaded group (control), compared to AM in the immediate loaded group (test). No other significant differences were found at any time point.

TNF-α No significant differences were found between machine-milled abutments and TiUnite™ abutments (AOX) and between immediately loaded (test) and conventional loaded (control) implants. During selective CF analysis in one test patient with two unstable implants at 90 days, TNF-α exhibited higher expression at this time point than at the earlier assessments (Figure 19).

OC At TiUnite™ abutments (AOX), conventional loaded implants (control) showed significantly higher OC expression than immediately loaded implants (test) at 14 days. In the control implants, OC expression was significantly higher at AOX abutment – compared to AM abutments in the test implants at 14 days.

ALP At TiUnite™ abutments (AOX), ALP showed a significantly higher expression in the conventional loaded (control) implants compared to AOX in the immediate loaded group after 90 days. No other significant differences were found for this gene.

CK Significantly higher expression of CK was detected for the AOX abutments in the conventional loaded group (control) – compared to AOX abutments in the immediately loaded group (test) at 14 days, while no other differences were found.

TRAP In the conventional loaded group (control), TiUnite™ abutments (AOX) showed significantly higher expression than machine-milled abutments at 14 days.

Table 5. Relative gene expression (Mean; SEM). All values are $\times 10^{-6}$ (values with $\leq \times 10^{-6}$ are omitted). n = *samples showing gene expression.*

		Test (immediate loading)								Control (delayed loading)							
Gene	**Surface**	**2 days**	***n***	**14 days**	***n***	**28 days**	***n***	**90 days**	***n***	**2 days**	***n***	**14 days**	***n***	**28 days**	***n***	**90 days**	***n***
IL-1β	AM	107.2 (105.7)	5	225.5 (225.2)	4	1140.1 (1041.7)	4	1453 (1173.5)	4	1272.5 (417.2)	8	477.4 (176.9)	8	659.3 (226.3)	9	675.6 (382.4)	7
	AOX	314.9 (287.8)	6	1341.1 (1341)	2	251.8 (126.7)	3	294 (99.6)	4	976.6 (427.3)	8	438.1 (168.7)	9	438.9 (151.5)	8	579.3 (144.6)	8
TNF-α	AM	59.4 (29.4)	5	12.5 (4.2)	4	17.7 (11.9)	4	12.1 (6.7)	3	87 (43.3)	8	11.9 (7.3)	8	13.7 (5.8)	9	5.1 (1.1)	7
	AOX	99.4 (56.8)	6	67.5 (40.9)	4	11.6 (7.8)	4	15.5 (13.1)	4	49.9 (30.7)	8	20.2 (7.5)	9	13.2 (4.8)	8	17.5 (8.2)	7
OC	AM	1.5 (0.4)	5	1 (0.2)	3	1.9 (0.5)	2	4.8 (2.6)	3	3.7 (1.4)	4	1.1 (0.3)	5	3.5 (1.0)	7	1.7 (0.3)	5
	AOX	2.6 (1.2)	4	0.8 (0.3)	2	1.5 (0.6)	3	2.6 (1.2)	4	1.7 (1.4)	3	9.2 (2.9)	9	3.1 (0.9)	5	43.6 (29.6)	5
ALP	AM	22.7 (0.8)	5	20 (11.5)	4	11.3 (9.7)	4	5.3 (3.6)	4	14.5 (4.3)	8	7.1 (1.4)	8	22.3 (15.8)	9	13.7 (9.9)	6
	AOX	32.8 (15)	6	7 (3.5)	3	13 (10.4)	4	4.5 (1.2)	4	13.5 (3.4)	8	15.9 (4.9)	9	8.8 (3.1)	8	27.2 (9.9)	8
CK	AM	2.1 (0.4)	4	11.9 (10.3)	3	36 (34.5)	3	18.9 (17.4)	3	3.6 (1.3)	3	20.1 (17.7)	3	2.1 (0.8)	5	7 (3.9)	5
	AOX	2.2 (0.8)	4	0.6 (0.2)	2	1.3 (0.5)	3	1.1 (0.4)	4	2.1 (0.2)	3	16.9 (12.2)	6	5.3 (2.3)	5	26.4 (22.9)	5
TRAP	AM	1.5 (0.7)	4	6.5 (0.4)	4	11.4 (9)	4	3.5 (1.5)	3	1.6 (0.7)	4	1.9 (0.5)	8	4.9 (3)	7	2.8 (0.7)	7
	AOX	2.6 (1)	6	3.1 (0.8)	4	1.7 (0.3)	3	1.2 (0.2)	4	3.1 (1)	7	15.8 (8.0)	9	2.6 (0.6)	8	13.3 (8.7)	6

AM = Machine-milled abutment surface; AOX = Oxidized abutment surface.

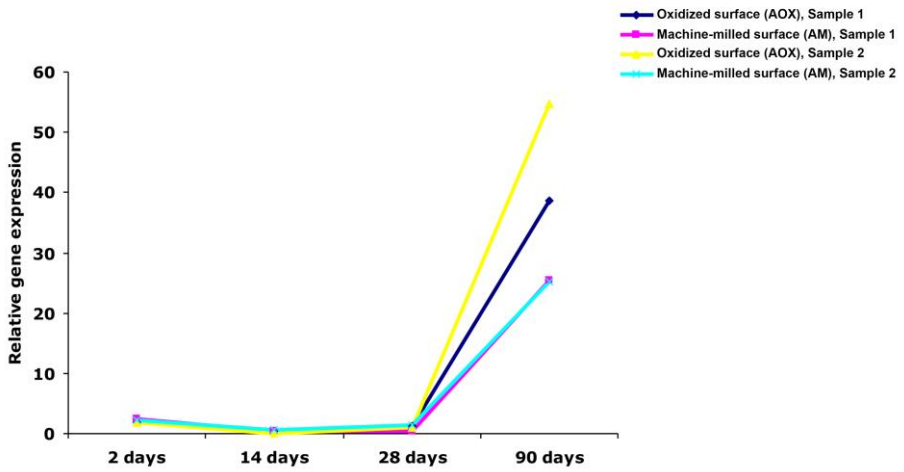

Figure 19: Gene expression of TNF-α at machine-milled (AM)and oxidized (AOX) abutments for one patient subjected to immediate loading. At 90 days, a sharp increase of TNF-α was found. Both implants were unstable and were subsequently removed. Two samples were collected at each time point.

