HADAR HALLSTRÖM

ON PREVENTION AND TREATMENT OF PERI-IMPLANT INFLAMMATION

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OF PERI-IMPLANT INFLAMMATION

Malmö University, Faculty of Odontology
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To Eva, Cecilia, Johanna, Viktor

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ABBREVIATIONS

BOP	Bleeding On Probing
CI	Confidence Interval
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GCF	Gingival Crevicular Fluid
GI	Gingival Index
PPD	Probing Pocket Depth
OHI	Oral Hygiene Instruction
Pi	Peri-implantitis
PI	Plaque Index
PiM	Peri-implant Mucositis
PISF	Peri Implant Sulcular Fluid
RCT	Randomized Clinical Trial
SD	Standard Deviaation
WHO	World Health Organization

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to by their roman numerals as listed below.

- I. Hallström H, Lindgren S, Yucel-Lindberg T, Dahlén G, Renvert S, Twetman S. Effect of probiotic lozenges on inflammatory reactions and oral biofilm during experimental gingivitis. *Acta Odontol Scand.* 2013; 71: 828–833.
- II. Hallström H, Lindgren S, Widén C, Renvert S, Twetman S. Probiotic supplements and debridement of peri-implant mucositis: A randomized controlled trial. *Acta Odontol Scand.* 2016; 74: 60-66.
- III. Hallström H, Persson GR, Lindgren S, Olofsson M, Renvert S. Systemic antibiotics and debridement of peri-implant mucositis. A randomized clinical trial. *J Clin Periodontol.* 2012; 39: 574–581.
- IV. Hallström H, Persson GR, Lindgren S, Renvert S. Open flap debridement of peri-implantitis with or without adjunctive systemic antibiotics: A randomized clinical trial. *J Clin Periodontol.* 2017; 44: 1285-1293.

THESIS AT A GLANCE

Study	Purpose	Study design	Sample	Outcome parameters	Main findings
I	To: evaluate whether daily oral administration of probiotic bacteria could influence the composition of the supragingival plaque, clinical parameters and levels of inflammatory mediators in an experimental gingivitis model	RCT Double blinded Placebo controlled Cross-over	18 females	GI PI BOP GCF Microbiota	Daily intake of probiotic lozenges containing two strains of <i>L. reuteri</i> did not seem to significantly affect the plaque accumulation, gingival inflammatory reaction or the composition of the supragingival plaque during conditions of experimental gingivitis as compared to the use of placebo
II	To: evaluate whether topical treatment and daily intake of probiotic lozenges as an adjunct to mechanical debridement and oral hygiene instructions in the treatment of peri-implant mucositis has any effect on the clinical, microbial and inflammatory outcome	RCT Double-blinded Placebo controlled Two arms	49 patients	PPD PI BOP GCF Microbiota	Topical treatment and daily intake of probiotic lozenges as an adjunct to mechanical debridement and oral hygiene instructions did not improve clinical, microbial or inflammatory variables of peri-implant mucositis as compared to the use of placebo.
III	To: evaluate whether systemic Azithromycin® as additive to mechanical debridement and oral hygiene instructions in the treatment of peri-implant mucositis has any effect on the clinical and microbiological outcome	RCT Examiner blinded Two arms	48 patients	PPD PI BOP Microbiota	The use of systemic antibiotics as additive to mechanical debridement and oral hygiene instructions in the treatment of peri-implant mucositis did not demonstrate better clinical results up to 3 months after treatment.
IV	To: evaluate whether systemic Azithromycin® as additive to open flap debridement in the treatment of peri-implantitis has any effect on the clinical and microbiological outcome	RCT Examiner blinded Two arms	39 patients	PPD PI BOP Microbiota Bone level	Surgical treatment of peri-implantitis with adjunctive treatment with azithromycin did not provide 1-year clinical benefits in PPD changes, microbiological changes or for the combined treatment outcome assessment in comparison with open flap debridement alone.

ABSTRACT

This thesis focuses on prevention and treatment of biological complications around dental implants.

Background

An increasing number of individuals have restorations that are anchored to the jaws by dental implants. Modern-day implants are a Swedish invention that became available to patients in the 1970s. Implant restorations are common and several patients have had their implants for more than twenty years. The long-time survival rate is good, but as with all treatments, complications do occur. These complications can be technical problems, like fractures of the framework, discolouring and wear of the prosthesis, or fractures of the actual implant. Biological complications appear initially as peri-implant mucositis (PiM) which is a bacterially induced inflammation of the soft tissue around an implant. PiM may progress into peri-implantitis (Pi) that involves the implant supporting bone and can ultimately lead to loss of the implant. The prevalence of complications has been debated. Depending on patient population and definition used, the prevalence has been reported in the range of 19-65% for PiM and 1-47% for Pi.

Treatment of peri-implant diseases consists of reinforcement of the patients' oral hygiene, non-surgical mechanical therapy and in cases with bone loss adjacent to the implant surgical interventions may be needed. Irrespective of treatment method complete resolution of the diseases is rare and even though no evidence exists that

the use of antibiotics improves the treatment outcome antibiotics are often prescribed. Frequent use of antibiotics is a main contributor to the development of bacterial resistance that has developed into a serious global problem. Probiotics are live bacteria that in adequate doses are beneficial to the host and under certain circumstances have demonstrated abilities to hamper inflammations.

Aims

The aim of the thesis was to clarify whether 1) probiotics administered in lozenges can prevent or lower the inflammatory reaction to plaque accumulation, 2) if the use of probiotics as an adjunct to mechanical treatment of peri-implant mucositis has a beneficiary effect, and 3) if systemically administered antibiotics given as additive enhance the outcome following the treatment of peri-implant mucositis and peri-implantitis.

Methods

Four studies were designed to fulfil the aim:

- a placebo controlled cross-over study testing whether probiotics can prevent or lower the inflammatory response to a bacterial challenge.
- a placebo controlled RCT study evaluating whether probiotics given as an additive to mechanical treatment of peri-implant mucositis provides any benefits.
- a RCT study evaluating whether systemically administered antibiotics given as an additive to mechanical treatment of peri-implant mucositis are beneficial.
- a RCT study evaluating whether systemically administered antibiotics given as an additive to surgical treatment of peri-implantitis are beneficial.

Clinical, microbiological, and immunological parameters were used to analyse study outcomes.

Results

- Daily probiotic lozenges had no significant effect on the inflammatory response in an experimental gingivitis model compared to placebo.
- Daily probiotic lozenges as additive to oral hygiene instructions and mechanical debridement had no significant effect on the clinical, microbiological, or immunological outcome in the treatment of peri-implant mucositis compared to placebo.
- Systemically administered antibiotics as additive to oral hygiene instructions and mechanical debridement had no significant effect on the clinical or microbiological outcome in the treatment of peri-implant mucositis.
- Systemically administered antibiotics as additive to oral hygiene instructions and surgical mechanical debridement had no significant effect on the clinical or microbiological outcome in the treatment of peri-implantitis.

Conclusions

Neither probiotics containing *Lactobacillus reuteri* or Azithromycin have any major effect on the treatment outcome of peri-implant mucositis or peri-implantitis when given as additive to conventional treatment. The use of daily lozenges containing *Lactobacillus reuteri* yields no benefits when it comes to preventing gingivitis under experimental gingivitis conditions.

