



A COMPARATIVE MICROBIOLOGICAL STUDY OF CHITOSAN AND CHITOSAN-CHLORHEXIDINE DRUG DELIVERY SYSTEMS ON COATED MODIFIED ABUTMENTS

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ABSTRACT Peri-implant diseases constitute a significant challenge for the clinician during the course of dental implant treatment. One of the principal factors associated with their development is bacterial accumulation, particularly involving the microgap located at the interface between the implant fixture and the prosthetic superstructure. Conservative management of peri-implantitis is unpredictable due to the difficulty in effectively controlling the bacterial biofilm on implant surfaces. Surgical treatment is technically demanding, may reduce patient motivation and trust, and increases treatment duration and cost. Therefore, priority is given to prevention and improving success rates in implant dentistry. **Aim:** To evaluate the potential for influencing bacterial colonization of the implant superstructure following the use of a modified abutment with a biocomposite coating based on chitosan and chlorhexidine. **Materials and Methods:** The study is randomized and conducted on 30 patients who received dental implants placed using a two-stage protocol in the mandibular premolar and molar regions. Modified abutments coated with 1% chitosan combined with 2% chlorhexidine (Group A) and 2% chitosan alone (Group B) were used to evaluate their effect on bacterial colonization. A microbiological analysis is performed prior to abutment placement and six months postoperatively to assess changes in the microbial profile. **Results:** At the six-month follow-up, both groups receiving implant crowns cemented with biocomposites demonstrated a statistically significant reduction in bacterial load compared to baseline. Patients treated with a 2% chitosan-based biocomposite showed a reduction across 17 microbial species, while those treated with a 1% chitosan and 2% chlorhexidine biocomposite exhibited a reduction across 19 microbial species identified at baseline.

KEYWORDS : Peri-implantitis, Bio-composite Coatings, Modified Abutments.

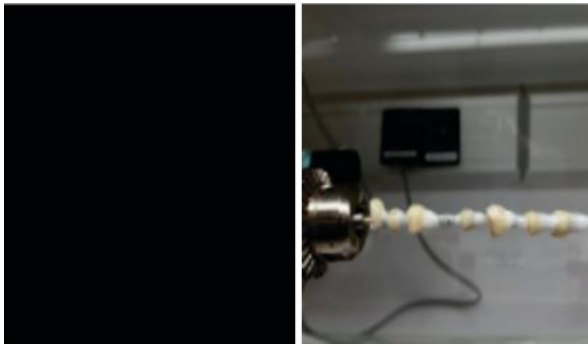
INTRODUCTION

Peri-implantitis is defined as an inflammatory reaction occurring around a functioning implant, accompanied by loss of the supporting bone (1,2). The pathogenic microflora plays a central role in the development of peri-implantitis (3). The oral biofilm is capable of adhering to any implant surface, thereby inducing inflammation of the peri-implant tissues (4). The quantitative and qualitative composition of the microbial flora is similar in peri-implant and periodontal pockets. The literature identifies several risk factors for the development of peri-implantitis, including periodontitis, poor oral hygiene, irregular maintenance following implant therapy, smoking, and diabetes (5). The accumulation of biofilm on the implant surface follows a sequence of bacterial colonization similar to that observed on natural teeth. The initial colonizers are predominantly aerobic and facultative anaerobic microorganisms, mainly Gram-positive cocci, including specific *Streptococcus* species capable of adhering to the hard tissues of teeth and implants, as well as to the surfaces of prosthetic constructions (6). An important secondary colonizer is *Fusobacterium nucleatum*, which facilitates subsequent microbial colonization. Various bacterial products can interact with epithelial cells and neutrophilic granulocytes within the gingival or peri-implant sulcus. If this biofilm is not adequately removed, it will lead to a host response in the connective tissue adjacent to the junctional epithelium. Contemporary strategies for the prevention of peri-implantitis are focused on inhibiting biofilm formation on the implant surface through the use of various biomaterials for implant surface modification (7). The development of bioactive coatings represents a significant area of interest in tissue bioengineering, playing a crucial role in the process of osseointegration (8,9). Among these materials, chitosan is incorporated into various compositions, forming a scaffold that serves as a platform for the delivery of therapeutic agents while simultaneously acting as a protective film covering the areas of interest (10,11). In the literature, chlorhexidine is also proposed as an antibacterial agent for the prevention of bacterial colonization on dental implants (12,13). It exhibits a broad spectrum of activity and is widely used in dental practice. One of the main limitations is its temporal instability; accordingly, various approaches to overcoming this drawback have been described in the literature (14). Against this background, the present study aims to evaluate the effect of modified implant abutments coated with chitosan alone and in combination with chlorhexidine on bacterial colonization, through microbiological analysis at baseline and six months after placement. In addition, the study seeks to assess the stability and applicability of these bioactive coatings when applied to the abutment surface using a rotational technique based on the film deposition method.

