Bioactive Healing Abutment as a Potential Tool for the Treatment of Peri-Implant Disease—In Vitro Study

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Abstract: The common use of dental implants for dental reconstruction poses new treatment challenges for physicians, peri-implantitis being a particularly difficult one. Micro-organisms, including drug-resistant Staphylococcus spp. strains, play a crucial role in the etiology of peri-implantitis. In this paper, the authors assess the efficacy of a bioactive healing abutment (BHA) of their own design for the local release of antibiotics as a potential tool for the treatment of peri-implant disease. BHA filled with a collagen material, combined with the antibiotics clindamycin and tetracycline, was tested in vitro by disk diffusion assay. Antibacterial activity was observed for the chosen Staphylococcus aureus and Staphylococcus epidermidis bacterial strains. In addition, the impact of titanium discs (which were used to make the BHA) was monitored. The results show that the zone diameter breakpoints for BHA are higher than those of standard absorbent paper discs in both tested strains for both tested antibiotics. This work demonstrates that the proposed BHA can serve as an effective and precise drug carrier. The release of antibiotics from the described implant device is easy to control and allows for an effective local antibacterial in vitro treatment. The procedure is inexpensive, easy to perform, and repeatable.

Keywords: dental implants; peri-implantitis; healing abutment

1. Introduction

Treatment with dental implants to replace missing teeth is currently a recognized and effective method with well-documented long-term survival rates and is constantly presenting new techniques [1,2]. One of the most commonly identified reasons for treatment complications is peri-implantitis. Occurrence of peri-implantitis has been described for 3% up to 15% of all implants in a ten-year observation. This means that the pathology may apply to as many as 250,000 implants annually [3].

As in periodontitis, peri-implantitis is described as an irreversible infection process causing bone tissue loss around the implant, gingival pockets, and exudate [4,5]. More advanced stages of peri-implantitis are manifested by redness and swelling of the soft tissue around the implant, gum recession, blood or purulent exudate, significant bone loss visible in radiology tests, and even implant stability loss [2,5]. Dysbiotic plaque biofilm plays a very important role in the development of peri-implantitis. During the course of peri-implantitis, many Gram-negative bacteria have been
detected, including red complex bacteria, which is a complex that is predisposed to periodontitis. This may explain the predisposition to peri-implantitis in patients positive for periodontitis [6–8]. In addition, peri-implantitis has been linked with *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter cloace*, *Eshericha coli*, *Helicobacter pylori*, *Petostreptococcus micra*, and *Candida* sp. [9,10].

*Staphylococcus* spp. bacteria are one of the leading causes of implant infections. These bacteria create a biofilm which impedes treatment. The occurrence of *Staphylococcus aureus* has been confirmed in 80% of bacterial samples from implant area pockets impacted by peri-implantitis. A lack of this micro-organism in the bacterial plaque has been confirmed for 90% of healthy areas [11,12].

Peri-implantitis treatment is an important clinical challenge with a significant need to control local infection, preserve soft and bone tissue in the area around the implant, and ultimately maintain the aesthetics and stability of the implants. Depending on the implant type and treatment strategy, only 34–79% of peri-implantitis cases are treatable. Prospective studies of peri-implantitis treatments longer than 1 year have shown many recurrences, even up to 100% with regards to certain treatment strategies. Taking this into account, peri-implantitis can be seen as a chronic disease requiring many treatment episodes [13–15]. Such an approach to peri-implantitis appears to be highly problematic, as the proposed treatment strategies are cumbersome and expensive. Treatment methods used for peri-implantitis can be divided into two main groups: surgical and non-surgical procedures [15,16].

Non-surgical procedures incorporate oral hygiene instructions and training the patient in teeth and implant dental plaque cleaning techniques. Rinses and irrigation with chlorhexidine, triclosan, or other disinfectants have been used. However, the efficacy of these procedures is restricted to early forms of peri-implantitis (called peri-implant mucositis) and has not provided satisfying results in fully developed peri-implantitis [2,15,16]. The main method of treatment for peri-implantitis is manual therapy using a teflon, carbon, plastic, or titanium current, as well as plaque removal procedures using ultrasonic scalers with titanium or plastic tips. It is advised to use pressure sandblasting machines with specially constructed nozzles. The effectiveness of these methods is comparable. Efficacy results for implant surface decontamination in vitro are very promising. Unfortunately, clinical results for the use of mechanical methods are unsatisfying. Peri-implantitis recurrence has been observed after these procedures [15–18].