INTRODUCTION

During the last decades, dental implants have become a frequently used method to replace missing teeth. Rehabilitation of partial and total edentulism with a fixed prosthesis attached to osseointegrated implants has been reported to have a long-term survival rate of 94.8-99.7% (Iniesta et al., 2012, van Velzen et al., 2015, Hjalmarsson et al., 2016, Park et al., 2017). Biological complications (peri-implant mucositis and peri-implantitis) are common findings usually occurring 5 to 10 years after the installation of dental implants (Mombelli et al., 2012). Peri-implant mucositis has been defined as a reversible inflammatory process with bleeding on gentle probing without signs of loss of supporting bone following the initial bone remodelling during the healing process. Peri-implantitis is an inflammatory disease adjacent to a dental implant and a condition with evidence of bleeding on gentle probing and with progressive loss of supporting alveolar bone beyond the biologic bone remodelling process following the initial healing phase (Zitzmann and Berglundh, 2008, Anonymous, 2013).

The reported prevalence of peri-implant diseases varies considerably. In a recent systematic review, the patient based prevalence of peri-implant mucositis and peri-implantitis were reported to be in the range of 19-65% and 1-47% respectively (Derks and Tomasi, 2015). The wide range in the reported prevalence may partly be explained by differences in study cohorts, follow-up time and case definitions. Implant removal and surgical procedures to handle biological complications at implants are an increasing clinical problem in patients supplied with dental implants resulting in an economic

burden on both society and the individual, and negatively affecting the patients' quality of life. Between 2009 and 2014 an increasing number of surgical interventions (operations and extractions) due to peri-implantitis was reported to the Swedish Social Insurance Agency (SSIA, Försäkringskassan) and at the same time the quota of implant extractions increased and 2014 comprised 28% of performed treatments (Sjödén, 2015). (Figure 1)

Figure 1. Number of implant extractions and operations due to peri-implantitis reported to the Swedish National Dental Insurance System during 2009-14.

Figure 2. Clinic, radiographic and histologic illustrations of successful implants.

Gingival and peri-implant tissues

The soft tissue barrier around implants resembles clinically and histologically the gingiva around teeth (Figure 2). Clinically, if placed in keratinised tissue, it has a firm consistence, is pink in colour and has a keratinised zone separated from the non-keratinised alveolar mucosa by a groove below the mucosal margin (Berglundh et al., 1991). Histologically, the tissue facing the implant has a shallow sulcus, a non-keratinised junctional epithelium of 1.5-2.0 mm and supra crestal 1.0-1.5 mm of connective tissue. There are collagen fibres originating from the periosteum and organised parallel with the implant (abutment) surface (Berglundh et al., 1991, Berglundh and Lindhe, 1996). The overall distance, bone to soft tissue margin is larger at implants than at teeth (Berglundh et al., 1991, Buser et al., 1992, Abrahamsson et al., 1996, Berglundh and Lindhe, 1996).

Factors affecting the peri-implant marginal bone level

Using bone level implants with an abutment to pass through the mucosa results in a gap between the two parts close to the marginal bone level. Bacteria may develop/reside at this connection resulting in local inflammatory responses (Abrahamsson et al., 1998, Passos et al., 2013). So-called tissue level implants where the mucosal passage constitutes of a collar in continuation of the endosse-

ous part of the implant does not show this phenomenon (Abrahamsson et al., 1998, Abrahamsson et al., 2002). Following the surgery when installing the implants, remodeling of the alveolar bone occur in the range of approximately 2 mm during the first year (Cochran et al., 2009). The amount of remodelling may vary and more bone loss has been reported at bone level implants with an external connection, compared to bone level implants with an internal connection, and in bone level implants with an internal connection compared with tissue-level implants (Laurell and Lundgren, 2011).

Microbiological aspects

The microbiota around dental implants with healthy soft tissue conditions are dominated by gram-positive facultative cocci and rods with gram-negative anaerobic rods present in small numbers (Mombelli et al., 1988, Mombelli and Decaillet, 2011). Thirty minutes after the installation of an implant there is a biofilm in place on the titanium surface (Fürst et al., 2007). Although the surface topography differs between machined and rough titanium surfaces the initial bacterial colonisation is similar (de Melo et al., 2016, Ferreira Ribeiro et al., 2016). Some studies comparing microbiological samples from healthy sites to samples from sites diagnosed with peri-implant mucositis and/or peri-implantitis did not find any significant differences in the microbiota (Renvert et al., 2007, Charalampakis et al., 2012). However, in a later study comparing the microbiome in 47 healthy and 166 peri-implantitis sites using the DNA-DNA checkerboard analysis with a panel of 73 species, a cluster of bacteria including *Tanerella forsythia* and *Staphylococcus aureus* was reported to be associated with peri-implantitis (Persson and Renvert, 2014). Furthermore, using 16S pyrosequencing to analyse subgingival and submucosal samples from healthy and peri-implantitis sites it was reported that the subgingival microbiome is more complex and differs significantly from the submucosal microbiome at implants both in health and disease (Kumar et al., 2012).

Figure 3. Inflamed gingiva (gingivitis) positive for bleeding on probing, BOP.

Gingivitis

In 1965 Loe et al. performed an experiment that has become known as “experimental gingivitis in man” (Figure 3). The authors demonstrated that if bacteria in the oral cavity were allowed to form along the gingiva for three weeks, gingivitis occurred and when oral hygiene was reinstituted the inflammation disappeared (Loe et al., 1965). Later studies have demonstrated that the severity and the time-point when the inflammation occurs may vary (Trombelli et al., 2006, Trombelli et al., 2008)

Figure 4. Clinical and radiographic picture of an implant diagnosed with peri-implant mucositis (PiM)

Peri-implant mucositis

The reaction to de novo plaque accumulation around implants and teeth is similar and results in an inflammatory reaction (Pontoriero et al., 1994, Zitzmann et al., 2001, Salvi et al., 2012). Peri-implant mucositis (PiM) is defined as a reversible inflammatory lesion limited to the surrounding soft tissues of an osseointegrated implant (Zitzmann and Berglundh, 2008, Lang and Berglundh, 2011, Figuero et al., 2014). Clinically peri-implant mucositis is evidenced by bleeding on probing without any bone loss beyond crestal bone level changes resulting from initial bone remodelling (Figure 4).

Figure 5. Clinical and radiographic pictures of implants diagnosed with peri-implantitis (PI)

Peri-implantitis

Peri-implantitis is defined as an inflammatory process around an osseointegrated implant in function, resulting in loss of supporting bone (Lang and Berglundh, 2011). Clinically, peri-implantitis is evidenced by presence of bleeding and/or suppuration on gentle probing and an increased probing depth compared to previous examinations. On radiographs bone loss beyond bone level changes resulting from initial post-installation bone remodeling is present (Figure 5).

Treatment of gingivitis, peri-implant mucositis and peri-implantitis

The treatment protocol for gingivitis, peri-implant mucositis and peri-implantitis all includes professional cleaning and training in self-performed dental hygiene procedures. Good daily plaque control is a prerequisite for a successful treatment outcome and participating in a professional maintenance program is beneficial (Pontoriero et al., 1994, Serino and Strom, 2009, Salvi et al., 2012, Costa et al., 2012).

Peri-implant mucositis can convert into peri-implantitis for which there exist no predictable and effective treatment (Jepsen et al., 2015a). Accumulation of plaque on the implant surface is an etiological factor. Self-administered mechanical plaque control is an effective treatment of peri-implant mucositis, professional oral hygiene instructions and debridement improve mucosal health (Jepsen et al., 2015a). Brushing with a dentifrice containing Triclosan during six months reduced the clinical signs of mucositis compared to the use of a regular fluoride dentifrice (Ramberg et al., 2009). Daily use of adjunctive chlorhexidine gel (0.5%) for four weeks after mechanical treatment does not enhance the result compared to a placebo gel (Heitz-Mayfield et al., 2011). A recent study, where chlorhexidine gel (0.2%) was used while brushing demonstrated a significant reduction of bleeding on probing compared to the use of a placebo gel (Hallstrom et al., 2015a).