CASE STUDY

The study included 30 patients with distal edentulous sites in the mandible, with sufficient bone volume for implant placement. All procedures were conducted in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice, and applicable Bulgarian regulations for clinical research involving human subjects. The study was approved by the Institutional Ethics Committee (ref. no. R-183). Anamnestic data were recorded using standardized case report forms. All patients underwent initial oral sanitation, including removal of dental calculus and biofilm, and received individualized oral hygiene instructions. Cylindrical conical dental implants (BT Konic, BTK Implant, Biotec srl, Italy) were used, with diameters of 3.25, 4, and 5 mm and lengths of 10, 11.5, and 13 mm. A two-stage surgical protocol was applied. After a three-month healing period, second-stage surgery included flap elevation under local anesthesia, removal of the cover screw, and placement of a healing abutment selected according to soft tissue thickness and occlusal conditions. Two weeks later, the healing abutment was removed and an intraoral scan was performed using a Trios 3Shape scanner (3Shape, USA). Digital impressions were exported in STL format for CAD/CAM processing. A screw-retained temporary crown was designed and fabricated using SLA 3D printing (*Formlabs Form 2, Formlabs, USA*) with PMMA Temporary CB Resin, and cemented extraorally to the prosthetic base using self-adhesive resin cement (*Breeze™, Pentron*). The definitive prosthetic phase included removal of the healing abutment, clinical try-in of the PMMA restoration, and final placement on a titanium base. The prosthetic screw access was sealed with Teflon tape and composite resin (*Breeze™, Pentron*). In the control group, abutments were used without coating, whereas in the test groups, abutments were coated with a nanocomposite polymer matrix incorporating antibacterial agents (chitosan alone or chitosan with chlorhexidine). Coating application was performed using a film deposition method. The abutments were mounted on a sterile metallic holder and placed on a high-speed orbital shaker (Witeg HS-100D, Witeg Labortechnik GmbH, Germany) inside a laminar flow cabinet equipped with HEPA filtration and UV sterilization to ensure aseptic conditions. For Group A, 2 g chlorhexidine was dissolved in 2% acetic acid under magnetic stirring at 200 rpm, followed by the addition of 1 g chitosan with continued stirring for 30 minutes. For Group B, 2 g chitosan was dissolved in 2% acetic acid under magnetic stirring at 200 rpm for 30 minutes.

Figure 1. Screw-retained temporary crowns before and after the application of the bio-composite coatings.



The first microbiological sample was collected during fabrication of the provisional restoration using a sterile paper point from the peri-implant sulcus. The second sample was obtained from the same site six months after placement of the definitive crown. The aim was to compare microbial load, assess the clinical efficacy of the bio-composite material, and evaluate its long-term stability and sustained release of antimicrobial agents. Classical microbiological methods were used, including cultures on blood agar, Levine agar, chromogenic *Candida* agar, and selective media for anaerobic bacteria (thioglycolate medium, Schaedler agar, Wilkins-Chalgren agar, etc.).



Figure 2. Digital impression and emergence profile of the screw-retained temporary crown with the modified abutment. Table 1. Positive microorganism findings at baseline and 6-month follow-up in Group A (chitosan 1% + chlorhexidine 2% biocomposite; n = 15)

Table 1. Positive microorganism findings at baseline and 6-month follow-up in Group A (chitosan 1% + chlorhexidine 2% biocomposite; n = 15)

#	Microorganism	Baseline	6 months
Streptococcaceae			
1	<i>Streptococcus mitis/oralis</i>	9 (60.0%)	9 (60.0%)
2	<i>Streptococcus gordonii</i>	3 (20.0%)	0 (0.0%)
3	<i>Streptococcus salivarius</i>	2 (13.3%)	2 (13.3%)
4	<i>Streptococcus constellatus</i>	1 (6.7%)	0 (0.0%)
5	<i>Streptococcus cristatus</i>	1 (6.7%)	0 (0.0%)
6	<i>Streptococcus parasanguinis</i>	1 (6.7%)	0 (0.0%)
7	<i>Enterococcus faecalis</i>	2 (13.3%)	0 (0.0%)
Actinobacteria / Rothiaceae			
8	<i>Rothia dentocariosa</i>	2 (13.3%)	1 (6.7%)
9	<i>Rothia mucilaginosa</i>	1 (6.7%)	0 (0.0%)
10	<i>Actinomyces oris</i>	1 (6.7%)	0 (0.0%)