Attempts to treat peri-implantitis with biostimulating lasers in photodynamic therapy have led to improved effectiveness, although only when combined with mechanical methods. As independent methods, they do not have a higher effectiveness rate and longer remission duration. However, these methods require specialist equipment and qualified personnel, as well as many repetitions, which raises the treatment cost [19,20].

One of the non-surgical peri-implantitis treatment methods, referring to the proposed bacterial etiology of this pathology, is systemic or local antibiotic therapy. The use of systemic, broad-spectrum antibiotics usually results in a slight initial improvement; however, it typically quickly deteriorates. Better results can be obtained when combining systemic antibiotics with surgical and mechanical methods or with phototherapy. Nevertheless, despite the significant impact on the body, the results remain unsatisfying. Instead, drug resistance occurs, and secondary infections of implant pockets with so-far non-pathogenic bacterial flora impede treatment [21–23]. The treatment of peri-implantitis has unpredictable results, while control of the bacterial count is crucial [24]. The use of local application of antibiotics as an adjunct to mechanical therapy is beneficial in the treatment of peri-implant lesions and can provide a sustained high dose of the antimicrobial agent precisely into the affected site for several days. To provide clinical improvements, the treatment may have to be repeated [25].

An important addition to non-surgical treatments of peri-implantitis are procedures. Of particular importance are flap procedures and guided tissue regeneration. Surgical methods are often combined with non-surgical ones in order to increase and prolong treatment results, which often is unsatisfying for the physician as well as the patient. Surgical procedures can mean pain and discomfort for the patient; therefore, there is a constant search for alternative, more effective methods [16,26].
The aim of this study was to establish the usefulness of a bioactive healing abutment (BHA) for the reduction of micro-organism growth in the active stages of peri-implantitis in laboratory conditions. The proposed solution is cost-effective and painless in application (also when repeated) and could provide a promising alternative (or addition) to the currently applied treatment strategies used for peri-implantitis.

2. Materials and Methods

2.1. Bioactive Healing Abutment

The experiment was conducted with the use of a bioactive healing abutment made with class V titanium (Ti6Al4V), manufactured by TAV Dental Israel (subject to patent application no. P.427453).

2.2. Bacterial Strains

Two *Staphylococcus* bacteria strains were used in the experiment: coagulozo-positive *S. aureus*, for which a study was conducted using fifteen clinical strains isolated from oral cavity patients (Culture Collection Department of Medical Biology, Medical University of Warsaw, Poland), and coagulozo-negative strain *S. epidermidis* ATCC12228 (EurofinsPanlabs, Inc. & Eurofins Pharma Bioanalytics Services US Inc., St. Charles, MO, USA). The *S. aureus* used in the experiment demonstrated an ability to produce a biofilm on surfaces—in particular, on titanium surfaces (unpublished data in preparation).

2.3. Reagents

*Staphylococcus* spp. were routinely cultured in Luria Bertani (LB) broth (10 g tryptone, 5 g yeast extract, and 5 g NaCl L$^{-1}$), Mueller–Hinton agar (MHA), and Chapman agar. In the experiment, the following antibiotics were used: clindamycin hydrochloride and tetracycline hydrochloride. All reagents and antibiotics were manufactured by Sigma-Aldrich Sp. z o.o. (Poznań, Poland), unless otherwise stated.

2.4. Medium and Bacterial Culture Preparation

Overnight cultures (1 mL) of *S. aureus* and *S. epidermidis* were collected and placed in a 50 mL test tube with LB until a solution of 1:50 was achieved. Then, it was incubated at 37 ± 1 °C with shaking (180–200 rpm) to obtain an optical density of 600 nm (OD600) of 0.5.

2.4.1. Disk Diffusion Assay

In the disk diffusion assay, 10 µL bacterial suspension ($1.5 \times 10^8$ colony-forming units [CFUs]·mL$^{-1}$) was evenly dispersed on Mueller–Hinton (M-H) plates. Antibiotic discs containing clindamycin (2 µg) or tetracycline (30 µg), in accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [27], were placed on the agar and the plates were incubated at 37 °C for 18 h, following which the diameter of the inhibition zone was observed. The disk diffusion assay was processed in triplicate.