Non-surgical treatment of peri-implantitis reduces bleeding on probing and probing pocket depths, but rarely result in control of the disease (Schwarz et al., 2015b). Adjunctive therapies such as the use of an Er: YAG laser, chlorhexidine chip, local antibiotics, glycerine powder air polishing, antimicrobial photodynamic treatment further improved the results of non-surgical therapy alone (Schwarz et al., 2015b).

As non-surgical therapy of peri-implantitis may not result in complete disease resolution, different surgical approaches have been suggested (Schou et al., 2004, Renvert et al., 2008, Renvert et al., 2009, Persson et al., 2010, Figuero et al., 2014). Treatment includes removal of the biofilm and creation of anatomical and prosthetic conditions that makes it possible to maintain oral health.

The surgical procedure may include bone resection, pocket reduction and/or augmentation of bony defects to accomplish this. To clean the surface, mechanical debridement combined with different chemical procedures has been tested (Carcuac et al., 2015, Carral et al., 2016, Hentenaar et al., 2017). At present no chemical treatment have demonstrated superiority (Claffey et al., 2008). Modifying a rough implant surface using burs and polishing devices (implantoplasty) may improve the treatment result (Romeo et al., 2005, Schwarz et al., 2013).

In connection to surgical therapy of peri-implantitis systemic antibiotics are often prescribed post-operatively although the evidence base is limited (Behneke et al., 2000, Leonhardt et al., 2003, Romeo et al., 2005, Roos-Jansaker et al., 2007, Roccuzzo et al., 2011, Roos-Jansaker et al., 2011, Serino and Turri, 2011, Aghazadeh et al., 2012, Heitz-Mayfield et al., 2012, Roos-Jansaker et al., 2014).

Probiotics

Probiotics (Greek: for life). At the beginning of the nineteenth century Elie Metchnikoff stated that “lactic bacilli are good for health”, and Henri Tessier, a paediatrician, claimed that *Bifidobacterium* could restore the balance in the gut microbiota after diarrhoea (Metchnikoff, 1907, Teughels et al., 2008). Parker in 1974 introduced the term probiotics, which means “for life”(Parker, 1974). Up until the past three decades treatment using live bacteria were sparsely used and reported treatment results were mostly of an anecdotal character. During the 1980s, the interest increased and in a review 1989 Roy Fuller claimed that a normal balanced gut flora protects against diseases and if disturbed it can be restored using probiotics (Fuller, 1989). During the last twenty-five years, the use of probiotics has become a commercial success and attracted increasing interest from researchers in different fields of medicine.

The Joint FAO/WHO Expert Consultation 2001 defined probiotics as ‘Live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (WHO). Under normal conditions, probiotics do not permanently colonise the host

and hence have to be supplied daily (Sinkiewicz et al., 2010). *Lactobacillus* and *Bifidobacterium* are lactic acid bacteria and commonly used as probiotics (Teughels et al., 2008). Probiotics can confer health by *a*) providing nutrients and co-factors *b*) compete with pathogens *c*) interact with virulence factors of pathogens and *d*) stimulate the immune response of the host (Yanine et al., 2013). Probiotics have mainly been used and suggested for treatment of different gut problems. However, during the last decade, the suggested treatment panorama has extended to involve both caries and periodontitis. Probiotics may cause interference with the growth of periodontal pathogens (Saier and Mansour, 2005, Stamatova and Meurman, 2009, Mayanagi et al., 2009). Also, the replacement of pathogens with beneficial bacteria may prevent colonisation by periodontal pathogens (Marcotte et al., 2006).

In-vitro studies have demonstrated that probiotics can inhibit or hamper growth of pathogens associated with periodontal disease (Teughels et al., 2011). Probiotic supplements have in some studies reported improved gingival and periodontal conditions and also altered inflammatory markers in gingival crevicular fluid (Krasse et al., 2006, Shimauchi et al., 2008, Staab et al., 2009, Twetman et al., 2009, Iwamoto et al., 2010, Vivekananda et al., 2010, Martin-Cabezas et al., 2016). In a randomised clinical cross-over study Flichy-Fernandez et al. demonstrated positive treatment effects of probiotics on peri-implant mucositis (Flichy-Fernandez et al., 2015). Commercially, probiotic bacteria are available in different products e.g. tablets, lozenges, chewing gums and in milk.

Antibiotics

Antibiotics (Greek: opposing life), kill or slow down the growth of bacteria. Bacteria organised in the submucosal biofilm have a higher tolerance towards antibiotics than bacteria in a planktonic phase (Larsen, 2002, Oettinger-Barak et al., 2013). Systemically administered tetracycline and azithromycin reach higher concentration in GCF and gingival connective tissue than in serum (Lavda et al., 2004, Lai et al., 2011, Raghunatha and George, 2013). Locally delivered antiseptics are at risk of being flushed away due to the flow of peri-implant sulcular fluid. Different slow release local antibiot-

ics have been developed to have a long-term effect in the treatment of peri-implant diseases (Mombelli et al., 2001, Renvert et al., 2006, Bassetti et al., 2014). There is a lack of scientific evidence whether the additive use of systemic antimicrobials should be recommended as an adjunct to surgical treatment of peri-implantitis and hence there is a need of randomised controlled trials (Sanz and Chapple, 2012). At present only one RCT has evaluated the use of systemic antibiotics in conjunction with surgical treatment of peri-implantitis (Carcuac et al., 2016). The additive use of systemic antibiotics did not improve the treatment result following surgical intervention at implants with a non-modified surface whereas an additive effect was reported at implants with a modified surface (Carcuac et al., 2016).

Rationale for the studies

As peri-implant mucositis is a common condition and may proceed to peri-implantitis, there is a need to evaluate methods to prevent and treat the condition (Ericsson et al., 1992, Derks and Tomasi, 2015, Jepsen et al., 2015a). Mechanical debridement and full mouth disinfection can result in clinical improvement evidenced by reduced pockets, decreased bleeding on probing and reduction of the microbiota over three months (Maximo et al., 2009, Thone-Muhling et al., 2010). Following non-surgical mechanical debridement with or without the addition of a chlorhexidine gel (0.5%) complete resolution of BOP at three months was achieved in 38% of the treated implants (Heitz-Mayfield et al., 2011). Accordingly, there is a need to evaluate other treatment modalities to improve these results. One option could be to use either antibiotics or probiotics in treatment of peri-implant mucositis. Due to the global problem of an increasing number of bacteria resistant to antibiotics, partially caused by the frequent use/misuse of antibiotics, there is a need to clarify whether antibiotics have any place in the treatment of peri-implant mucositis and peri-implantitis (Laxminarayan et al., 2013).

To reduce the use of antibiotics an effective alternative to antibiotics is important. Probiotics have been demonstrated to have beneficial effect, alone or in combination with scaling in the treatment

of gingivitis and periodontitis (Krasse et al., 2006, Vivekananda et al., 2010, Maekawa and Hajishengallis, 2014). Hence, probiotics might be a candidate to prevent and treat peri-implant mucositis, thereby decreasing the incidence of peri-implantitis.

AIMS AND HYPOTHESIS

The objectives of the series of studies presented below were to evaluate the adjunctive effect of antibiotics in the treatment of peri-implant mucositis and peri-implantitis and to evaluate the impact of probiotics in preventing and treating peri-implant mucositis.