5 DISCUSSION

5.1 Discussion of materials and methods

This thesis is based on a prospective, randomized, double-blinded, parallel-arm, longitudinal, clinical trial. Random clinical trials (RCTs) are often used to test efficacy or effectiveness of various interventions; they also provide information about adverse effects and are often considered the gold standard for clinical trials.[309]

In the present RCT, two experienced specialists treated patients to evaluate two different treatment protocols. Consequently, the research setting, with properly selected participants, under controlled conditions, provides a high level of internal validity but may jeopardize the study´s effectiveness and external validity.[310] The documentation of treatment outcomes in this field is mostly confined to small patient groups and *efficacy* evaluations *i.e.*, the probability of an intervention being beneficial to patients under ideal conditions.[311] Multicenter studies with different categories of clinicians (*e.g.* specialists and general practice dentists) often include many participants, different geographic locations, a wide range of population groups, and the ability to compare results among centers. All of these factors increase the generalizability of a given study. It should be kept in mind that the study design can induce a gain or a loss of power.[312]

The patients in the present study were monitored via a strict follow-up regime. A prosthodontist, periodontist, and dental hygienist registered all clinical parameters; they have extensive experience with patients rehabilitated with implant treatment. High levels of inter- and intra-class correlation coefficients were shown and thus high levels of inter- and intra-examiner agreement.
A clinical RCT study investigates a treatment outcome and provides knowledge that cannot be otherwise obtained. The prospective study design is considered more powerful than the retrospective – due to elimination or minimization of unconscious bias. However, it has been proposed that large, retrospective evaluations of treatment outcome provide the only hope for realizing significant answers concerning implant prosthodontics.[313] If only prospective, controlled clinical trials have to be used to answer specific questions, such questions may never be answered.

Intra-oral radiographs using a paralleling technique were obtained on the day of surgery and after 1, 3, and 5 years. The measurements and interpretations

of the radiographs were performed by a single specialist in oral and maxillofacial radiology blinded to whether patients belonged to the test or control group. The participating radiologist has previously participated in many similar studies and evaluations, revealed consistent measurements with good agreement.[314]

Radiographs are useful in identifying osseointegration loss but the uncertain diagnostic accuracy cannot be discounted. So fixed prosthesis removal might be needed for definitive confirmation of osseointegration loss. In the present study, it is an advantage that all superstructures were removed after 2 days, 2 weeks, 4 weeks, 3 months, 6 months, 1 year, 3 years, and 5 years and, hence, the implant stability could be evaluated clinically.

Previously, a high positive predictive value (83%) was demonstrated for radiographically identified failing implants, only 5% were clinically found to be failing without having been detected radiographically.[315] This indicates that the radiographic identification of unstable implants is reliable when performing annual examinations and routinely examining patients over the long term. However, it is also emphasized that intraoral radiographs cannot be relied on as the sole diagnostic test and the removal of superstructure is still mandatory.[227] Despite relatively good diagnostic accuracy, the probability of predicting clinical implant instability from a radiographic examination can be low in populations with a low prevalence of implants displaying clinical instability.[316]

5.1.1 Study group, sample size

A power analysis with the primary outcome being peri-implant marginal bone loss (MBL) determined the number of patients. A difference of 0.4 mm between the groups, with a standard deviation of 0.8 mm, 80% power and $\alpha<0.05$ yielded a necessary sample size of 63 implants in each group.

Fifty patients (150 implants) were included to allow reliable interpretation of results (studies I, III, IV). Allocation and randomization were computerized. A proper randomization in RCTs eliminates bias in treatment assignment – particularly selection bias and confounding factors.

Although RCTs should produce the most reliable data, as mentioned before, RCTs often include small samples that may reduce their scientific value. A systematic review reported poor quality of RCTs in the implant dentistry field: a risk of bias was associated with higher risk of reporting statistically significant results.[317]

A subsample of 18 patients was consecutively recruited to a pilot study (study II) on sampling and molecular analysis of peri-implant crevicular fluid. The sampling technique may represent the lower limit of what can be measured, and the various observations may represent a natural biological variation of gene expression during wound healing among individuals. Some genes correlated with clinical findings. However, further studies are needed to refine and optimize the sampling process, to find the appropriate panel and to validate gene expression for monitoring implant healing during clinical conditions. In a larger perspective, qPCR monitoring may be useful in the clinic for early detection and prevention of implant failure or implant-related diseases.

It is concluded that the present RCT provided sufficient power. Experienced clinicians treated well-maintained patients, adhering to a strict follow-up maintenance protocol, which most likely influenced the successful outcomes. However, further studies with a multicenter approach and even larger cohorts are desired.

5.2 Discussion of results

5.2.1 Implant survival

This study found no difference in loss of immediately loaded implants as compared to conventionally loaded implants. The present data on implant survival showed that 6 implants out of 150 were lost in 5 patients, resulting in 5 year cumulative survival rates of 93.9% and 97.0% for immediately and conventionally loaded implants, respectively. This important finding is consistent with results in other published reports of immediate implant loading in partially edentulous individuals.[83,169,318]

Implant failure and complications have multifactorial causes and tend to cluster in patients with common risk profiles.[319] In general, patient-related factors appear more critical in determining implant-failure risk than factors associated with the implant itself.[319] Further, several risk factors can be modified. For example, the patient can modify smoking and the clinician can modify implant selection, site preparation, and loading strategy.[319] No statistical difference in the mean survival rates between immediate and conventional loading were found for partially edentulous patients in the posterior zone as long as strict inclusion and exclusion criteria were followed.[50] In agreement with this result, other authors reported survival rates

of more than 95% for immediately, early and conventionally loaded implants in non-comprised patients.[147,170,320-322]