2.4.2. Antimicrobial Effect of Bioactive Healing Abutment

To examine the antimicrobial effect of BHA, first, the 10 µL bacterial suspension ($1.5 \times 10^8$ colony-forming units [CFUs]·mL$^{-1}$) was evenly dispersed on Mueller–Hinton (M-H) plates. BHA filled with a heterogeneous bovine collagen sponge (Biokol, Stalmed, Poland), with antibiotics clindamycin (2 µg) or tetracycline (30 µg), were placed vertically in the medium until the openings were in line with the medium. All dishes were kept for an hour at room temperature and placed and incubated at 37 °C under aerobic conditions for 18 h to observe zone inhibition diameter. The assessment of class V titanium (Ti6Al4V) toxicity was performed with disks (10 mm diameter and 2 mm height)
manufactured by TAV Dental Israel. All experiments were processed in triplicate and values were averaged from three independent experiments.

2.4.3. Statistical Analysis

All experiments were performed in triplicate wells for each of the fifteen samples retrieved from patients and repeated at least three times. Results were expressed as mean value ± standard error of the mean (SEM) of growth inhibition zone diameters. All experimental data were compared using Student’s t-test, and \( p < 0.05 \) was taken as the level of significance. All statistical analyses were performed using Statistica 13.3 (StatSoft, Tulsa, OK, USA).

3. Results

We explored the possibility of using the bioactive healing abutment (BHA) as an antibiotic carrier. The functional idea of the proposed BHA is based on the slow release of the substance on an appropriate carrier, deposited in the abutment chamber to the surrounding environment through the adjacent side openings. The standard healing abutment is commonly used in dental implantology for the healing of gums surrounding an implant bed, in order to allow attachment of the implant superstructure. However, it is in one piece, solid, and has a purely mechanical function—blocking and shaping of gum epithelialization over the implant bed after it has been uncovered. In the proposed version, the BHA, made with class V titanium (Ti6Al4V), has an empty core and two parts: a corpus and a top lid. The corpus has screws allowing for attachment of the implant main body and a chamber for drug deposition. This chamber is connected with the external environment by eight equally distributed openings on the side walls, each with a diameter of 1 mm. The abutment is 5 mm long and the attachment screws and base diameter are adjusted to the TAV Dental Silhouette (Israel) implant bed with a diameter of 3.75 mm. The achieved capacity of the BHA chamber used in the experiment is, after the lid is screwed on, 0.019764 mL each (Figures 1–3).

![Bioactive healing abutment scheme.](image1)

**Figure 1.** Bioactive healing abutment scheme.

![Bioactive healing abutment idea of action.](image2)

**Figure 2.** Bioactive healing abutment idea of action: (A) treatment with bioactive healing abutment; and (B) implant restoration after treatment.
Figure 3. Bioactive healing abutment structure and dental implant fixation: 1. Drug chamber vertical view; 2. Corpus and lid horizontal view; 3. Bioactive healing abutment with closed lid; 4. Bioactive healing abutment with closed lid mounted on the dental implant.

We first selected two antibiotics—clindamycin and tetracycline—to examine the antibacterial activity in *S. aureus* and *S. epidermidis*, respectively. The antibacterial activity of antibiotics was analyzed by disk diffusion assay in two trials with BHA and paper discs (Figure 4). Table 1 lists the inhibition zone diameter of clindamycin and tetracycline for *S. aureus* and *S. epidermidis* in the first trial (paper discs). In Table 1, a mild increase in the inhibition zone diameter can be observed in clindamycin and tetracycline in the second trial with BHA: 27.4 ± 2.5 mm and 18.8 ± 3.4 mm, respectively, for *S. aureus* and 34.7 ± 2.3 mm and 13 ± 3.0 mm, respectively, for *S. epidermidis*. In the obtained results, statistically significant differences were observed for the individual study groups for the different antibiotic carriers (paper disc or BHA) in the case of the *S. aureus* strain.

![Experimental scheme for the disk diffusion assay.](Image)

Table 1. Comparison of median zone diameter breakpoint using a paper disc and bioactive healing abutment depending on antibiotic type and bacterial strain. Zone diameter breakpoint, mm; n = 3.

| Antibiotic          | Clindamycin 2 µg | Tetracycline 30 µg |
|---------------------|------------------|--------------------|
| Bacterial strain    |                  |                    |
| Paper disc (mm)     | 19.9 ± 2.3       | 15.2 ± 2.7         |
| Bioactive healing abutment (mm) | 24.4 ± 2.5 | 18.8 ± 3.4         |
| Difference (mm)     | 4.5 ± 0.1        | 3.7 ± 0.7          |
| p                   | 0.0000043        | 0.6164             |

The control study group, based on the antimicrobial activity of a titanium disc for individual bacteria cultures, showed homogenous and undisturbed micro-organism growth. No zone diameter
breakpoint was observed and the cultures grew evenly on the surface around the titanium disk (Figure 5).

