Microbiological Status and Clinical Outcomes in Peri-Implant Mucositis Patients Treated with or without Adjunctive Bioadhesive Dental Gel

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Abstract

Aim: Biological complications of restored dental implants share similarities with the biofilm infections of natural dentition. Mechanical non-surgical therapy could be effective in the treatment of peri-implant mucositis lesions. The objective of this study was to assess the efficacy of a commercially available dental gel containing 0.05% cetylpyridinium chloride and essential oils for controlling established peri-mucositis.

Methods: A double-centre, double-blind, randomized, parallel group clinical trial was conducted for a period of 6 weeks. Microbiological changes were also recorded.

Results: The overall effect of the subgingival application of a bioadhesive dental gel, was statistically significant for both changes in Probing Depth (PD) (p=0.016) and Bleeding on Probing (BoP) (p=0.001) but not for microbial loading.Mean PD decreased from 4.6 mm at baseline (T_0) to 3.2 mm at 6 weeks (T_1), Clinical Attachment Level (CAL) from to 3.1 mm at baseline (T_0), were reduced to 2.7 mm. In both treatment groups, Plaque Index score (PI) showed a trend towards reduction at 6 weeks from 55.4% to 30.2%. In peri-implant mucositis patients without pus formation, all parameters decreased and no additional surgery was necessary.

Conclusions: Both treatment modalities led to an improvement of the clinical parameters and a temporary reduction ofmicroflora at implants with mucositis. Within the limits of the present study, the results indicate that, compared with supportive therapy alone, adjunctive subgingival application of a bioadhesive dental gel, associated to strict home care regimen could contribute enhancing outcomes of implant therapy as regard PD and BoP.

Key Words: Nonsurgical periodontal treatment, Mucositis, Peri-implantitis, Dental gel, Microbiological evaluation, Cetylpyridiniumchloride, Essential oils.

Introduction

Dental implants have been reported to achieve long-term success, however they are not guarantee from potential complications, due to improper treatment planning, surgical and prosthetic execution, material failure, and maintenance [1]. Included in the latter are the biologic complications of peri-implant mucositis and peri-implantitis, inflammatory conditions in the soft and hard tissues at dental implants [1], requiring management through several strategies applied at different stages

To date, evidence suggests that peri-implant mucositis can be successfully treated, if they are early detected and when combined with effective nonsurgical efforts [1-3]. Careful monitoring and preventive care of peri-implant tissue health, during maintenance, is of paramount importance [4]. The long-term outcomes of implant therapy appear to be enhanced by supportive periodontal treatment for patients who are periodontally compromised [5], but not in those who are not compliant [6].

Proper maintenance is imperative, since implants, like teeth, are susceptible to bacterial plaque accumulation and calculus formation, and thus at risk of developing peri-implant mucositis or peri-implantitis [7]. The dental team must play a critical role in educating patients to control plaque-biofilm associated with peri-implant tissues and associated restorations [8,9].

Since tooth-paste/gel is the standard medical device used for in house plaque control and it potentially have positive effect in controlling peri-implant mucositis. Recently a new dental gel was introduce in Italian market, called Hobagel (Hobamasrl, Milan – Italy).

The gel consists of an original mixture of various compounds. Some of those have a specific adhesive function (poli-vinil-pirolidone copolymer, cellulose gum hydrated silica), while other substances (Cetylpyridinium chloride and triclosan) have an antiseptic action. Certain essential oils (Melaleuca alternifolia, thymus vulgaris and commiphora myrrha) offer antioxidant and antiphlogistic properties, Sodium hyaluronate have strong hydrating and healing capacity. Bisabolol and Vitamin E, in microcapsules, can relieve pain.

Methods

Study design

This study was designed as a double-centre, double-blind, randomized, parallel group clinical trial. The blind was maintained since a number was given to each patient by two clinicians (M.R. and G.G.) whereas clinical and microbiological data were elaborated by a third author (F.C.).

Microbiological changes were also recorded.

Subjects were well informed of the study protocol and objectives, and gave their written consent before participation. The study was conducted according to the European directives and ICH Harmonised Tripartite Guideline E6: Note for Guidance on Good Clinical Practice, CPMP/ICH/135/95 Step5 (http://www.ema.europa.eu)

Study population

Consecutive subjects (38 subjects) were screened and enrolled

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in this clinical trial if they fulfilled the following criteria:

Inclusion criteria

• ≥ 18 years old.