Many authors have highlighted the importance of high primary implant stability (high value of insertion torque) for a successful outcome of immediately loaded implants.[170,323,324] Consequently, high primary stability was included as one of the inclusion criteria in the present study. Despite these strict inclusion criteria, early examinations found rotational instability at two test and four control implants. Both test implants were unloaded and became stable. One of the control implants was lost, and the other three were stable at the 3-month examination. Most likely, the action of unloading aided in stabilizing these implants and contributed to the comparable survival rates between the groups. Calandriello and colleagues described a similar approach in a 5-year study on posterior single implants.[325]

The role of implant surface properties for osseointegration development has been evaluated in several experimental[156,326-328] and clinical studies_ENREF_329.[329-331] _ENREF_50 Previous data indicates that oxidized implant surfaces rapidly promote a high degree of mineralized bone apposition.[156] Compared with machined implants, a higher degree of mineralized bone and more intensive bone remodeling was found in contact with oxidized implants, thus accelerating bone-implant interface maturation.[155] Subsequently, these material surface-induced cellular responses resulted in a stronger implant-bone interface compared to machined implants.[269] Previous trial outcomes[83,158-160] and the present study confirm and extend results of experimental data.

As per a Lekholm and Zarb definition[305], type-4 bone quality correlates with implant failure.[332] In the present RCT, type-3 bone quality was mostly prevalent (73%), while 15% type-4 sites were recorded. All but one of the lost implants in the present study was categorized with type-3 bone quality, while no implants in type-4 sites were lost. Compared to findings by Herrmann and co-workers[332], the present study found no correlation between type-4 bone quality and implant failure or MBL. One possible explanation for this finding is the difficulty in surgically discriminating between type-3 and type-4 bone.[333] In the present study, the most common bone volume was A or B.[305]

Surgical trauma, together with the anatomical conditions, is believed to be the most important etiological factors for early implant losses.[276] A retrospective study by Han et al. claimed inflammation as the main cause of early implant failure.[334] Furthermore, these factors triggered significantly higher implant failure rates: position (in the anterior maxilla), poor primary implant stability,

machined surface, length exceeding 15 mm, implants placed with a reconstructive procedure, and two-stage surgery.[334] Most implants in the present study were placed in the posterior maxilla, which represents a vulnerable position that is exposed to high occlusal and lateral forces.[335,336] The superstructures were designed with freedom-in-centric and avoided steep cuspal inclinations and extreme lateral contacts. Most likely, these precautions were beneficial to the study outcome.

Besides biological and biomechanical aspects, operator-related factors may influence short- and long-term implant-treatment success. Early implant failures are complex, multifactorial problems associated with many surgical procedure factors, and a variation of failures for individual surgeons have been observed over the years.[337] Surgeons reduced their failure rates when using implants with oxidized implant surfaces, but the relationship of failure rate among surgeons was maintained.[337,338] The use of oxidized implants with moderately rough surfaces and treatments performed by an experienced specialist might have influenced implant survival rates in the current study.

To conclude, multifactorial causes for implant failures probably exist, and no studies have found a single parameter to determine treatment outcome.

5.2.2 Tissue reactions, loading times and abutments

5.2.2.1 Marginal bone loss (MBL)

In this study, no difference was found after 1, 3 and 5 years between immediately loaded and conventionally loaded implants in regards to MBL. These findings are aligned with two reviews, which suggest that different loading protocols do not influence MBL.[339,340]

The technique of connecting the superstructure directly at implant level with no abutment has been used for several years. It did not belong to the original Brånemark concept and has still not been evaluated scientifically in RCTs until now. In the present study, a major observation after 1, 3 and 5 years was significantly more MBL when connecting the superstructure directly at the implant level compared to the use of a machine-milled abutment. After 1 year, but not at 3 and 5 years, a similar observation was also found when connecting the superstructure directly at the implant level compared to the use of an abutment with an oxidized surface. Taken together, these findings suggest that it is advantageous to use an abutment. The impact on long-term (> 5 years) success and the mechanism for the increased MBL when placing the superstructure directly on implant compared to use of a machine-milled

abutment remains to be established. Albeit speculative, from a biological viewpoint, exclusion of an abutment may cause a potential microbial challenge at the superstructure-implant interface close to the bone, which leads to inflammation and bone resorption.[189,341]

Another possible explanation is the differences in manufacturing methods, *e.g.*, between a factory-made abutment compared to a hand-made superstructure (closer to the bone) processed by a dental technician in a dental laboratory. Moreover, another possibility is that the smooth surface of the machined abutment is beneficial for hindering establishment of microbes in the peri-implant compartment. Observations that different surface characteristics of abutment made of commercially pure titanium failed to influence plaque formation and establishment of inflammatory cell lesions in the peri-implant mucosa[144] partially contradict the latter explanation. Additionally, surface roughness reduction, below Ra 0.2 microns, had no further effect on quantitative or qualitative microbiological adhesion or colonization – neither supra- nor sub-gingivally.[342]

Another important issue is whether the present study design might have affected differences in MBL between implant-level and machined abutments. In ordinary cases, the superstructure is not regularly removed at follow-up visits. In the present study, clinical assessments necessitated superstructure/healing abutment removal. It cannot be excluded that repetitive superstructure/healing abutment removal damaged the soft tissue. Based on animal experiments, such assumption is both supported[343] and refuted.[344,345] Further, results of clinical studies support[137,182,346,347] and negate[180,181] this contention. Consequently, the implant-level site might have been affected the most due to a higher soft tissue passage.

To our knowledge, this RCT-study is the first to report that abutment (machine-milled) use reduces the risk of MBL for Brånemark implant system. But longitudinal observations are desired to analyze if abutment exclusion represents a true risk factor for MBL in the long term. Further, the role of various abutment surface properties for soft tissue and bone response, in association with bacteria, remains an important future research topic in basic and clinical studies.