Study I

Effect of probiotic lozenges on inflammatory reactions and oral biofilm during experimental gingivitis - a crossover study

Aim: to evaluate whether daily oral administration of probiotic bacteria improves clinical parameters of gingivitis by influencing the composition of the supragingival plaque, clinical parameters and levels of inflammatory mediators in an experimental gingivitis model.

We tested the null hypothesis of no differences in the microbiological profile of supragingival samples or the concentrations of selected cytokines/chemokines in gingival crevicular fluid between the test and the placebo group.

Study II

Probiotic supplements and debridement of peri-implant mucositis - a randomised clinical trial

Aim: to evaluate the effect of probiotic bacteria, applied as topical oil in the gingival sulcus and lozenges for three months, on clinical variables adjacent to implants with a clinical diagnosis of peri-implant mucositis. Secondary end-points were effects on inflamma-

tory mediators in gingival crevicular fluid and composition of the subgingival microbiota.

We tested the null hypothesis of no differences in clinical parameters, microbiota or crevicular fluid between implants treated with probiotics supplemented with lozenges and oil or placebo as an additive to mechanical treatment?

Study III

Systemic antibiotics and debridement of peri-implant mucositis - a randomised clinical trial

Aim: to study the clinical and microbiological results during six months after generalised treatment with azithromycin prescribed during four days as an adjunct to mechanical therapy at implants with a clinical diagnosis of peri-implant mucositis.

We tested the null hypothesis of no differences in clinical and microbiological outcomes over six months between treatment with non-surgical debridement alone, or in combination with systemic administration of azithromycin for four days.

Study IV

Open flap debridement of peri-implantitis with or without adjunctive systemic antibiotics - a randomised clinical trial

Aim: to evaluate the clinical and microbiological efficacy over 12 months following open flap debridement of implants with the diagnosis of peri-implantitis with or without systemic azithromycin.

We tested the null hypothesis of no differences in clinical and microbiological outcome over 12 months between peri-implant surgery with or without systemic azithromycin during five days after the intervention.

MATERIAL AND METHODS

Subjects

The Regional Ethics Committee at Lund, Sweden approved the four studies. The studies were performed at The Maxillofacial Unit, Halland Hospital, Halmstad between 2008 and 2013. The 18 participants in study I were recruited among staff members at the Maxillofacial Unit at the Halland Hospital, Halmstad, Sweden. Study individuals in studies II-IV (49, 48 and 39 respectively) were recruited among patients referred to the clinic for the treatment of PiM (study II and III), and for the treatment of Pi (study IV).

Case definitions

Individuals recruited to Study I presented with healthy gingiva adjacent to teeth included in the study. Healthy conditions were defined as no pockets > 3mm, no BOP, and no bone loss as documented on bite-wing radiographs. The participants were shown pictures of different types of probiotic products and were instructed to abstain from using such products during the study. Before initiation of the study, the participants documented their food intakes during a week in an attempt to control if they used any probiotics. Before study start, the participants had their teeth professionally polished Monday through Friday for two weeks to obtain healthy gingival conditions. Eighteen females participated in this cross-over study.

In studies II and III, peri-implant mucositis was defined by the presence of probing pocket depths ≥ 4 mm, bleeding and/or pus on probing using a force of 0.2N, but with <2mm of bone loss as as-

sessed from freshly taken intra-oral dental radiographs in comparison to bone level on radiographs obtained at delivery of the prosthetic reconstruction.

In study IV, peri-implantitis was defined by the presence of inter-proximal marginal bone loss of ≥ 2 mm at screening radiographs compared to inter-proximal bone levels on radiographs obtained one year following implant reconstruction combined with probing pocket depth ≥ 5 mm and with bleeding and/or suppuration on probing. If radiographs from the time of superstructure placement were not available the presence of a distance between implant platform to bone level ≥ 3 mm bone loss identified from peri-apical radiographs at one year was used. Only one implant per individual was used for study purposes.

Study design

Study I, is a prospective randomised double-blinded placebo controlled clinical crossover study. Study II is a two-arm prospective double-blinded placebo-controlled randomised clinical trial. Studies III and IV are two arms prospective operator blinded randomised clinical trials.

Study intervention

In study I, the individuals refrained from oral hygiene of the test teeth for three weeks. During this period lozenges containing *L. reuteri* (ATCC55730 and ATCC PTA5289) or placebo were taken twice a day until day 21. After a two-week extended wash-out period, the placebo and test intervention was exchanged between the groups and a new test period started lasting for 21 days.

In studies II and III (peri-implant mucositis) the participants received an individualised oral hygiene instruction, and the selected implants were mechanically debrided with titanium curettes and polished using rubber cup and polishing paste. In study II the treatment session at base-line was finished with the application of a droplet of oil containing probiotics (test) or placebo (control). Lozenges containing either probiotics or placebo were delivered, and the participants were instructed to use the lozenges twice daily for the coming three months. The active oil and lozenges contained

two *Lactobacillus reuteri* strains (DSM 17938 and ATCC PTA 5289) (Prodentis, BioGaia AB, Lund, Sweden). In study III, following the non-surgical treatment, the test group received Zithromax® 250 mg x 2 at the day of intervention, and 250 mg x1 per day for four additional days. The control group did not receive any placebo.

In study IV a mucoperiosteal flap was raised, granulomatous tissue removed, and the selected study implant was cleaned using sterile curettes and saline-soaked cotton gauze. The flaps were repositioned, and neither pocket reduction, nor bone resection was performed. Postoperatively, the test group received Zithromax® 250 mg x 2 starting on the day of surgery, and 250 mg x1 per day for four days. The control group did not receive a placebo medication.

Outcome parameters and analyses

Clinical data

The intervention was in all four studies evaluated using plaque index (PI), bleeding on probing (BOP) and probing pocket depth (PPD). The change in the supragingival microbiota was analysed in study I, and in study II, III and IV the effect of treatment on the subgingival microbiota were evaluated. In study, I and II effect on the total amount of crevicular fluid and selected cytokines in the crevicular fluid was analysed.

In study I, at baseline (day 0) and at follow-up (day 21) registrations were made of PI, gingival index (GI), and BOP.

In study II and III, the following clinical data were collected at baseline and 4, 12, and 26 weeks: full mouth plaque index, local plaque index (presence of dental plaque along the mucosal margin at included implants), probing pocket depths and bleeding on probing graded as; (1) no bleeding; (2) point of bleeding; (3) line of bleeding; and (4) drop of bleeding at the experimental implants.

In study IV, PI, BOP and PPD scores were recorded at baseline and after 4 and 12 months. All registrations were performed at four sites of the included implant.

Analyses of microbiological data

In study I supra-gingival plaque collected with sterilised wooden toothpicks at baseline and at follow-up were analysed with the DNA-DNA checkerboard technique with microbial probes for *Porphyromonas gingivalis*, *Prevotella intermedia*, *Porphyromonas endodontalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, *Parvimonas micra*, *Campylobacter rectus*, *Streptococcus intermedia*, *Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus mutans*, *Veillonella parvula*, *Actinomyces naeslundii*, *Filifactor alocis*, *Lactobacillus reuteri* and *Lactobacillus fermentum*.

In study II subgingival bacteria were sampled using paper-points inserted to the bottom of the implant pocket at baseline, 1, 2, 4, 12, 26 weeks and analysed for the prevalence of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, *Parvimonas micra*, *Campylobacter rectus*, *Porphyromonas endodontalis*, *Filifactor alocis* and *Prevotella tannerae* utilizing the DNA-DNA checkerboard technique.