• Systemically healthy, but mild diabetes.

• Presence of at least 2 evaluable prosthetically restored implants in different quadrant.

• Light/Moderate peri-implant mucositis (≥40% bleeding on BoP) (Van der Weijden et al. 1994a).

• Absence of probing pocket depths (PPD ≥7 mm).

• Subjects willing to participate and comply with the objectives of the study.

Exclusion criteria included:

(1) Pregnancy

(2) An history of taking antibiotics or using antibacterial mouth rinses for past 6 months,

(3) Drug or alcohol abuse,

(4) An ongoing dental or medical treatment,

(5) Allergy to previously used oral hygiene products or any known allergy to any of the ingredients of the study products.

Twenty four female and 14 males were enrolled. Four have diabetes, 30 do not smoke whereas four, two and two smoke less than 5, 10 and 15 cigarettes per days, respectively. The mean age was 58.8 ± 8.3 years. Seventeen maxillary and 22 mandibular implants supporting prostheses were evaluated, in the following sites: 4 incisors, two cuspids, 12 premolars and 20 molars.

Clinical methods

In each patient, implants with perimplant mucositis were randomly assigned to test or control treatment, according to a split mouth design (76 sites).After microbiologic diagnosis, all patients were treated at baseline and received individualized home oral hygiene instructions. Non-surgical periodontal instrumentation was performed with hand instrumentation (*Figure 1*), utilizing a titanium curette (Roncati implant care, by Martin KLS, Germany) and piezoelectric ultrasonic device with plastic fused to a metal insert (Piezon Master 700, EMS, PI insert) as needed. Test implants received adjunctive antimicrobial treatment by locally delivered bio-adhesive dental gel (HG) (*Figure 2*).

Measurements

At baseline and 6 weeks, the following measurements were taken at 6 sites per implants:

- Modified Plaque Index (PI) (Mombelli A, et al. 1987)

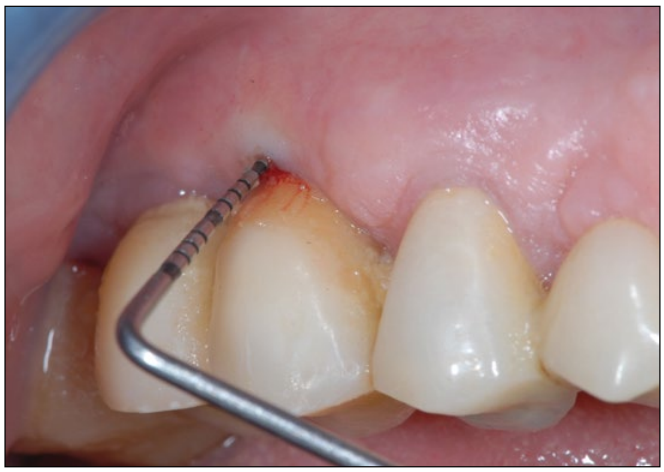

***Figure 1**. Probing depth (PD) is about 5 mm, buccally on posterior maxillary implant, BoP positive.*

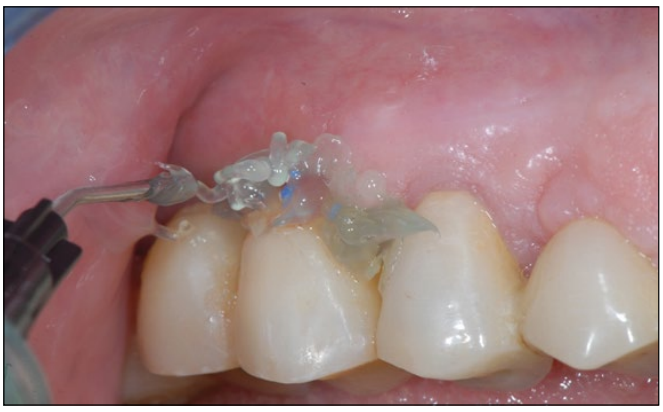

***Figure 2**. The image illustrates the adjunctive antimicrobial treatment by locally delivered bio-adhesive dental gel.*