The present study could not corroborate the original contention that an abutment with an oxidized, moderately rough surface could improve soft-tissue adhesion and seal and protect bone from the surrounding oral environment. The assumption that a roughened abutment surface interacts with the peri-implant mucosa to provide a seal against the contaminated environment of the oral cavity is derived from observations that the surface

texture of implants affected the orientation of collagen fibers at the implant surface[138] and that oxidized and acid-etched mini-implants exhibited a longer zone of connective tissue seal and a shorter epithelial attachment compared with machined surfaces.[348] This implies a strengthened mucosal attachment that may prevent bacterial colonization and subsequent MBL. In contrast, an experimental study in dogs found that the attachment between the peri-implant mucosa and titanium abutments with either a turned or an acid-etched surface was similar. The attachment comprised a barrier epithelium and a zone of connective tissue of similar dimension.[142] In the present study, no difference in MBL could be demonstrated after 5 years between machine-milled and oxidized abutments. Hence, our study failed to support findings in a human study by Schupbach and co-workers[138] and in an animal study by Albouy and colleagues.[98]

After 3 years, in the present RCT, similar MBL was found around implant sides irrespective of facing a tooth, an implant, or an edentulous area. In contrast, a clinical study has reported lower MBL at implant facing a tooth than at implant facing a neighboring implant.[349] In addition, significant predictors for loss in proximal bone crest level were horizontal inter-unit distance and peri-implant bone-level change.[349,350] This could not be shown in the present study, presumably due to the meticulous compliance with the surgical protocol that prescribed a 7 mm center-to-center distance.

After 5 years, the following independent variables explained about 27% of MBL variation: health deterioration, high blood pressure medication, periodontal disease experience, smoking (≤10 cigarettes/day), and PPD. Interestingly a change in general health during the study period and also medication for high blood pressure influenced MBL. This novel finding should be interpreted with caution because the data is based on self-reported patient health; hence no data on duration and dosage could be ensured.

Nevertheless, these findings deserve further exploration in new studies. This is supported also in a recently published systematic review, which claims the need for evidence for potential risks associated with implant therapy in patients with systemic diseases and conditions.[293] Periodontal disease experience and smoking are well-known risk factors in implant treatment[204,291], and these were seen in the present study.

5.2.2.2 Soft tissue

The present observations on changes of peri-implant soft tissue dimensions are essentially in agreement with results of studies reporting that the dimensions of the peri-implant soft tissues around early or immediately loaded implants are similar to those around conventionally loaded

implants.[135,136] An exception is the proximal site, where the retraction was most pronounced in the control group (statistically significant at 1 year but not thereafter). Despite higher initial soft tissue height in the control group at implants with abutments, the difference leveled out after 1 year through larger retraction in the control group. The present study also demonstrated that most peri-implant soft tissue changes occurred during the early follow-up phase, while only minimal changes were found after 1 year. Similar findings were demonstrated in another study in which most recession occurred within the first 3 months between implant placement/provisionalization and definitive restoration.[134] Conflicting opinions exist about the role of the soft tissue biotype (thick or thin) dimension, and its importance for soft tissue stability. A recently published review showed that pre-operative tissue biotype did not significantly influence soft tissue and esthetic outcomes around implants in the anterior maxilla.[136]

Between 1 and 3 years, minor retractions of the buccal soft tissue height were found. At the proximal sites, higher plaque and bleeding indices over time (resulting in slight swelling of the interproximal soft tissue) might explain minor, increased soft-tissue height at proximal sites after 1 year.

The present observation that retraction was more pronounced at buccal sides than at proximal sides stands in contrast to results that revealed no buccal retraction and papilla loss during 3-month follow-up period when immediate loading was performed.[119] Neither the Covani study nor the present study could detect any significant differences regarding loading protocol. Conflicting opinions occur in the literature regarding papilla loss (proximal soft tissue loss) due to immediate implant restorations.[170] For example, the immediate rehabilitation favors the early generation of proximal soft tissue height (papilla) but no significant intergroup difference was revealed after 1.5 years between early or delayed loading protocol of single tooth treatment.[120] Chang and coworkers observed about 0.6 mm soft tissue margin retraction at buccal implant sites, while a mean increase was observed at tooth-facing proximal sites (1.1 mm) and no change at inter-implant sites during the first 6 months after the one-stage implant placement surgery.[349] Between 6 and 36 months, no further significant soft or hard tissue changes were observed.[349]

Soft tissue margin change is most likely associated with the primary healing phase. This results from peri-implant soft tissue remodeling, and subsequent adaptation to adequate biological dimensions as per the *biological width* concept.[125] These soft tissue alterations, after the placement of superstructure, may affect the esthetic appeal of the restorative therapy.[351] In particular, in esthetically sensitive cases, a temporary restoration may be indicated. Ross

and co-workers found that use of a customized anatomic provisional abutment reduces the amount and frequency of recession.[134]

As mentioned above, immediately loaded implants appear to result in a soft tissue reaction comparable with those that are conventionally loaded. Experimental and clinical studies report that a soft tissue seal of about 3–4 mm in height is established around the transmucosal part of the implant unit, independent of implant geometry and treatment protocol.[124-128] Further research remains to be done on soft tissue aspects of implant therapy, in general, and immediate loading, in particular, and more well-designed long-term studies are recommended.[168]

5.2.3 RFA

RFA was primarily introduced as a diagnostic tool for scientific purposes to study implant integration and primary stability[232], but has also been used by clinicians to monitor osseointegration in predominantly soft bone qualities. RFA has been thoroughly studied and compared with removal torque testing in *in vitro*[352,353], animal[354-356] and clinical[357,358] studies. It is considered a helpful diagnostic tool for measuring implant stability and detecting circular bone loss[237] and demonstrates a high degree of interoperator reliability and repeatability.[238]

In the present study, the mean ISQ decreased between 2 and 4 weeks in the control group, while the mean ISQ in the test group was roughly the same from surgery until 4 weeks postoperatively but with a significantly lower ISQ in the test group compared to the control group at 2 and 4 weeks. Both groups demonstrated improving stability up to three years and thereafter they exhibited stable values. As discussed above, some implants showed a decrease in ISQ during initial healing. Unloading these implants subsequently improved the stability, which demonstrates the benefits of RFA, especially when performing immediate and early implant loading or treating compromised implant cases.[239,240]

The dip in implant stability during the first postoperative weeks is most likely related to inflammatory, resorptive, and remodeling activities during bone healing, as demonstrated in different animal models.[359,360] While new implant surface modifications have improved osseointegration, the initial implant stability dip is still present and remains a challenge for future research and development. As judged by the decreasing ISQ values in the test group from 2 days to 2 weeks, immediate loading seems to act in concert with the biological activities that maintain a period of low stability.