In study III subgingival bacteria were sampled at baseline, 1, 2, 4, 12, 26 weeks and in study IV at baseline, 2, 6, 12, 26 and 52 weeks using paper-points inserted to the bottom of the implant pocket and analysed with the DNA-DNA checkerboard technique towards a panel of 73 selected bacterial species.

Analysis of gingival crevicular fluid

In study I gingival crevicular fluid samples (GCF), were collected at baseline and at follow up and in study II at baseline, 1, 2, 4, 12 and 26 weeks with the aid of two separate periopaper strips inserted into the gingival/mucosal sulci for 20s, following gentle air dry-

ing. The fluid volumes were recorded using a Periotron 8000 (ProFlow, Amityville, NY, USA), and expressed in μL . In study I, the concentration of IL-1 β , IL-6, IL-8, IL-10, IL-18, TNF- α , and MIP-1b and in study II, the levels of IL-1 β , IL-1RA, IL-4, IL-6, IL-8, IL-17A, CCL5, TNF- α IFN-g and GM-CSF were determined. In both studies, commercial Bio-Plex Cytokine Assay kits (Bio-Rad Laboratories, Hercules, CA) were used according to the manufacturer's instructions for the xMAP technology with multiple beads. The cytokine concentrations were expressed as pg/mL.

RESULTS

Study I

Clinical data

All subjects exhibited a local accumulation of supragingival plaque and all but one developed gingivitis at the selected test sites during the intervention periods. No significant differences in PI, GI or BOP were displayed between the probiotic test and placebo groups.

Microbiological findings

Increasing bacterial counts were identified in supra-gingival plaque samples. *S. oralis* and *A. naeslundii* were the most prevalent species at baseline and follow-up. *T. forsythia*, *S. mutans* and *L. fermentum* were infrequently identified in all samples. No significant differences were obtained between the groups concerning the microbial species studied. The counts of *F. nucleatum* and *V. parvula* increased significantly in both groups during the development of gingivitis ($p < 0.05$). The counts of *S. oralis* increased only in the probiotic group. Most subjects harboured *L. reuteri* at baseline in both groups, but neither the number of subjects nor the bacterial counts changed markedly during the intervention for *L. reuteri*.

Inflammatory mediators in GCF

The volume of gingival crevicular fluid increased in both groups during the experimental periods but was significant ($p < 0.05$) only in the placebo group. The mean concentrations of TNF- α , IL-6 and IL-10 were not significantly altered between baseline and follow-

up, whereas IL-1 β and IL-18 significantly ($p < 0.05$) increased at follow-up both in the test and placebo groups. Conversely, the mean concentrations of the chemokine IL-8 and MIP-1b were significantly ($p < 0.05$) lower at follow-up compared to baseline.

Conclusion

Daily intake of probiotic lozenges did not significantly affect plaque accumulation, the composition of the biofilm or the inflammatory reaction during experimental gingivitis.

Study II

Clinical data

A general improvement was identified both in the test and in the placebo group at the follow-ups in comparison with baseline, but no significant differences were displayed between the groups. The change in PPD at deepest site at the implant ranged from 0.7–1.2 mm in both groups. The PPD at three months was significantly lower compared to baseline ($p < 0.05$). Similarly, the plaque index and bleeding on probing were reduced, with more than 50% in both groups. The clinical improvements (BOP and PPD) persisted three months after the intervention was terminated.

Microbiological findings

The most prevalent bacterial species were *F. nucleatum*, *P. micra*, *P. intermedia* and *P. nigrescens*. The same four species also displayed the highest counts. Likewise, *F. alocis* was often prevalent, but only in low counts. *P. gingivalis*, *A. actinomycetemcomitans* and *T. denticola* were sparsely detected in all samples. No significant alterations over time or differences between the groups were recorded.

Inflammatory mediators in GCF

The concentrations of the selected cytokines and chemokines in gingival crevicular fluid reflected the improved clinical health. The volume of GCF decreased in both groups during the intervention

and at the follow-up ($p < 0.05$) compared to baseline. There was a tendency of reduced levels of the pro-inflammatory cytokines during the intervention period in both groups compared with baseline, but there were no statistically significant differences between the groups. After four weeks, the levels of IL-1RA, IL-8, CCL5, TNF- α and GM-CSF were significantly lower than at baseline ($p < 0.05$).

Conclusion

Mechanical debridement and oral hygiene reinforcement resulted in clinical improvement of peri-implant mucositis and a reduction in cytokine levels. Probiotic supplements did not provide added benefit to placebo.

Study III

Clinical data

Mean PI, BOP and PPD were reduced compared to baseline in both groups at all occasions. There were no statistically significant study group differences at 1st and 3rd month while at six-month registration significantly lower scores were found for full mouth PI ($p < 0.01$), mean gingival score (%) at implants ($p < 0.02$), and for PPD at worst site of implant ($p < 0.05$) in the test group.

An intent to treat analysis failed to demonstrate study group differences for any of the clinical parameters at baseline or 3rd month after treatment. At the 1st month examination gingival scores (%) at implants was lower in the test group ($p < 0.05$). At the 6th month significantly lower scores were found in the test group for full mouth plaque scores ($p < 0.01$), mean gingival score (%) at implants ($p < 0.01$), full mouth gingival index ($p < 0.05$) and for probing pocket depth at worst site of implant ($p < 0.05$).

Microbiological data

The Kolmogorov-Smirnov test identified that none of the microbial variables presented with a normal distribution pattern. At baseline, the highest counts as determined by mean and median values were found for the following 15 species in decreasing order: *Tan-*

nerella forsythia, *Fusobacterium nucleatum* spp. *naviforme* (selected to represent *Fusobacterium*), *Campylobacter showae*, *Campylobacter rectus*, *Parvimonas micra*, *Aggregatibacter actinomycetemcomitans* (Y4), *Eikenella corrodens*, *Streptococcus pneumoniae*, *Staphylococcus haemolyticus*, *Pseudomonas aeruginosa*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Treponema socranskii*, *Staphylococcus aureus*, and *Porphyromonas gingivalis*.

At all time-points, using either the per-protocol analysis or the intent to treat protocol, statistical analysis failed to demonstrate significant study group differences in bacterial counts for all bacterial species studied. Statistical analysis also was unable to confirm study group differences in the changes of bacterial counts between baseline and the 3rd, and the 6th months respectively. Furthermore, statistical analysis by intent to treat failed to identify within-group differences comparing baseline data with all other time points (1,2, 4, 12 and 26 weeks). At four weeks after treatment, trends ($p < 0.05$) of lower counts were found for *F. nucl* spp. *naviforme*, *S. pneumoniae*, *P. intermedia*, *T. forsythia*, and *T. denticola* but only at this time point and just for the test group.

Conclusion

No short-term differences were found between study groups. The present study does not provide evidence for the use of systemic antibiotics in treatment of peri-implant mucositis.

Study IV

Clinical data

At baseline, all implants bled on probing. At six-months 7% in the test group and 6,3% in the control group demonstrated bleeding on probing. Corresponding figures at 12 months were 13,3% in the test group and 37,5% in the control group. Statistical analysis failed to demonstrate differences in bleeding on probing by study group assignment ($p=0.1$).

Results from analysis between study groups by independent t-test (equal variances not assumed) of probing depth and radiographic bone levels are reported. Data analyses based on mean probing pocket depth, and radiographic bone level values failed to show group differences at time points assessed. Hence, data analysis was unable to demonstrate study group difference for the primary outcome measure.