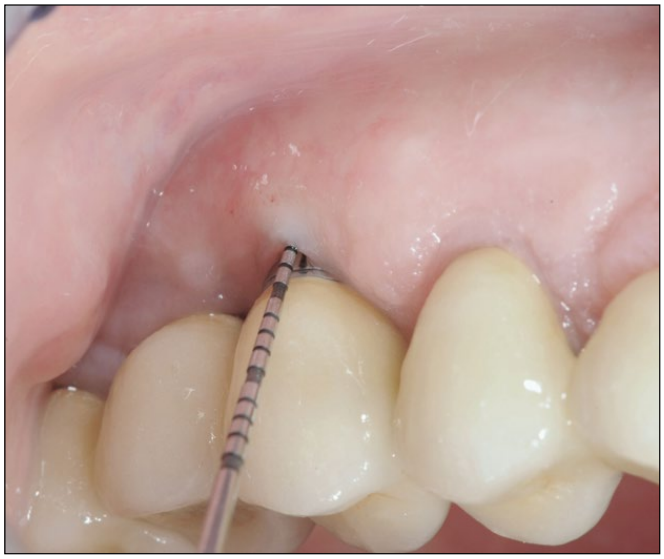

***Figure 3.** The same site, as in Fig.1, 6 weeks later. The peridontal probe measures 2 mm PD. The tissue surrounding the implant seems to offer better clinical stability, compared to initial clinical evaluation.*

- Bleeding on Probing (BoP) (Van der Weijden, et al. 1994a).
- Clinical Attachment Level (CAL), using the top of the implant abutment as a reference point.

For bacteria analysis, sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of subgingival samples and were immediately transferred to microbiological lab for processing. Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Campylobacter rectus and total bacterial loading were evaluated.

Real-time polymerase chain reaction

Probes oligonucleotides were designed basing on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA Ref Seq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequence or less conservative spots. Three real-time Polymerase Chain Reaction (PCR) runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching a highly conservated sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria, i.e. P. gingivalis, T. forsythia and T. denticola, in a multiplex PCR. The third reaction detected

and quantified Aggregatibacter actinomycete mcomitans, Fusobacterium nucleatum, Campylobacter rectus in a multiplex PCR. These two reactions included a total of six primers and three probes that were highly specific for each species.

Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile were initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All these experiments were performed including non-template controls to exclude reagents contamination. Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same amount of plasmids, in serial dilutions ranging from 10^1 to 10^7 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions (data not shown). The copy numbers for individual plasmid preparations was estimated using the Thermo Nano Drop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of relative amount of species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling was performed in a separate laboratory with dedicated pipettes.

After six-weeks microbiological samples were collected again from both sites in each patient (*Figure 3*) and the analysed with RT-PCR method.

Statistical analysis

SPSS program and paired simple statistic *t*-test were used to detect statistical significant differences between groups.

Results

All 38 patients (76 sites, 38 test and 38 control) completed the study.

The overall effect of the subgingival application of a BIOADHESIVE DENTAL GEL, Hobagel, HobamaSrl, Milan, Italy (HG), was statistically significant for both changes in Probing Depth (PD)(p=0.016) and Bleeding on Probing (BoP) (p=0.001). No statistical significant differences occurred for microbial loading changes when test were compared with control sites.Mean PD decreased from 4.6 mm at baseline (T_0) to 3.2 mm at 6 weeks (T_1), Clinical Attachment Level (CAL) from to 3.1 mm at baseline (T_0),were reduced to 2.7 mm. In both treatment groups, Plaque Index score (PI) showed a trend towards reduction at 6 weeks from 55.4% to 30.2%.

Statistical significant improvement was detected on BoP and PD by using HG in association with standard methods. Clinical parameters improved in both groups.

Table 1 reports mean values and statistical significance.

In test implant, combination of non-surgical periodontal treatment and adjunctive topical application of micro-granular dental gel resulted in a reduced BoP trend (from about 90% to 16%, bleeding teeth percentage compared to total tested teeth).

Table 2 reports mean values and statistical significance.

Discussion

The prevalence of peri-implant complications will increase as dental implant-retaining prostheses become worldwide. Peri-implant diseases are present in two forms: peri-implant mucositis and peri-implantitis. Plaque-induced mucositis is a reversible inflammation of the peri-implant gingiva. The

Table 1. *Clinical parameters. Group 1 – Treated without Hobagel Group 2 – Treated with Hobagel, SD – Standard deviation.*