![Antimicrobial effect of bioactive healing abutment, paper disc, and titanium disc: (A) clindamycin; (B) tetracycline.](image)

Figure 5. Antimicrobial effect of bioactive healing abutment, paper disc, and titanium disc: (A) clindamycin; (B) tetracycline.

The average values for the zone diameter breakpoint with the use of clindamycin for *S. aureus* were approx. 4.5 mm higher in the experiment with the use of BHA, compared to the results for the test using paper discs. The average values for the zone diameter breakpoint with the use of clindamycin for *S. epidermidis* were approx. 3.7 mm less, compared to BHA. The average growth inhibition results with the use of tetracycline, for both *S. aureus* and *S. epidermidis*, applied on a collagen carrier in BHA, were higher than the zone diameter breakpoints created around paper discs soaked with the same antibiotic doses. The difference was, accordingly, 3.7 mm for *S. aureus* and 1.7 mm for *S. epidermidis*.

4. Discussion

Complete elimination of bacterial biofilms from the surface of an implant in peri-implantitis is difficult to achieve. Surgical therapeutic approaches such as mechanical treatment, antiseptic treatment, and antibiotic treatment to eliminate infectious bacteria in the surrounding peri-implant tissues, as well as photodynamic treatment and laser application of peri-implantitis in humans, have shown some positive results, but a long-term evaluation has yet to be determined. In peri-implantitis treatment, it has become standard practice to locally administer antiseptics and/or antibiotics to patients with
mild–moderate infections, which are largely the most frequent in clinical practice. Antiseptic or antibiotic drug-loaded devices, such as gels, chips, polymeric fibers, or microcapsules, can be used to treat infections. Various antibiotics and antiseptics have been incorporated into these devices, including tetracycline, gentamycin, doxycycline, chlorhexidine, and metronidazole.

Most recent reports have suggested the possibility of incorporating tetracycline antibiotics on the surface of titanium dental implants with the use of polylactide nano-fibers or gentamicin with the use of nanoparticles of Fibroin protein derived from silkworm larvae. Slow release and local antibacterial properties for antibiotics used this way have been proven. The used surface engineering methods did not interrupt the osteoblast adhesion processes on the implant surface. Unfortunately, a high torque of 35 N/cm² and friction during the embedding of the implant into the bone can lead to destruction and inefficiency of the created surface. Similarly, antibiotic release using this method does not extend beyond 7 days and there is no possibility of reapplication [28,29].

The surfaces of titanium implants have been coated, by some authors, with non-specific antibiotic products, which are supposed to disrupt the sedation of planktonic bacteria in the implant surface. The antibacterial effect of silver nanoparticles in chitosan and hyaluronic acid can last up to 14 days in laboratory conditions; however, unsatisfactory mechanical properties of the described surfaces have been reported, as well as the cytotoxic effects of silver particles, the release of which can disrupt implant osseointegration [30]. Rat studies have shown a significant reduction of the growth of S. aureus strains when using titanium implants with a gel coating containing vancomycin, with lower osteolysis observed around the implant. This tendency was sustained for up to 3 weeks [31]. Compared to the described techniques, use of the proposed BHA is practically unlimited when it comes to duration and it can be repeated frequently, even with a change in treatment factor. Peri-implantitis mostly develops not in the first days after implantation but more often as a complication after the implant has integrated. Thus, the described solution has more benefits than implant surface engineering.

Successful attempts at implant surface engineering have been described by the modification of implant connectors. Doxycycline applied electrochemically on the connector surface reduced both biofilm deposition and S. epidermidis culture growth for 24 h [32]. Implant connector modification methods are, in the opinion of the authors, the closest method to the one proposed. BHA is a type of healing abutment called a healing cap. In the case described by Xing R. et al. [33], the cap was covered with doxycycline. Despite the theoretical possibility of unscrewing a cap covered with doxycycline, it is not possible to change the type or dose of the drug. It is not possible to reapply the product unless the cap is replaced. Reapplication requires the antibiotic to be reapplied electrochemically, which is not possible in clinical conditions. The proposed BHA can act as a carrier of many antibiotics used in clinically appropriate concentrations, with the possibility of easy reapplication.

