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Research Article

Microbiological Effectiveness of Anti-Bacterial Agents Used Inside Implants

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Abstract

To prevent bacterial contamination between implant/abutment, different types of gels and ointments inside of implants are commonly used. This *in vitro* study aimed to evaluate, the anti-bacterial effectiveness of different concentrations of chlorhexidine and tetracycline gels; Neosporin® and Proheal® ointments. The anti-bacterial activity was determined by inhibition zones through agar diffusion method in plates previously inoculated with different bacteria: *F. nucleatum, P. nigrescens*, (obligatory anaerobic bacteria) and *E.coli, S. sanguinis* (Facultative anaerobic bacteria). The plates were prepared in triplicate for each type of bacteria. The diameter of microbial inhibition were measured (mm) and statistically analyzed (One-way ANOVA, α=0.05). The greatest inhibition halos against anaerobic bacteria were produced by Proheal® (85.69 mm) which was significantly greater than 2.5%, 2% and 1% tetracycline gels (63.09 mm), followed by 2.5%, 2%, 1% chlorhexidine gels and Neosporin® (19.72 mm). For aerobic bacteria the greatest halos were produced by 2.5% and 2% tetracycline (36.05 mm), which were significantly superior than 1% tetracycline (30.02 mm) followed by 2.5%, 2% and 1% chlorhexidine (17.75 mm), and these were statistically different from Neosporin® (10.98 mm) and Proheal® (6.22 mm). Although Proheal® presented the greatest halos of inhibition against anaerobic bacteria. Due to its effectiveness for all bacteria tested, the tetracycline gel seems to be the most indicated.

Keywords: Anti-Bacterial agents, Dental implant-abutment interface

Introduction

It is already known that a microgap exists in the Implant/Abutment interface (IAI) [1]. This misfit creates bacterial niches that may develop an inflammatory tissue near the IAI [2]. Micro movements caused during the loading of dental implants [3], loss of preload of abutment screws [4] and the misfit between the IAI provide bacterial microleakage between the components of the implanted prosthesis; being the main cause of a bone loss and inflamed tissue near the implant/Abutment junction [5]. Several antibacterial agents have been used within the implants in order to prevent bacterial microleakage, the most common antibacterial agents are tetracycline gel, chlorhexidine gel, Neosporin® ointment and Proheal® Ointment [6,7]. Koutouzis et al. (2013) analyzed the influence of Chlorhexidine 0.12% in the micro leakage through the IAI in Morse taper junction, however in this concentration the chlorhexidine cannot preclude that the cytokine leakage through IAI, in other study Paolantonio et al. (2008) found that the chlorhexidine can alleviate contamination and decrease the count of bacteria inside the implant.

Few studies have evaluated the efficiency of various antibacterial gels and its various concentrations. Therefore, this study aims to evaluate the antibacterial efficiency between gels with different concentrations of chlorhexidine and tetracycline and also the Neosporin® and ProHeal®. In this way, can determine which substance has the best antibacterial effect and which concentration should be used inside implants to avoid bacterial growth.

Materials and Methods

For this study, the following substances were analyzed: Chlorhexidine (Group Cl) and Tetracycline (Group Te) (Pharmus, Uberlândia, Brazil), at concentrations of 1%, 2% and 2.5%, and ProHeal® (Biomacmed ointments, Juiz de Fora, Brazil) (Ph group) and Neosporin® (Johnson & Johnson Consumer Companies, Inc., USA) (Ne group) and control group as the pure thickening gel-natrosol (GC group) (Table 1). These substances were selected with the different concentrations available on the market.

Agar diffusion method

Facultative anaerobic bacteria grown in aerobic atmosphere: Escherichia coli (ATCC35218), Streptococcus sanguinis (ATCC 10556) cultivated in BHI culture (HIMEDIA, Mumbai, India). And the obligate anaerobic bacteria: *Fusobacterium nucleatum* (ATCC 25586) *Prevotellanigrescens* (ATCC 33563) grown in Schaedler Broth (HIMEDIA, Mumbai, India) supplemented with 5% defibrinated sheep blood, 1% hemin and 1% menadione, held in an anaerobic Workstation (Whitley DG250, Don Whitley Scientific, West Yorkshire, England).

To confirm the purity of the bacteria, all microorganisms were grown previously in petri dishes and then cultured in their respective environments. Aerobic bacteria were incubated for 24 hours in BHI agar and then removed for inoculation into a test tube containing 3ml of BHI. After that, they were incubated again for 18 hours in aerobic environment. Anaerobic bacteria were incubated for 72 hours in

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Table 1: Gels and ointments used in this study.