Variale	Group	Meanvalue ± SD T_0	Meanvalue ± SD T_1	Statistical significant differences
Plaque Index	1	57.2 ± 21.1	57.2 ± 21.1	No
	2	30.1 ± 9.0	30.1 ± 9.0	No
Probing	1	4.5 ± 0.8	4.9 ± 0.6	No
	2	3.5 ± 0.6	2.6 ± 0.8	p=0.016
Bleeding	1	0.8 ± 0.4	1.0 ±0.1	No
	2	0.3 ± 0.4	0.1 ± 0.1	p=0.001
CAL	1	3.4 ± 1.5	3.4 ± 1.9	No
	2	3.2 ± 1.2	2.6 ± 1.6	No

Table 2. *Bacteria loading.*

Variable	Group	Meanvalue ± SD T_0	Meanvalue ± SD T_1	Statistical significant differences
Aggregatibacter Actinomycetemcomitans	1	677 ± 2926	766 ± 3270	No
	2	21 ± 91	167 ± 732	No
Treponemadenticola	1	2899 ± 9811	5499 ± 15584	No
	2	2752 ±7137	1652 ± 5475	No
Tannerella forsythia	1	1779 ± 2758	2499 ± 4451	No
	2	22342 ± 89628	487 ± 889	No
Porphyromonasgingivalis	1	802 ± 1571	1222 ± 3328	No
	2	23654 ± 77149	1495 ± 3999	No
FusobacteriumNucleatum	1	66308 ± 134292	188548 ± 517020	No
	2	157332 ± 385878	57896 ± 137154	No
Campylobacter Rectus	1	3926 ± 8605	13014 ± 40877	No
	2	12535 ± 40217	958 ± 1886	No
Total bacterial loading	1	1201689 ± 2173052	1659060 ± 3205363	No
	2	1881258 ± 3344905	806839 ± 1794476	No

description of the inflammatory process of peri-implant mucositis around an implant is quite similar to gingivitis around natural teeth [4]. It is generally accepted that mucositis will eventually give rise to peri-implantitis, with inflammation encroaching on the alveolar support. Long-term maintenance care for high-risk groups is essential to reduce peri-implantitis prevalence [10]. Periodic evaluation of implants, surrounding tissues and oral hygiene are vital to the long-term success of the dental implant [11].

Reviewing the literature, the available evidence for non-surgical treatment of peri-implant mucositis and peri-implantitis is scarce [12].

Peri-implant mucositis and peri-implantitis differ with respect to treatment [1]. Depending on the severity of the peri-implantitis lesion, surgical or nonsurgical procedures should be implemented [10].

It was observed that mechanical non-surgical therapy could be effective in the treatment of peri-implant mucositis lesions [12]. Non-surgical treatments are also recommended for peri-implant defects with less than 2 mm destruction [13] or as initial treatment, prior to surgical management [10].

The outcome of nonsurgical periodontal treatment of peri-implantitis is inconsistent and unpredictable [14]. The decision as to whether a questionable implant should be treated and maintained non-surgically or surgically is complicated, due to variables related to patient behavior. Nonsurgical periodontal treatment is indicated when a patient has medical or psychological contraindications. In peri-implant infections, 5 and 6 mm probing depths are frequently found and initially treated non-surgically [10].

As peri-implant diseases are initiated and exacerbated by bacteria then microbiota and their products removal becomes essential. Commonly used approaches for non-surgical implant surface detoxification are both mechanical and chemical methods [15].

Combination therapy i.e. non-surgical periodontal treatment plus adjunctive antimicrobial treatment by locally delivered bio-adhesive dental gel showed a trend in reducing BoP scores in peri-implant mucositis. These outcomes may improve patient's comfort and the ability to perform proper oral hygiene. Improved peri-implant mucosal health may also be associated with a reduced risk for the development of peri-implantitis, therefore having a secondary preventive effect [16].

HG, in association with standard treatment for peri-implant mucositis seems to be effective in reducing clinical Data (PD) and gingival Bleeding scores (BOP) due to its antiphlogistic and antiseptic properties, enhanced by the high bio-adhesive capability. The limited antibacterial effect on specific microflora can be the result of a limited antimicrobial agent volume in HG, in 4-6 mm peri-implant pockets depth.

The scientific evidence supports the adjunctive use of local antimicrobials to debridement in deep or recurrent periodontal sites [17]. Employing local antimicrobials, as an adjunct to mechanical treatment, in case of peri-implant mucositis demonstrated mean BoP and PD improvements, but this therapy did not resolve the lesion in all cases [12]. The addition of chlorhexidine to mechanical debridement did not enhance the results, on peri-implant mucositis, as compared to mechanical debridement alone [7].