Microbiological findings

At twelve months, 8/31 (25.8%) individuals presented with no detectable level of nine bacterial species included in the cluster of bacteria explicitly associated with peri-implantitis (Persson and Renvert, 2014). Statistical analysis by Mann Whitney-U tests failed to identify differences between test and control groups for the total bacterial load (sum of DNA of selected bacterial species in the cluster group of bacteria) at all time points (p-values varying between 0.51 to 1.0). When changes in bacterial load over time were compared to the baseline values, data analysis failed to demonstrate group differences in bacterial load. The microbiological analysis by per protocol and intent to treat gave similar results. Trends of decreasing bacterial loads were found between baseline, two and four weeks in both the experimental and the control groups. Such reductions were not retained at later time-points. Based on successful clinical outcome, defined as PPD $\leq$ 5 mm, no suppuration, no BOP at the implant sites, and bone loss $\leq$ 0.5 mm between baseline and year one, no differences in changes of bacterial load were found between successfully and non-successfully treated implants.

Conclusion

Surgical open flap debridement of peri-implantitis with adjunctive systemic azithromycin did not provide any one-year clinical benefits in comparison to those only receiving open flap debridement.

DISCUSSION

The rationale for the studies in this thesis was: (I) the reported high rate of soft-tissue complications around dental implants, (II) recent data suggesting benefits of probiotics in periodontal therapy, and (III) the frequent use of systemic antibiotics when treating peri-implant inflammation without clear evidence of treatment efficacy.

Sites having a clinical probing depth ≥ 4 mm in conjunction with bleeding and/or suppuration on probing using a probing force of 0.2 N, and bone-loss ≤ 2 mm on present radiographs compared to radiographs taken at the time of prosthetic delivery was diagnosed as peri-implant mucositis (studies II and III). At healthy periodontal sites, using a probing force of 0.2 N the probe tip reaches the most apical part of the junctional epithelium (Lang et al., 1994). When gingival and peri-implant tissues are inflamed, the probe penetrates deeper than at healthy sites and deeper into peri-implant tissue compared to gingiva (Ericsson and Lindhe, 1993, Lang et al., 1994, Schou et al., 2002). Bleeding on probing (studies II-IV) has been used and recommended as a discriminating factor assessing health and disease at dental implants (Jepsen et al., 2015a). Bleeding can, however, be initiated by the trauma induced by probing of the peri-implant tissues and may not necessarily be associated with disease (Ericsson and Lindhe, 1993). A graded bleeding index may, however, improve the evaluation of BOP values. To assess differences between peri-implant mucositis and peri-implantitis changes in bone levels beyond the initial bone remodelling is considered as a discriminating factor (Lang and Berglundh, 2011).

In studies II-IV, radiographs were used to study alveolar bone levels at implants to assess the presence and extent of bone loss. Bone-loss beyond the radiological measurement error in combination with inflammation has been proposed to define peri-implantitis (Sanz and Chapple, 2012). Radiographs taken at the time of prosthesis delivery should be used as reference for future assessment of implant conditions and confirm a diagnosis of peri-implantitis or not (Lindhe et al., 2008). The actual bone loss at dental implants is, however, under-estimated when assessed from peri-apical radiographs (Serino et al., 2017, Christiaens et al., 2018). Newer methods, using three-dimensional (3D) image superimposition, measuring volumetric bone changes around implants has however demonstrated a correlation between direct measures on periapical radiographs and volumetric measurements on Cone Beam Computed Tomography (Villarinho et al., 2018). In the present study, intra-oral conventional long-cone X-rays over time was used to assess bone level changes.

The association between the presence of plaque and the development of peri-implant mucositis has been demonstrated (Lindquist et al., 1988, Ericsson et al., 1992, Pontoriero et al., 1994, Zitzmann et al., 2001, Lang and Berglundh, 2011). An increased amount of plaque has also been reported at sites diagnosed with peri-implant mucositis and peri-implantitis. Since bacterial plaque is an essential factor for the development of peri-implant diseases microbiological analyses were included in the thesis (Studies I-IV). The peri-implant microbiota was assessed before and after study intervention. The supragingival bacterial samples in study I was collected with sterilised wooden sticks. In study II-IV, microbial samples were collected with paper strips. Paper strip harvesting of sub-gingival microorganisms has been shown to give a higher variation and magnitude of microorganisms compared to the use of curettes (Gerber et al., 2006). In all studies, the microbial samples were analysed with the checkerboard DNA-DNA hybridisation technique either at the microbiology laboratory at the University of Gothenburg, Sweden (Study I, and II), or the microbiology laboratory at the University of Bern, Switzerland (Studies III, and IV). Studies have shown that the checkerboard method has a high level

of agreement with conventional anaerobic and aerobic culture methods (Moncla et al., 1994, Socransky et al., 2004).

In a study testing the reproducibility, the authors concluded that subsequent subgingival samples analysed with checkerboard DNA-DNA hybridisation displayed an acceptable reproducibility between repeated samples (Hallstrom et al., 2015c). The checkerboard DNA-DNA hybridisation technique allows identification of both dead and alive bacteria whereas culture methods only detect living organisms. Checkerboard analyses only identify the presence of DNA-DNA signals against reference strains whereas culture methods are specific to culturing methods, i.e. growth media. Today there are methods to analyse the biofilm sample on all its nucleic acid sequences and then by comparing the found sequences with e.g. the Human Oral Microbiome Database identify all bacteria in the biofilm, and thereby overcome some of the limitations associated with culturing and checkerboard analyses (Kilian et al., 2016). In addition to the microbiological assays, levels of pro-inflammatory cytokines in peri-implant crevicular fluid samples were studied using multiplex technology (studies I, and II).

Results from a data analysis showed that levels of pro-inflammatory cytokines might predict peri-implantitis (Wang et al., 2016a, Wang et al., 2016b), and levels of interleukins IL-1 β , IL-6, TNF- α , have been linked to the clinical response (Ata- Ali et al., 2015). In studies, I, and II the levels of cytokines were used to assess clinical responses to the study interventions.

An experimental gingivitis study was chosen to evaluate the effects of additive probiotics on the development of gingival inflammation. By inviting the clinical staff of the department to participate in this study we tried to minimise the impact of lousy compliance, and using a custom-made splint also lowered the risk for accidental brushing. Tissue reaction to plaque accumulation is reported to be different between individuals (Trombelli et al., 2004) and we, therefore, used a crossover study design.

Probiotics have been tested both as primary and secondary prevention for oral diseases.

In Study I, no significant differences in PI, GI or BOP were found between the two tested modalities. The volume of gingival crevicu-

lar fluid increased in both test and control groups, but only statistically significant in the placebo group. The concentration of TNF- α and IL-1 β also increased with increasing gingival inflammation. These findings are in agreement with another study (Offenbacher et al., 2010).

In a previous study using chewing gums containing two strains of *Lactobacillus reuteri*: ATCC 55730 and ATCC PTA 5289, a significant reduction of TNF- α and IL-8 levels ($p < 0.05$) were reported in adults with moderate gingivitis (Twetman et al., 2009). Such effects could not be identified in the experimentally induced gingivitis study (Study I). It is possible that the use of chewing gums is more efficient than the distribution of probiotic microorganisms via lozenges. It is also possible that differences in the strains used in the two products could affect the results. In Study, I, DSM 17938 and ATCC PTA 5289 strains were used whereas in the study by Twetman et al. (2009) different lactobacilli (strains ATCC 55730 and ATCC PTA 5289) were used. Furthermore, the studies on the effects of probiotics to control inflammation in established gingivitis lesions may be entirely different from the effects of an experimental gingivitis model that resembles the development of an acute inflammatory process.