Bacillaceae / Other gram-positive			
11	<i>Paenibacillus provencensis</i>	2 (13.3%)	0 (0.0%)
12	<i>Staphylococcus warneri</i>	1 (6.7%)	0 (0.0%)
13	<i>Bacillus altitudinis</i>	1 (6.7%)	0 (0.0%)
14	<i>Kocuria rhizophila</i>	1 (6.7%)	0 (0.0%)
Gram-negative bacteria			
15	<i>Bordetella bronchiseptica</i>	1 (6.7%)	0 (0.0%)
16	<i>Neisseria mucosa/sicca</i>	1 (6.7%)	0 (0.0%)
17	<i>Neisseria flava</i>	1 (6.7%)	0 (0.0%)
18	<i>Moraxella catarrhalis</i>	1 (6.7%)	0 (0.0%)
19	<i>Lactobacillus curvatus</i>	1 (6.7%)	0 (0.0%)
Species distribution per patient			
1	1 species detected	3 (20.0%)	12 (80.0%)
2	2 species detected	10 (66.7%)	0 (0.0%)
3	3 species detected	1 (6.7%)	0 (0.0%)
4	4 species detected	1 (6.7%)	0 (0.0%)
100%	negative	0 (0.0%)	3 (20.0%)

Values expressed as n (%). n = 15 per group.

Table 2: Positive microorganism findings at baseline and month 6 in Group B (chitosan 2%) n = 15

No.	Microorganism	Baseline n (%)	Month 6 n (%)
1.	<i>Streptococcus mitis/oralis</i>	11 (73.30%)	8 (53.30%)
2.	<i>Actinomyces ursingii</i>	3 (20.00%)	0 (0.00%)
3.	<i>Rothia dentocariosa</i>	3 (20.00%)	0 (0.00%)
4.	<i>Streptococcus salivarius</i>	2 (13.30%)	0 (0.00%)
5.	<i>Streptococcus vestibularis</i>	2 (13.30%)	0 (0.00%)
6.	<i>Veillonella atypica</i>	2 (13.30%)	0 (0.00%)
7.	<i>Neisseria flava</i>	2 (13.30%)	0 (0.00%)
8.	<i>Enterococcus faecalis</i>	2 (13.30%)	0 (0.00%)
9.	<i>Streptococcus sanguinis</i>	2 (13.30%)	1 (6.70%)
10.	<i>Staphylococcus warneri</i>	1 (6.70%)	0 (0.00%)
11.	<i>Streptococcus constellatus</i>	1 (6.70%)	0 (0.00%)
12.	<i>Actinomyces naeslundii</i>	1 (6.70%)	0 (0.00%)
13.	<i>Rothia mucilaginosa</i>	1 (6.70%)	0 (0.00%)
14.	<i>Rothia aeria</i>	1 (6.70%)	0 (0.00%)
15.	<i>Leuconostoc mesenteroides</i>	1 (6.70%)	0 (0.00%)
16.	<i>Enterobacter hormaechei</i>	1 (6.70%)	0 (0.00%)
17.	<i>Lactobacillus pentosus/plantarum</i>	1 (6.70%)	0 (0.00%)
TOTAL			
o	+ 1 microorganism species	5 (33.30%)	7 (46.70%)
o	+ 2 microorganism species	3 (20.00%)	1 (6.70%)
o	+ 3 microorganism species	4 (26.70%)	0 (0.00%)
o	+ 4 microorganism species	1 (6.70%)	0 (0.00%)
o	+ 5 microorganism species	2 (13.30%)	0 (0.00%)
o	100% negative	0 (0.00%)	7 (46.70%)

DISCUSSION

For Group A *Streptococcus mitis/oralis* was the most frequently detected species, identified in 9 patients (60.00%) at baseline and in the same proportion at the six-month follow-up. Among the initially positive patients, two became negative at six months, representing an improvement of 13.30%. However, two previously negative patients demonstrated the presence of *Streptococcus mitis/oralis* at follow-up; consequently, the overall proportion of positive findings remained unchanged at 60.00%. *Streptococcus gordonii* was detected in 3 patients (20.00%) at baseline and was absent at the six-month follow-up. *Streptococcus salivarius* was identified in 2 patients (13.30%) at baseline; at six months, one case resolved while the other persisted, with one new positive case maintaining the overall proportion.

Paenibacillus provencensis and *Enterococcus faecalis* were each found in 2 patients (13.30%) at baseline and were absent at six months. *Rothia dentocariosa* was detected in 2 patients (13.30%) at baseline, with one case remaining positive and one becoming negative at follow-up. Overall, at the six-month follow-up, a reduction in bacterial load was observed in 12 patients in Group A (80.00%), while the remaining 3 patients (20.00%) showed no change. No new microbial species were detected in any of the patients.