After 3 years, but not after 5 years, ISQ after 2 days was one of the independent variables which significantly could explain MBL in the multiple regression analysis. At present, we have no explanation for this finding. Further, correlation was revealed with gene expression and RFA at different time points (study II). Nevertheless, these results should be interpreted with caution due to the relatively small sample size.

Before using RFA as a standard tool for clinical follow-ups, several limitations of the method and the device must be solved. Firstly, removal of the superstructure before applying the measuring device at each implant is time consuming in a clinical set-up. Secondly, prospective clinical studies are needed to ensure the method´s internal and external validity.[233,242,243]

5.2.4 Plaque, mucosal bleeding, PPD and BoP

At baseline, average plaque and mucositis scores were low in the present study. Significantly higher plaque and mucosal bleeding scores were found in the test group at 3 months and this may be related to the temporary bridge having a porous acrylic surface that facilitates plaque formation. Over time, plaque and mucosal bleeding increased in test and control groups despite strict patient monitoring. The importance of providing proper oral hygiene instructions to patients rehabilitated with dental implants has previously been stressed. Further, the need for proper prosthetic constructions that allow accessibility for oral hygiene around implants has been emphasized.[288] Although no association was demonstrated between plaque or mucositis and MBL in the present study, it may be anticipated that further decline of long-term oral hygiene standards may jeopardize peri-implant health.[361] But no evidence exists on effects of optimal oral hygiene self-care around dental implants; consequently, urgent need exists for academic institutions and industry to initiate and support high-quality RCTs on this topic.[362]

The connective tissue zone for an implant has only two fiber groups (parallel and circular) and neither of them inserts into the implant. As a result, when measuring pocket depth at implant sites, the probe goes beyond the sulcus, through the junctional epithelial attachment and through most of the connective tissues and reaches closer to the bone.[213] Under healthy conditions, peri-implant, soft-tissue crevice depth is about 3-4 mm, although probing force and angulation, diameter of the probe tip, inflammatory status, and soft tissue firmness can trigger higher values.[208] A clinical study by Mombelli et al. showed that peri implant pocket probing is more sensitive to force variation than periodontal pocket probing.[217]

The present study demonstrated a PPD range between 2 and 4.5 mm over the course of the follow-up (Figure 14). It can be argued that the vertical positioning of an implant obviously leads to variations in pocket depth.[363] In the present study, all PPD recordings were performed after superstructure removals, so the superstructure did not restrict correct, reproducible probe placement. PPD for the merged test and control groups showed only minor changes at all implant sites up to 3 years for all superstructure connection types. Thereafter, a marked increase in PPD occurred for proximal sites of all three groups. At 3- and 5-year time points, a significantly higher proximal PPD was found at IL compared with AM and AOX, which suggests that the soft tissue in some way adheres better to an abutment, resulting in a decreased pocket depth. Nevertheless, the proximal PPD alteration between 3 and 5 was not statistically significant compared with the proximal PPD alteration between 1 and 3 year for any connection types.

A gradual increase in probing depth over time is a more significant indicator of pathology than a single probing depth unrelated to a time interval.[208] Increasing pocket depths should be monitored regularly to rule out ongoing pathology. Reinstruction of proximal hygiene measures particularly seem to be indicated because access is more limited at these sites. Consequently, buccal sites, which generally allow easier access for proper hygiene measures, underwent the smallest changes in PPD.

Interestingly, PPD (along with periodontal disease experience, health deterioration, medication for high blood pressure, and smoking) was one of the parameters which correlated with MBL in the 5-year regression analysis. So increasing PPD could indicate progressive MBL and pathology, in combination with bleeding, indicates need for additional radiographic examination.[284] In agreement, another study has also demonstrated a correlation between PPD and MBL.[363] On the contrary, Lekholm and co-workers found that accelerated MBL did not accompany presence of deep pockets.[364]

In peri-implantitis, the probe may penetrate beyond the connective tissue onto the alveolar bone.[208,213] From a clinical perspective, repeated PPD recordings over time are recommended as a monitoring tool that aims at early detection of peri-implant attachment loss.[208] It has been demonstrated that any probing-triggered disruption of the soft-tissue-implant interface will result in formation of new epithelial attachment within 5 days.[365]

Probing also reveals tissue consistency, bleeding and exudate. Therefore, probing is important for (*i*) measuring increasing sulcus depth and (*ii*)

evaluating other peri-implant parameters. BoP alone is indicative of soft tissue inflammation, and additional findings such as suppuration (pus) support the suspicion of a pathological process[223,290,366] and warrant further investigation. BoP has been shown to mirror inflammatory activity in the gingival pocket in cases with periodontitis, although sensitivity over time is as low as 29%. However, specificity has been shown to be around 88% and therefore, absence of BoP is regarded as a safe tool to monitor periodontal health.[367] Jepson and co-workers confirmed this and reported that high negative predictive values characterize BoP – and thus negative scores can serve as indicators for stable peri-implant conditions.[220] So high BoP assessment accuracy at implant sites was demonstrated.[221]

In the present study, no statistical differences with respect to BoP and type of superstructure connection were found. Moreover, the regression analysis after 3 years (but not after 5 years), showed that abundant BoP was significantly associated with MBL. Similar to Renvert and colleagues[368], the present RCT attempted to discriminate between traumatic probe penetration-triggered bleeding and bleeding as an indication of inflammation (no bleeding, minute bleeding, and abundant bleeding). In our study, all analyses were based on registration of abundant BoP due to difficulties of interpretation of minute bleeding.