Care should be exercised in the use of acidic chemicals, to detoxify implant surfaces, as surface alterations in the titanium oxide layer might jeopardize reattachment [18] Product safety and efficacy are highly required. The US Food and Drug Administration (FDA) non-prescription drugs advisory committee divided antimicrobials in three categories [19]. Among the ingredients reviewed, only two single active ingredients were recommended as Category I both for safety and efficacy: cetylpyridinium chloride (rinse) and stannous fluoride (dentifrice).

Microbiological analysis, performed by RT-PCR, assessed how bacteria load at implants and teeth sites could decrease 24h after treatment; however, such reduction was not significant at 8 months follow up [20]. Chlorhexidine is still considered "gold standard" of antimicrobials, even if recent trials in peri-implant mucositis show some conflicting results [21]. In a randomised controlled clinical trial, chlorhexidine gel application after debridement did not enhance clinical results in comparison with the mechanical cleansing procedure alone. Moreover, the use of an air-abrasive device or carbon curets, in association with chlorhexidine digluconate, resulted in a comparable, but limited CAL gains at 6 months. The air-abrasive device was associated with significantly higher BoP reductions than chlorhexidine alone [22].

Intensive application of chlorhexidine containing chips in sites with peri-implantitis after debridement resulted in a substantial improvement of clinical attachment levels [15], with a 50% reduction in BoP [23]. Mechanical non-surgical therapy could be effective in the treatment of peri-implant mucositis lesions. Furthermore the adjunctive use of antimicrobial mouth rinses enhanced the outcome of mechanical therapy of such mucositis lesions [24].

It has been suggested that the adjunctive use of either CHX gel or minocycline microspheres would improve both clinical and microbiological parameters following treatment [24]. Patients with a history of periodontitis presented, who did not completely adhere to the Supportive Periodontal Therapy (SPT), were found to present a higher implant failure rate. This underlines the value of the SPT in enhancing the long-term outcomes of implant therapy, particularly in subjects affected by periodontitis, in order to control reinfection and limit biological complications [25]. Consequently, it seems reasonable the employment of other effective periodontal substances as essential oils, triclosan and cytilpyridinium chloride. The adjunctive use of these substances provides a clinically significant and additional benefit in reducing plaque and tissues inflammation [26-28].

In a recent double-blind randomized clinical trial, Pasini and coworkers [29], treated a group of periodontal adult patients with professional mechanical instrumentation followed by a three months application of a gel (HG) containing a mixture of cytilpyridinium chloride, triclosan and essential oils, for daily use, in comparison with a placebo control group. Small but significant additional benefit in reduction of plaque and gingival index scores were noted at one month visit, slightly decreasingup to to3 months follow up period.

10% PI and 35% BoP reduction were recorded in test group. In the HG gel formula, specific essential oils are included; which have shown healing and antioxidant activity,

quick absorption and richness in monoterpens, sequiterpens and thrichetons [30-32]. For long term application, essential oils might be a reliable alternative to chlorhexidine, in case of gingival inflammation [33].

Conclusion

Maintenance of implants is imperative, since implants, like teeth, are susceptible to bacterial plaque accumulation and calculus formation, and thus at risk of developing peri-implant mucositis or peri-implantitis. Reduction, of the bacterial load to a level allowing healing is difficult to accomplish with mechanical means alone. Therefore, adjunctive therapies like antiseptic treatments have been proposed in order to improve the non-surgical treatment options of peri-implant mucositis. In the present study, both treatment modalities led to an improvement of the clinical parameters and a temporary reduction of the microflora at implants with mucositis, but without significant inter-group differences after one month.

Combination therapy i.e. non-surgical periodontal treatment plus adjunctive antimicrobial treatment, by locally delivered bio-adhesive dental gel showed small but significant additional benefit, with a trend in reducing gingival index scores, peri-implant mucositis.

In summary, clinical trials evaluating the treatment of peri-implant mucositis provide a variety of effective protocols for reducing peri-implant tissue inflammation and therefore the clinician should select those that adapt better to the specific patient's need[15].Randomized controlled clinical studies with sufficient statistical power are required to determine the optimal therapy for peri-implant pathology.

Acknowledgments

The participation of the subjects in this study are greatly appreciated and acknowledged.

NDHRA

This study supports the objective: Assess strategies for effective non-surgical approach, in case of peri-implantitis mucositis.

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