Anti-bacterial gels and ointments	Manufacturer	Composition
Chlorhexidine	Pharmus, Uberlândia, Brazil	Tetracycline 1%, 2% or 2.5%, natrosol
Tetracycline	Pharmus, Uberlândia, Brazil	Tetracycline 1%, 2% or 2.5%, natrosol
Neosporin®	Johnson & Johnson Consumer Companies, Inc., EUA	Bacitracin, neomycin, polymyxin, cocoa butter, olive oil, cottonseed oil, sodium pyruvate, vitamin E, white petrolatum
Proheal®	Biomacmed, Juiz de Fora, Brazil	Triiodo Methane (Iodoform) 15.5%,Calendula Oil 0.5%,Lanolin Anhydrous 74%,Beeswax 10%,nipazol 10.05%

Table 2: Mean and Standard Deviation (SD)of induced inhibition halos related to analyzed bacteria and different antibacterial agents - different letters mean statistically significant differences in lines between the antibacterial substances used with p <0,05.

	Cl1%	Cl2%	Cl2,5%	Te1%	Te2%	Te2,5%	Ph	Ne
Fusobacterium nucleatum	19,04 ± 0,05 C	20,33 ± 0,86 C	20,73 ± 0,98 C	55,81 ± 1,23 B	55,31 ± 0,96 B	59,23 ± 1,23 B	103,71 ± 11A	12,67 ± 0,1C
PrevotelLa nigrescens	23,83 ± 0,24 B	25,08 ± 1,16 B	24,92 ± 0,86 B	66,46 ± 2,35 A	71,81 ± 0,02 A	69,90 ± 2,7 A	67,66 ± 15,33 A	11,15± 0,99 B
Escherichia coli	18,05 ± 0,24 C	19,62 ± 0,77 C	20,37 ± 0,21 C	31,95 ± 0,15 B	39,67 ± 0,33 A	39,84 ± 0,86 A	6,00 ± 0,01 D	12,95 ± 0,26 D
Streptococcus sanguinis	15,17 ± 1,56 C	16,11 ± 0,65 C	17,16 ± 0,62 C	28,09 ± 2,33 B	32,39 ± 1,59 A	32,29 ± 1,46 A	6,46 ± 0,02D	8,99 ± 0,95 D

Schaedler Agar. Each colony was removed and then inoculated into a test tube containing Schaedler broth supplemented with hemin 0.1% and menadione 0.1%. After that, they were all incubated again for 72 hours in anaerobic Workstation. After bacterial growth in the tubes, a bacterial solution with 1 MacFarland scale was prepared corresponding to approximately $3x10^{8}$ colony forming units per ml (UFC/ml). Using a precision pipet 100µl, the solution was collected and pipetted into on Schaedler agar for the anaerobic bacteria and BHI to bacteria under aerobic conditions, and seeded using a polypropylene handle drigalski until the bacterial suspension is spread across the surface of the medium. Two sterile metal tubes with an internal diameter of 4 mm and a height of 6 mm were added to the medium and opposite sides and filled with the tested antibacterial substance and the other with the control group, chlorhexidine gels and Neosporin® ointment. For the tetracycline gels and ProHeal®, the bacteria were grown in aerobic environment and pipe inserted in four equidistant points from each other. A filter paper disc was placed on the board and then, closed and involved with Para-film. Three specimens were prepared for each group. The plates were incubated for 72 hours for the anaerobic bacteria and 24 hours for bacteria grown in aerobic environment. After the incubation period, the plates were opened and the diameters of the inhibition halos were measured using a digital caliper (Mitutoyo, Japan) by three different observers and the average of the diameters conducted to determine the extent of each inhibitory halo.

Statistical analyses

Data was analyzed using SPSS 15.0 for Windows (SPSS Inc, Chicago, Illinois, United States) program. The data was initially submitted to normality and homogeneity of variance, in this case using Shapiro-Wilk and Levene's test respectively. Then, because of the samples normality and homogeneity One-Way ANOVA-test was used to identify differences between mean inhibitory halos to each bacterium. For multiple comparison of means between groups, the Turkey test was used. All tests were applied with a probability level of 95% (α= 0.05).

The null hypothesis is that all antibacterial gels show no statistical difference between them.