In the present study, the frequency of BoP increased over time: at 3 and 5 years, minute BoP was found in 30–40% of the sites while abundant BoP occurred at about 20% of the sites. This highlights the importance of pretreatment patient selection and the close posttreatment monitoring.[369-371] However, it should be noted that daily hygiene procedures cannot clean a sulcus greater than 2 mm.[372] Consequently, deep PPD with pathology may require gingivectomy or bone revision surgery to enable the patient to carry out effective daily hygiene.

5.2.5 CF and qPCR analysis (Study II)

In the present study, the potential application of qPCR for a large-scale molecular analysis of the peri-implant crevicular fluid was evaluated. Crevicular fluid sampling and subsequent proteomic analysis (*e.g.* with ELISA) of factors related to tissue healing and/or destruction have received large attention in the recent years.[265-267] However, given the complexity of the healing processes and the involvement of multiple cytokines and biological mediators during the various osseointegration phases, a robust and highly sensitive analytical tool that can measure multiple factors in a critically low amount of biological material (*e.g.* CF), is still required. To our knowledge,

this pilot study is the first attempt to apply qPCR gene expression analysis of peri-implant crevicular fluid in an RCT.

The morphological analyses of the paper strips revealed that the retrieved CF was predominated by mononuclear leukocytes and granulocytes, yet, larger cells with mesenchymal cell-like appearance were also detected. Subsequently, using qPCR, these different cell populations were homogenized and analyzed for a panel of selected genes that represent inflammation, bone formation and remodeling, which are major biological bone-healing and osseointegration processes.[84]

Overall, peri-implant CF molecular-analysis feasibility was confirmed, and despite the low number of retrieved cells, sufficient RNA was extracted, which enabled analysis of the selected genes. However, it was evident from the large inter-subject variations that further optimizations are required, which is discussed later in this section.

In the present study, an interesting observation was the significantly higher expression of both bone formation (OC) and bone remodeling (TRAP) genes at the oxidized versus the machined abutment, particularly in the unloaded group at 14 days. This finding is at least partly in line with previous experimental studies, during unloaded healing conditions, where oxidized implants rapidly promoted coupled increase in the expression of bone formation genes, including OC, and bone remodeling genes, including TRAP, compared to machined implants.[155,156] In this clinical study, because all implants had an oxidized surface, it is possible that during unloaded conditions the detected transient coupled increase of OC and TRAP was an add-on effect from oxidized surface of the abutment in addition to the implant.

Regarding inflammatory cytokines, the expression of TNF-α was generally higher during early days after implantation and was reduced to lower levels at 28 and 90 days after implantation. This is aligned with the transient nature of inflammation, irrespective of abutment surface type or loading protocol. Interestingly, for one patient, who had unstable implants at 90 days, TNF-α was exceptionally higher at 90 days than at earlier assessments (Figure 19), which indicates a predictive potential for TNF-α as a molecular indicator of reduced osseointegration. The correlation analyses partially supported this assumption, where TNF-α and IL-1β and ALP, showed the most common correlations with the clinical parameters. In addition, TNF-α expression, at 2 and 14 days, also showed the strongest correlations with complications at 90 days.

Otherwise, it was evident that significant differences of gene expression were only found at some occasions, whereas no specific pattern was revealed comparing oxidized and machined abutments or immediately loaded and unloaded implants. Some findings were difficult for interpretation, for instance, significantly higher expression was found for IL-1β at 2 days between the test and control group for the machined abutments. No possible explanation for this finding was found because no loading had been instituted before sampling at the 2-day period.

Taken together, whereas the present pilot study confirms the suitability and high sensitivity of qPCR for analyzing temporal molecular activities in the peri-implant CF, further optimization, however, is still required. From a biological viewpoint, the present analysis was basically performed on the total cell populations in the CF, where no cell type-specific gene expression can be determined. It would be of great interest to first separate the different cell phenotypes in the CF before the subsequent analysis of the gene expression. To achieve this, the retrieved CF can be streamed in fluorescence assisted cell sorting (FACS), whereby different cell phenotypes can be determined, and then sorted for subsequent cell-specific gene expression analysis. Based on the microscopic analysis in this study, paraffin embedding and sectioning of the sampled paper strips might be possible, which can then be subjected to immunohistochemistry and laser micro-dissection of specific cells that can be analyzed thereafter with qPCR.

From a technical viewpoint, several optimization steps might be considered. Firstly, with respect to the reference genes, it is now will established that the stability of reference genes used for normalization might vary among individuals and experimental conditions. To compensate for this, it is now recommended to screen a panel of species-specific reference gene panel to determine the best stable reference gene(s) to be used for normalization before running the sharp qPCR analysis.

Secondly, when designing the primers for the genes-of-interest, it is now recommended to validate and confirm the specificity of these primers prior to the sharp analysis. Finally, because RNA inhibition is a potential risk that may compromise the qPCR results, it is now recommended to include a synthetic RNA Spike in the analysis, which allows for detecting and solving any RNA inhibition that might happen during sampling or qPCR procedures. These guidelines were recently published[373] to facilitate and improve qPCR data quality and reproducibility, which is particular important when diagnostic potential is considered in qPCR studies.