The development of nanotechnology and tissue engineering creates possibilities for local application of surface-absorbed antibiotics or even inside scaffolds used for guided bone regeneration [34]. The antibacterial efficiency of microporous hydroxyapatite scaffolds impregnated with vancomycin particles has been proven in vitro for the S. aureus strain. This effect has been observed up to 5 days after application. In the period of increased vancomycin release, a lower proliferate osteoblast response has been observed, which returned to typical values for clean hydroxyapatite scaffolds after the antibiotic stopped being released [35]. The capability to locally release vancomycin, rifampin, and linezolid from silica-based mesoporous material has been confirmed in laboratory conditions for a 24 h period [36]. Progress in the scope of biofunctional material synthesis with a controlled nanostructure and functionalized surface has opened new possibilities of manufacturing implantable scaffolds with required antibacterial content and their bioavailability. The use of such modified implants lowers the number of complications resulting from antibiotic use, as well as increasing their bioavailability in the expected location. The negative aspect of these strategies is mostly a lower implanted material regeneration potential and the need for a surgical procedure to apply an antibiotic. Additionally, an important downside to the use of drug-exuding scaffolds is their
price and high specificity, expressed by the lack of modification possibilities of drug type and dose; furthermore, they are restricted to one-time use [37].

Treatment effectiveness of bacteriostatic materials, in the form of washings or swabs, has been assessed differently, depending on the author. In the scientific literature, we can find information indicating the effectiveness of antibacterial use in peri-implantitis treatment in the form of washings [38].

At the same time, there are reports suggesting that the same product does not improve peri-implantitis treatment when used in the form of swabs. It appears that the difficulty of repeating application in the form of washings and swabs is a major issue. Additionally, there is an inability to produce a repetitive and stable concentration of the active substance using these methods in local application to gum pockets [39].

Application of antibacterial products directly to gum pockets allows their more precise dosage and a stable release process. Currently, there exist antibiotics, as well as antibacterial products, for applicable carriers for direct gum pocket application. Studies using those products have consistently shown the statistically important effectiveness of restricting gum pocket depth and reducing bleeding during probe exams. This effect has also been obtained when the treatment was combined with professional oral hygiene procedures. Treatment can be treated as supportive and can be independently managed; however, it does not improve clinical parameters in periodontium disease and peri-implantitis [40–43].

The aim of this study was to assess the impact of the active substance distribution with the use of the proposed bioactive healing abutment. The experiment has shown bacterial growth inhibition in the form of a circular sphere, which indicates an even antibiotic diffusion to the environment. The width of the sphere allows us to conclude that the achieved concentration may have a beneficial effect on clinical conditions.

The in vitro tests of clindamycin and tetracycline release on selected strains (i.e., *S. aureus* and *S. epidermidis*) described in this study demonstrate the potential use of an applicator [44] in the treatment of a difficult medical problem such as peri-implantitis. Distribution of oral drugs in the treatment of oral infections, including implant area infections, is low. Concentration of the chosen drugs in gum areas is not always effective in the eradication of pathogenic micro-organisms. Therefore, systemic and local treatment is necessary. Use of a local drug carrier can significantly increase drug concentration in the infected area, allowing for the effective elimination of pathogens.

The use of a bioactive healing connector, as in the presented construction, can allow effective treatment of implant area infections at an early stage and can find use as a supportive treatment in known peri-implantitis treatment methods.

Further studies on the use of a bioactive healing connectors in the treatment of implant area infections, including clinical studies on the use and efficacy of the proposed treatment method, should be performed. It is necessary to determine the impact of the effectiveness of antibiotic distribution on the formed biofilm.

5. Conclusions

The proposed bioactive healing abutment is inexpensive to manufacture and easy to apply. In clinical conditions, placement and removal require a short and painless procedure for the patient. The chamber of the bioactive healing abutment can be filled with a medicinal product appropriate to the expected treatment result, through the use of a compatible carrier. The proposed device can, therefore, serve as an effective drug carrier. Antibiotic release from the described implant device is easy to control and allows for effective local antibacterial effects in vitro. The procedure is inexpensive, easy, and can be repeated.

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