All remaining microbial species (No. 7–19) were detected in one patient (6.70%) at baseline and were completely absent (0.00%) at the

six-month follow-up. Overall, at baseline, 3 patients (20.00%) were positive for one of the 19 detected microbial species, 10 (66.70%) for two species, 1 (6.70%) for three species, and 1 (6.70%) for four species, with no completely negative cases.

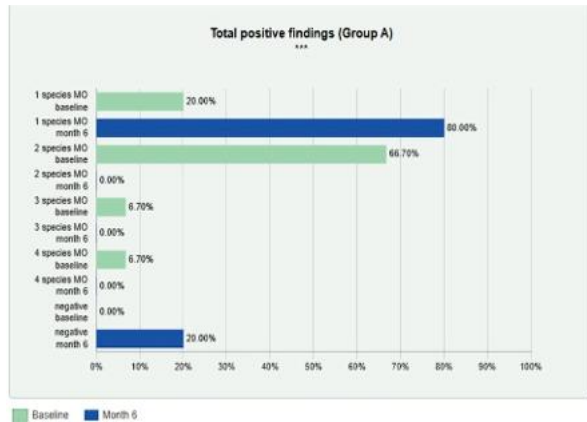


Figure 3. Total positive microbial findings at baseline and at the six-month follow-up in Group A, according to microorganism species.

In Group B the microbiological analysis revealed the presence of 17 microbial species. The number of affected patients and their corresponding proportions out of the total of 15 patients are presented for each microorganism in Table 2.

In Group B, *Streptococcus mitis/oralis* was the most frequently detected species, identified in 11 patients (73.30%) at baseline and in 8 patients (53.30%) at the six-month follow-up. Among the initially positive cases, four patients became negative, corresponding to a 26.60% improvement. One initially negative patient became positive at six months, while three patients remained positive throughout the observation period. *Actinomyces ursingii* and *Rothia dentocariosa* were detected in 3 patients (20.00%) at baseline and were absent at six months. Five additional species (*Streptococcus salivarius*, *Streptococcus vestibularis*, *Veillonella atypica*, *Neisseria flava*, and *Enterococcus faecalis*) were identified in 2 patients (13.30%) at baseline but were not detected at follow-up. *Streptococcus sanguinis* was found in 2 patients (13.30%), with one remaining positive and one becoming negative at six months.

All remaining species (n = 10–17) were detected in a single patient (6.70%) at baseline and were absent at follow-up. Overall, at baseline, 5 patients (33.30%) were positive for one microbial species, 3 (20.00%) for two species, 4 (26.70%) for three species, 1 (6.70%) for four species, and 2 (13.30%) for five species, with no completely negative cases. At six months, a statistically significant change was observed ($p = 0.001$), with 7 patients (46.70%) showing complete microbial elimination, 7 (46.70%) presenting only one detected species, and 1 patient remaining positive for two species. A reduction in bacterial load was observed in 86.70% of patients, while 13.30% showed no change in bacterial burden.

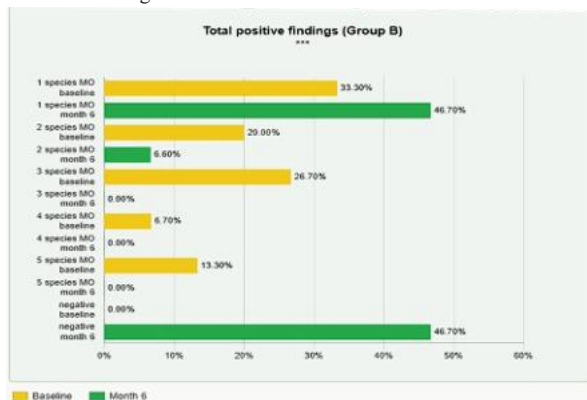


Figure 4. Total positive microbial findings at baseline and at the six-month follow-up in Group B, according to microorganism species.

CONCLUSIONS

Both experimental groups demonstrated a statistically significant reduction in bacterial load following the application of modified abutments. This effect was maintained over the six-month observation period, indicating a sustained antimicrobial performance of the tested biocomposite coatings. The results suggest that both chitosan-based formulations, with and without chlorhexidine, may contribute to improved microbial control in the peri-implant environment. These findings highlight the potential of bio-tissue engineering approaches in enhancing implant surface properties and promoting long-term implant success. Overall, the study underscores the growing importance of biomaterial-based strategies in contemporary dental implantology.

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