Several studies have been performed aiming at reducing and changing the biofilm on the implant surface preventing the reestablishment of a pathologic biofilm (Salvi et al., 2012, Pontoriero et al., 1994, Zitzmann et al., 2001, Schwarz et al., 2015a). Non-surgical debridement and oral hygiene instructions result in reduced bleeding on probing even if it does not entirely resolve the inflammation (Heitz-Mayfield et al., 2012, Maximo et al., 2009, Thone-Muhling et al., 2010) which was confirmed in this thesis (Studies II, and III). Non-surgical debridement alone is considered as the golden standard of initial therapy to which any additive interventions or home care anti-infective substances should be evaluated. Among tested additives to non-surgical therapy are minocycline microspheres, chlorhexidine (rinsing or gel), tetracycline fibres, air polishing with glycine powder (Figuerio et al., 2014). None of these additive treatment modalities has demonstrated statistical-

ly significant advantages compared to standard of care (Schwarz et al., 2015a). One study using tooth brushing with a chlorhexidine gel reported a substantial reduction of BOP while another similar study did not (Heitz-Mayfield et al., 2011, Hallstrom et al., 2015a). Data also suggest that a dentifrice containing triclosan can reduce the extent of bleeding on probing compared to using a sodium fluoride dentifrice (Ramberg et al., 2009).

There are some studies on probiotics in the treatment of gingivitis and periodontitis demonstrating significant reductions in clinical inflammatory parameters (Krasse et al., 2006, Vivekananda et al., 2010). An effect on the subgingival microbiota without any significant impact on the inflammatory response has been reported (Iniesta et al., 2012, Montero et al., 2017). As of March 2018, a search on PubMed on “peri-implant mucositis and probiotics” yielded five studies (Flichy-Fernandez et al., 2015, Hallstrom et al., 2015b, Mongardini et al., 2017, Tada et al., 2017, Galofre et al., 2018). The results are difficult to compare since the design, inclusion and diagnostic criteria and parameters used for evaluation differ. In Hallstrom et al. (2015) study II all subjects received subgingival mechanical debridement with titanium curettes, OHI and were polished. In the studies by Flichy-Fernandez et al., (2015) and Galofre et al. (2018) only supragingival debridement were performed. In the study by Tada et al. (2017) supragingival debridement was combined with the use Azithromax® and in the study by Mongardini et al. (2017) supragingival debridement was supplemented with photodynamic biolight (Table 1). Subgingival debridement and OHI reduces BOP in PiM cases but does not lead to complete resolution of inflammation (Heitz-Mayfield et al., 2011). Studies on experimentally induced PiM have shown that reinstituted oral hygiene result in resolution of inflammation (Pontoriero et al., 1994, Salvi et al., 2012).

Table 1. Treatment outcome measured as bleeding index and group differences. “Yes” means a significant reduction in bleeding index or difference between test and control ($p<0.05$).

Author, year Treatment at baseline	Diagnosis	Index	Probiotics reduction	Placebo reduction	Group difference
(Galofre et al., 2018) Supra gingival prophylaxis	PiM	BOP	Yes	No	Yes
(Tada et al., 2017) OHI, supra gingival scaling, Azithromax	Pi (mild)	mBI	No	No	Yes
(Mongardini et al., 2017) OHI, plaque removal photodynamic therapy	PiM Experim.	BOP	Yes	Yes	No
(Hallstrom et al., 2015b) OHI, mechanical debridement	PiM	BOP	Yes	Yes	No
(Flichy-Fernandez et al., 2015) Supra gingival prophylaxis	PiM	mGI	No	No	No

In study II, the results failed to identify differences between active and placebo groups regarding the microbiota and clinical outcome measures. However placebo should not be confused with no treatment as there is solid evidence that placebo can have an effect even on physiological data as well as the fact that just participating in a study can alter disease parameters. It has been demonstrated that the additive of a tasty non-effective placebo to an immunosuppressing medication can “teach” individuals to suppress the immune response and later exposition to the placebo alone will lower the immune response measured as T-cell activity (Owens et al., 1997, Feil et al., 2002, Albring et al., 2014).

Systemic administration of Azithromycin® (Sandoz AS, Copenhagen, Denmark) has proven useful in the treatment of adult periodontitis (Smith et al., 2002, Mascarenhas et al., 2005, Haas et al., 2008, Yashima et al., 2009). At the time of initiation of Study III, no data were available on the additive effects of Azithromycin® in the treatment of peri-implant mucositis. Therefore, the impact on

the management of peri-implant mucositis using adjunct Azithromycin® was studied. At three months, a trend towards lower bacterial counts was found in the test group. At six months, however, there was no difference between study groups. A general trend of increasing microbiological numbers was, however, identified over time following an initial reduction shortly after antibiotic intake. Similar results have been determined elsewhere in the treatment of periodontitis (Yashima et al., 2009). At six months, the test group demonstrated more significant reduction of bleeding on probing and probing pocket depth compared to the control group. This difference between groups, observed at six months, was not found earlier in the study and could be explained by the superior plaque control in the test group rather than the effect of antibiotic treatment six months before registration. Thus, the adjunct therapy with systemic antibiotics in the management of peri-implant mucositis considering the overall risks for development of antibiotic resistance is not warranted.

There is a correlation between clinical signs of bleeding on probing and the histological level of mucositis and therefore BOP is considered a key parameter to distinguish between health and disease at implants (Lang et al., 1994, Lang and Berglundh, 2011, Tonetti et al., 2015). Hence, reporting and evaluating the treatment results from peri-implant mucositis studies, bleeding on probing is often used as the primary outcome parameter. BOP is often reported as the proportion of bleeding sites at baseline and follow ups. The four study groups in study II and III demonstrated, measured on implant level 100 % BOP at BL and reduction to 80-90% at 26 weeks and at surface level 80% BOP at BL and reduction to 30-45% at 26 weeks. If a point of bleeding is interpreted as disease 80-90% of the implants would be classified as diseased compared to 30-45% of the surfaces. (Figure 6 and 7). The dichotomous character of BOP registrations shows the extension of inflammation but says nothing about the severity. By grading the bleeding 0=none, 1=point, 2=line and 3=drop, (Mombelli et al., 1987). Information about the severity is added to the status (Figure 8).

Figure 6. BOP expressed as proportion (%) of surfaces (S) and of implants (I) at BL and follow ups 4, 12 and 26 weeks. (Prob=probiotics; Plac=placebo)

Figure 7. BOP expressed as proportion (%) of surfaces (S) and of implants (I) at BL and follow ups 4, 12 and 26 weeks. (Ab=antibiotics; Cont=control)

Figure 8. Bleeding on probing (%) at BL, 4, 12 and 26 weeks. (0) no bleeding, (1) point of bleeding, (2) line of bleeding and (3) drop of bleeding. Plac=placebo, prob=probiotic.

The optimal goal of the treatment of peri-implantitis would be to change the biofilm at implants to what might be associated with healthy conditions, reinstituting clinical health, and allow regeneration of lost bone adjacent to the implant. A more realistic goal may be to resolve the inflammation preventing the progression of the disease (Lindhe et al., 2008, Heitz-Mayfield et al., 2012). Available data suggest that non-surgical treatment of peri-implantitis has a low efficacy and that surgical treatment is considered the method of choice (Persson et al., 2010, Persson et al., 2011, Roos-Jansaker et al., 2015).