5.2.6 Risk factors and complications

It is well known that implant-supported prostheses generate more complications than tooth-supported prostheses.[294,295] A review by Pjetursson et al.[72] reports that only 66.4% of patients were free of any complication (biological and technical) after 5 years. However, another systematic review indicated a positive learning curve in implant dentistry; higher survival rates and lower complication rates occur in newer studies – compared with older studies.[273] The author highlights need for early identification of problems and their etiology for better treatment outcomes. Costs associated with implant complications are high for the patient, the clinician and health care, but patient suffering is of course the worst. In order to minimize patient suffering and cost, it is imperative that failures and complications be reduced as much as possible. Various causes that may influence treatment outcome are discussed in the literature, and a multifactorial background is likely.[274,374] Concerning systemic diseases, the level of evidence indicative of absolute and relative contraindications for implant therapy, due to systemic diseases, is low.[375,376] The results of the present study indicate the need for understanding the role of systemic health and disease for oral implant-associated complications.

Frequently reported criteria for implant pathology are mobility, pain, peri-implant bone loss and radiolucency. Regarding the peri-implant soft tissue, signs of pathology are abundant bleeding and/or suppuration. Nowadays, prosthetic aspects (technical complications/prosthetic maintenance, adequate function, and esthetics) and patient satisfaction (discomfort and paresthesia, satisfaction with appearance, and ability to chew/taste) are emphasized as important criteria for success. Implant dentistry should ideally evaluate a long-term primary outcome of the implant-prosthetic complex as a whole.[270] Over the years, many authors have tried to define success criteria.[290,377,378] Varying thresholds for acceptable bone loss are suggested. Traditional implant-treatment-success criteria are time-dependent and might be less appropriate for use in everyday clinical practice. So in the present study, we emphasized implant survival, implant failure, and other clinical parameters. The consequences of a complication mean more for the patient than the definition itself. Therefore, complications should always be taken into account despite the generally high success rate of dental implants.

5.2.6.1 Biological complications

The most serious biological complication is, of course, implant loosening, generally termed as failure; see section 5.2.1 on "Implant survival".

A recently published systematic review and meta-analysis concluded that implant therapy must not be limited to dental implant placement and restoration but to implementation of peri-implant maintenance measures to potentially prevent biologic complications and hence to heighten the long-term success rate. Despite the establishment of proper peri-implant maintenance, biological complications might occur.[379] In the present study, patients were followed up many times during the first year and then annually up to 5 years. Despite thorough follow-ups and maintenance throughout the study, plaque and mucosal bleeding increased with time and maintenance efforts were apparently insufficient.

A serious biological complication is peri-implantitis. Today, peri-implant disease is considered the result of imbalance between bacterial overload and host defense.[380] Bacteria generally cause peri-implant mucositis and peri-implantitis. While the inflammatory lesion of peri-implant mucositis resides only in soft tissue, peri-implantitis also affects supporting bone.[381] A review identified strong evidence that poor oral hygiene, cigarette smoking, and a history of periodontitis are risk indicators for peri-implant disease.[382] But the complete biological background behind the pathogenesis of peri-implantitis is not fully understood. Many known (and unknown) general, patient-associated factors cannot be controlled for (*e.g.*, anatomy, genetics, systemic health, host immune responses and inflammatory responses), and these factors are considered important for determining implant loss or peri-implantitis risk.[383-385] Nevertheless, these factors deserve further investigations.

In the present study, peri-implantitis was found in 9.1% of the patients and at 4.0% of the implants. Several researchers have reported differences in peri-implantitis prevalence.[386,387] Tomasi and co-workers expressed need for improved reporting of peri-implant diseases via epidemiological studies.[388] A consensus definition of peri-implantitis criteria remains to be agreed upon. So reported peri-implantitis prevalence tends to vary among studies due to varying diagnosis criteria. Derks and co-workers reported that moderate or severe peri-implantitis (BoP or suppuration and >2 mm bone loss) was diagnosed in 14.5% of the patients. Higher odds ratios were identified for patients with (*i*) periodontitis, (*ii*) ≥4 implants, (*iii*) certain brands of implants, and (*iv*) prosthetic therapy delivered by general practitioners. Higher odds ratios were also identified for implants installed (*i*) in the mandible and (*ii*) with crown restoration margins positioned ≤1.5 mm from the crestal bone at baseline.[387]

A recently published review concluded that scientific articles on prosthetic risk factors for peri-implantitis are scarce.[389] Excess cement seems to be associated with mucositis and possibly with peri-implantitis, especially in

patients with a history of periodontal disease.[390-392] In the present study, all superstructures were screw-retained and cement problems are therefore not relevant for this study.

Note, however, that bone loss due to bacterial infection is to be discriminated from bone loss due to remodeling, such as in instances in which implants are placed too deep. [393] Conflicting opinions exist regarding early MBL detection and progress over time.[386,394,395] Jemt et al. claimed that a single-minded explanatory model for bone loss around implants is not acceptable. They also consider that MBL around implants is a complex phenomenon caused by several varying factors that are not yet fully understood.[396]

Different experimental and clinical studies have explored the contribution of implant surface properties to MBL, peri-implantitis, or even total loss of osseointegration (implant failure).[337,397,398] The present study results are not in agreement with experimental studies in a peri-implantitis model, which showed that oxidized implant surfaces caused a higher degree of marginal bone resorption, presumably due to increased harboring of bacteria in association with the oxidized surface. [397,399,400] A clinical study in partially edentulous patients also demonstrated that implants with a rough surface display higher peri-implantitis rates and late failures than implants with moderately roughened or turned surfaces.[401] In contrast, a review by Renvert and co-workers reported no evidence that implant surface characteristics affect the initiation of peri-implantitis.[402] Yet need exists for well-designed observational studies that provide better evidence for identifying risk factors for establishment and progression of peri-implant diseases.[403]

Not surprisingly, some of the parameters (periodontal disease experience, pocket depth, health deterioration, and smoking), which correlated with MBL in the 5-year regression analysis in the present study, are also strongly implicated in peri-implantitis.[288,404,405] For example, the combination of a history of periodontitis and smoking has been shown to increase implant failure risk and peri-implant bone loss.[27,406] Besides the above-mentioned parameters, the biomolecular background behind peri-implantitis pathogenesis is not fully understood. Due to the relatively low prevalence of peri-implantitis in the present study, peri-implantitis was not included in the regression analysis.