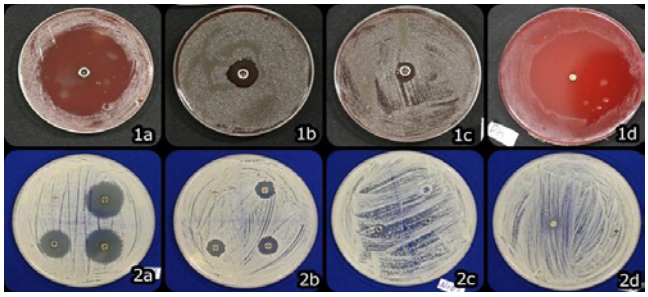

Figure 1: Inhibitory halos in the plates, seeded with bacteria *Fusobaterium nucleatum (1) e Streptoccoccuss anguinis* (2), produced by the antibacterial Tetracycline (a), Chlorhexidine (b) Neosporin® (c) e Proheal® (d).

Results

The results of agar diffusion test are presented on Table 2. All antibacterial agents used inside implants induced inhibition zones, except for Proheal® Ointment against aerobic bacteria. (Figure 2)

Discussion

Different antibacterial gels show different antibacterial effectiveness. Several methods are used for reduction of bacterial contamination between the implant/abutment interface and inside the implant [6-9]. Physical methods such as sealing the inner space with the use of silicone does not exhibit effectiveness in preventing bacterial microleakage6 chemical methods using anti-bacterial not of a comparative study on the various materials used. Different concentrations and substances used are using randomly [6,9]. In clinical studies, the used chlorhexidine gels with the concentration of 1% and 0.2% have decreased CFU/ml. Even though, it do not prevent bacteria from entering the interface P/I [7,10]. It also decreased the inflammatory tissue and bacterial contamination in the peri-implant sulcus [11]. However, the presence of periodontal bacteria does not necessarily imply peri-implant bone loss; it can cause it when associated to local or systemic factors [12].

The present study evaluated the *in vitro* antibacterial efficiency of various concentrations and substances used inside the implants against various bacteria in studies collecting bacteria inside present

high concentration of species used13. Isolated bacteria are not able to colonize implant grooves and anaerobic bacteria and the red complex are more common in peri-implant pockets and are always associated with the presence of *Fusobacterium nucleatum* [14]. This study used facultative anaerobic bacteria and obligate anaerobe bacteria to be highly present in the peri-implant sulcus, within the framework of implants and peri-implant tissue [7,13], showing that some products are effective against some bacterial species and may not be as effective against bacteria with different metabolism. The gel or solution form can influence the agar diffusion method presenting different solubility and diffusivity due to the fact that the agent efficiency depend on the diffusion of the substance through the Agar plate. (Amorim, 2006) However, a study of various concentrations of antibacterial agents in various media showed no statistical differences [15]. Siena (2013) evaluating 0.2% solution or gel 1% chlorhexidine in the treatment of periimplantitis found no statistical difference between the analyzed substances even in different concentrations. Therefore, this study used the gel due to ease of handling for use within implants [16].

The use of tetracycline and chlorhexidine by most dentists should be the indication of these substances to other treatments. Evaluating to decontaminate for the treatment of peri tetracycline proved very effective in reducing inflammation and bone loss [17]. Inhibitory halos in test chlorhexidine have good antibacterial activity and when used inside the root canal has kept alive for long periods [18]. However, in other studies, chlorhexidine was not able to completely eliminate the bacteria within the root canal and neutralize the endotoxin produced by these bacteria [19], and also showed effectiveness against *P. aeruginosa, B. subtilis* or a mixture of several bacteria20. In this study, all the two substances showed good effectiveness against the bacteria tested.

Laboratory tests using the agar diffusion method may not show the full effect of antibacterial used also depend on the diffusion and solubility of the agent [20]. In order to avoid patient discomfort during removal of the abutment due to this bacterial colonization within the implant, a substance having antibacterial high efficiency and good substantivity should be used. The ProHeal ointment has as major antibacterial agents as the iodorform and Calendula officinales [21,22]. An iodoform-based paste shows good antibacterial effectiveness in relation to anaerobic and aerobic bacteria [23]. However in some studies evaluating the antibacterial effects of essential oils, the calendula oil showed the lowest result. On the other hand, it is still effective against periodontal bacteria [24] and presents good effectiveness, reducing gingivitis and plaque accumulation when used in tooth brushing [25]. In this study, the ProHeal ointment showed greater antibacterial effect against bacteria of anaerobic red complex, which are major in the interior of implants [7,26] and peri-implant grooves [11] (Van Assche et al. 2011) but low effectiveness against aerobic bacteria.

In the present study, Tetracycline showed excellent antimicrobial efficiency and seems to be the best choice among the tested substances. To prove the efficiency of antibacterial tetracycline in long term and in clinical situations, further laboratory and clinical studies should be conducted.

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