Surgical treatment of peri-implantitis usually includes open-flap debridement and decontamination of the implant surface (Sanz and Chapple, 2012). Systemic administration of antibiotics is commonly used in combination with surgical treatment of peri-implantitis (Graziani et al., 2012, Renvert et al., 2012). Approximately 70% of American periodontists have reported that they used systemic antibiotics in some cases when treating peri-implant mucositis, and in about 90 % in the treatment of peri-implantitis (Papathanasiou et al., 2016). Recently it was, however, demonstrated that additive systemic antibiotics might only improve treatment results when performing peri-implantitis surgery on implants with a modified surface while there was no such beneficial effect at “non-modified surface implants” (Carcuac et al., 2016).

Reductions in bacterial loads at three and six months after surgery were obtained in both test and control groups. At twelve months, however, increasing bacterial counts were identified in both study groups. Thus, the data determined that the antibiotic prescription accordingly failed to prevent recolonization following therapy suggesting that the administration of adjunctive antibiotics provides no added values compared to surgical intervention alone. The fact that bacterial colonisation after intervention occurred are consistent with six month data reported in a previous study (Renvert et al., 2016). The clinical results of surgical intervention at peri-implantitis sites as reported in study IV are consistent with data reported in recent reviews (Renvert and Polyzois, 2015, Ramanauskaite et al., 2016).

CONCLUSIONS

Daily intake of probiotics does not affect supragingival plaque accumulation compared to placebo administration during the development of experimental gingivitis

Probiotic supplements do not add benefits to placebo regarding the impact on the subgingival microflora, on pro-inflammatory cytokines or oral clinical parameters during the development of experimental gingivitis

Adjunctive systemic administration of azithromycin to non-surgical debridement and oral hygiene instructions in the treatment of peri-implant mucositis does not provide additional clinical benefits when compared to routine non-surgical debridement without the use of systemic antibiotics

Systemic intake of azithromycin as adjunct to surgical open flap debridement in the treatment of peri-implantitis does not provide additional clinical benefits at twelve months when compared to routine surgical debridement without the use of systemic antibiotics

FUTURE RESEARCH

There is an urgent need for additional clinical studies on the prevention and treatment of peri-implant inflammations. Currently, few randomised clinical trials exist. Additionally, it is important to further elucidate risk indicators for peri-implant infections. This should be done in order to allow clinicians to minimise the risk of future peri-implant diseases by proper patient selection, choice of implants, surgical method and in the design of the prosthetic reconstruction. There is a further need to explore the possibilities of developing the prosthesis and other hardware products in a way that they may delay or even prevent the development of peri-implant diseases, e.g. the development of antibacterial properties on dental implant surfaces.

Even if the addition of probiotics did not result in a beneficial treatment effect in our studies, the additive use of probiotics in the treatment of infections is a promising field of research where others have shown interesting results. There are other ways of administering probiotics and also other bacterial strains that could be used than the ones used in this thesis.

CLINICAL RECOMMENDATIONS

As peri-implant mucositis is common and precedes the development of peri-implantitis future treatment strategies and research should be oriented towards prevention and treatment of peri-implant-mucositis.

For clinicians, it is therefore important to;

- Design the prosthetic construction so that it facilitates a good plaque control
- Involve implant patients in a structured maintenance program
- Register bleeding on probing and pocket depth at least once yearly
- Perform non-surgical therapy at sites demonstrating bleeding on gentle probing
- Reinforce patients' oral hygiene procedures

POPULÄRVETENSKAPLIG SAMMANFATTNING

Ett ökande antal individer har artificiella tänder som är förankrade till implantat i käkarna. Den moderna tandimplantatsbehandlingen är en svensk upptäckt som blev tillgänglig för patienter under 1970-talet. Detta tillsammans med det faktum att behandlingen ingår i det allmänfinansierade tandvårdsstödet har inneburit att det i Sverige är vanligt med implantat och att många patienter har haft sina implantat under lång tid.

Långtidsresultaten för implantatbehandling är goda och många har haft sina konstruktioner i tiotals år. Men som med all behandling förekommer det komplikationer. Dels tekniska komplikationer, vanligtvis frakturer eller lossade skruvar i tandersättningsdelen, men också frakturer av implantaten som är förankrade i käken och bär dessa konstruktioner. Biologiska komplikationer förekommer framförallt i två former, peri-implantär mukositis och peri-implantit. Peri-implantär mukositis är en inflammation i slemhinnan runt ett implantat. I en del fall utvecklas denna inflammation till peri-implantit som innebär att inflammationen också involverar och bryter ner käkbenet vilket på sikt kan leda till att implantatet lossnar. Förekomsten av biologiska komplikationer ökar desto längre implantaten varit i funktion och beroende på detta, samt hur man väljer att definiera peri-implantär mukositis och peri-implantit så är den rapporterade förekomsten 19–65% respektive 1–47%.

Behandlingen av inflammationer kring implantat består framförallt av att tandvårdspersonal ger hygieninstruktioner till patienten samt avlägsnar bakterier och tandsten på implantaten. I de fall där

käbbenet blivit involverat så krävs ofta dessutom kirurgi. Inte sällan kompletteras behandlingen med antibiotika. Oavsett behandlingsform så uppnås sällan fullständig utläkning och trots att antibiotika ofta används är det inte belagt huruvida detta förbättrar behandlingsresultatet. Utvecklingen av bakterier som är resistent mot antibiotika är ett stort och globalt problem. Frekvent användande av antibiotika är en faktor som ökar resistensutvecklingen i samhället och därför ska antibiotika användas endast när det erfordras och är effektivt. Probiotika är levande bakterier som, givet i adekvat dos, har en positiv inverkan på den behandlade individen, det vill säga en hämmande eller läkande effekt på inflammationer kring implantat.

Målet med denna avhandling är att klargöra huruvida systemiskt administrerad antibiotika förbättrar behandlingsresultatet vid behandling av peri-implantär mukosit och peri-implantit och om probiotika kan förebygga uppkomsten av inflammationer kring implantat eller förbättra behandlingsresultatet.

Avhandlingen består av fyra studier som på patient undersökt,

- om sugtabletter med probiotika kan hindra eller mildra uppkomsten av inflammation i tandköttet när man avstår från att borsta tänderna
- om sugtabletter med probiotika förbättrar resultatet vid behandling av peri-implantär mukosit
- om antibiotika givet i tablettform förbättrar resultatet vid behandling av peri-implantär mukosit
- om antibiotika givet i tablettform förbättrar resultatet vid kirurgisk behandling av peri-implantit

Avhandlingsarbetena visar,

- att dagligt intag av sugtabletter med probiotika inte hämmar eller förhindrar uppkomsten av inflammation i tandköttet när man avstår från att borsta tänderna
- att dagligt intag av sugtabletter med probiotika som tillägg till munhygien instruktion och mekanisk rengöring av implantat vid peri-implantär mukosit inte förbättrar behandlingsresultatet

- att tabletter med azitromycin som tillägg till hygieninstruktion och mekanisk rengöring av implantat vid peri-implantär mukosit inte förbättrar behandlingsresultatet
- att tabletter med azitromycin som tillägg till hygieninstruktion och operation med mekanisk rengöring av implantat vid peri-implantit inte förbättrar behandlingsresultatet
- att hygieninstruktion och mekanisk rengöring vid peri-implantär mukosit leder till minskad inflammationsgrad
- att hygieninstruktion och operation med mekanisk rengöring vid peri-implantit leder till minskade fickdjup och viss återväxt av käkben

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