Early complications in the test group, such as dehiscence, hematoma, and tender implants, are very likely related to difficulties in managing prosthetic interventions in a newly operated area.

5.2.6.2 Technical complications

Technical complications in implant dentistry are frequently reported.[271] The present study revealed few technical complications during the follow-up period and lower frequencies than other studies.[407] Although no complete superstructure fractures were noted, minor porcelain chipping was observed in both groups in the later follow-up phase. In general, technical complications were of minor severity and patients were unaware of fractures that were detected at follow-up visits, and they could often be polished or left untreated.

In comparison to the present results, Pjetursson and co-workers[72] found a higher rate of fractures in a systematic review. They reported fractures of veneering material as the most frequent complication over a 5-year observation period with 13.5% prevalence.[72] Svensson and co-workers suggested that absence of mechanoreceptors in the peri-implant bone results in inadequate sensory information for low-contact and high-biting forces, which in turn may lead to unfavorable forces and thereby prosthesis complications.[299] Another explanation for this complication may be technical difficulties associated with the technique of fusing porcelain to the titanium core.[408,409] Despite potential risks, the present knowledge is sufficient to conclude that veneering titanium with low-fused porcelain for crowns and fixed partial dentures can be recommended for routine clinical use.[409] The anatomical design of the core/framework is important for supporting the brittle veneering material. In addition, the dental technician's experience and material management capabilities – and those of the dentist – are important factors that may influence the final result.[274] Having close relationships with dental laboratories and clinicians is a critical success factor for ensuring that prostheses have successful outcomes. Technical complications can also be related to the nature and amplitude of the mastication loads and parafunctional habits and bruxism/clenching may increase implant or prosthesis stress – leading to mechanical complications.[410] But known bruxism was an exclusion criterion in the present study.

Many other authors reported veneering material fractures as the most common complication encountered and the similar results are seen for both for conventional and CAD/CAM technology.[407,411-414] In the present study, almost all titanium frameworks were manufactured with CAD/CAM technology and only minor problems with porcelain chipping were observed.

In the present study, complications such as misfit, loosening of abutment screws, and temporary bridge fracture, were mainly associated with the immediate loading concept (test group). This result is contradictory to other

studies that show similar technical and biological complications for immediate and conventional loaded implants.[323,415,416] Further, abutment screw loosening has been reported as the most frequently occurring technical complication.[114] In the latter study, a relationship was found between screw loosening and connection type: a higher frequency of loose screws was demonstrated for externally connected implant systems.[114] The present study used an external connection system which could be a reason for some abutment screw loosening.

The long-term clinical success of a restoration is attributable to diverse factors that can be grouped into three general categories: patient, clinician, and restorative material. Success or failure may well be due to various combinations of these factors and the relative contribution of each factor has not been clarified.

6 SUMMARY AND CONCLUSIONS

In this randomized prospective 5-years clinical trial, similar implant survival and marginal bone loss was demonstrated, irrespective of immediate or delayed loading protocol. The results of this study show that higher marginal bone loss occurred when superstructures were directly connected at the implant level in comparison with the use of machine-milled abutment.

Furthermore, the results reveal a relationship between the marginal bone loss and health deterioration, high blood pressure medication, periodontal disease experience, light smoking (≤10 cigarettes/day) and proximal pocket depth.

For all connection types and loading regimes, the most pronounced retractions in the soft tissue occurred during the first year, and thereafter only minor changes at proximal and buccal sites were found. After 5 years, no differences in soft tissue height were demonstrated between immediate and delayed loading, whereas a significantly greater soft tissue height was recorded for the superstructure placed directly on the implant level in comparison with the machine-milled and oxidized abutments.

Relatively few biological and technical complications were reported for the immediately loaded and conventionally loading regimes. The highest number of complications occurred during the first 3 months. Porcelain chipping was the most encountered technical complication. Among the biological complications, peri-implantitis was first detected after 3 years. After 5 years, peri-implantitis was found in 9.1% of the patients (4.0% of the implants).

In a sub-sample of patients, the present results confirmed the feasibility of qPCR molecular analysis of the peri-implant crevicular fluid. Despite a low number of retrieved cells, sufficient RNA was extracted, allowing the analysis of the selected genes.

In conclusion, the present 5-year results have demonstrated similar implant survival and marginal bone loss, irrespective of loading protocol. The use of a machined abutment should be preferred when it comes to marginal bone stability over time. Scientific support for placing superstructures directly on the implant is still unclear. As judged by regression analysis, factors related to systemic health and medication as well as periodontal disease experience and smoking, are associated with marginal bone loss. Peri-implantitis was found in 9.1% of the patients. Further studies are needed to refine and optimize the sampling process, to find the appropriate panel and to validate gene expression for monitoring implant healing.

7 FUTURE PERSPECTIVES

In the following, future challenges, research and educational projects are listed:

Acquiring more knowledge about the importance of the host response per patient – to select and treat with good prognosis.

Educating dentists in implant dentistry – especially regarding treatment planning and patient selection.

Developing methods to forecast existing implants, *e.g.*, to develop diagnostic potential regarding qPCR.

Further studying the importance of using an abutment in implant treatment.

Exploring the role of abutment surface properties for tissue responses.

Studying soft tissue aspects of implant therapy, in general, and immediate loading, in particular.

Exploring multifactorial causes and risk factors for implant failures and complications, *e.g.*, peri-implantitis.

Developing good, predictable peri-implantitis treatment for creating stable healthy tissues and for minimizing risk for recurrence of disease and possible implant loss.

Developing better dental technology to prevent veneering porcelain fractures and further educating dental technicians in implant prosthetics.

Developing digital technologies for implant treatment, *i.e.*, for planning, implant placement, intraoral scanning instead of impression, and individual digital production of dental restorations.

Standardizing documentation of implant components and individually produced dental products, *e.g.*, by simple scanning of products and direct data entry into medical records – traceability is a critical success factor.

Studying implant-treatment utility on aging patients regarding quality of life and nutritional capabilities.

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