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ANALYSIS OF MICROBIAL GROWTH IN HEALINGS, THROUGH THE APPLICATION OF CHEMICAL SUBSTANCES

Nicole Macedo de Paula
Graduated in Dentistry from the University: Cidade de São Paulo (UNICID)
http://lattes.cnpq.br/2211717278845285

Tarcila Triviño
Associate Professor at the Faculty of Dentistry of the University: Cidade de São Paulo (UNICID)
http://lattes.cnpq.br/8126848955427533

Abstract: The osseointegrated implant system is composed of two phases: the first is surgical, for the installation of the implants. The second phase, reopening for removal of the cover screw, installation of the healer, for gingival modeling and, finally, the making of the implant supported prosthesis. The healing device is intended to guide the proper healing of the peri-implant gingival tissue, shaping the space of the dental prosthesis in the patient's gingiva. **Goal:** to analyze and compare the antimicrobial effects of 0.12% chlorhexidine solutions and iodoform paste on the surface of dental implant healing. **Material and Method:** In the same patient, a minimum of 3 healers were placed in each arch, and one implant received no surface treatment (control group), a second implant will be soaked in 0.12% chlorhexidine solution (group 1) and in the third will be applied the iodoform paste (group 2). **Results:** In method one, when we evaluated the efficacy of the substances during the 15 days of healing, there was no decrease in antimicrobial activity. In method 2, chlorhexidine exerted a bacteriostatic effect on microorganisms, while the control group with iodoform was ineffective. In the third method, the Disc diffusion method, chlorhexidine showed strong antimicrobial activity. As for iodoform, there was microbial resistance. **Conclusion:** Chlorhexidine 0.12% and iodoform paste did not demonstrate antimicrobial efficacy in the 15-day healing protocol. However, when placed in contaminated culture medium, chlorhexidine demonstrated strong antimicrobial activity, while iodoform remained ineffective in both methods.

Keywords: Chlorhexidine, Iodoform, implants, healer

INTRODUCTION

Rehabilitation with integrated bone implants is today an extremely advantageous treatment alternative for edentulous patients. Since the discovery of bone integration, dentistry has reached high predictability in its treatments.[1]

The term "periodontal disease" defines various diseases associated with the periodontium.[2] It is a morbidity that affects the supporting structures of the teeth, namely the periodontal ligament, cementum, alveolar bone and gingiva; as well as implants and their supporting structures.[3] It affects virtually the majority of the world's population, being the biggest source of tooth loss after the age of 25.[4]

A system of integrable bone implants was then presented, which consisted of two phases: the first surgical phase, for implant installation, was maintained for a period of six months in the maxilla for bone integration and in the mandible for four months. The second phase, reopening for removal of the cover screw, installation of the healer, for gingival modeling and, finally, the making of the implant-supported prosthesis.[5]

The purpose of using the cover screw is to protect the internal region of the implant, preventing bone growth inside it or soft tissue invagination.[6]

1. Faverani, Leonardo Perez *et al*. Implantes ósseo integrados: evolução sucesso. *Salusvita*, Bauru, p. 47-58, 2011

2. Steinberg, D.; Friedman, M. Sustained release drug delivery devices for local treatment of dental diseases. In: TYLE, P. (ed.). *Drug Delivery Devices***.** New York: Marcel Dekker, p. 491-515, 1988.

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The healer is used in the second phase of the surgery, with the purpose of guiding the proper healing of the peri-implant gingival tissue, shaping the space of the dental prosthesis in the patient's gum and fixing it manually with the aid of a digital key.[7]

The loss of implants can occur due to bacterial contamination during the surgical procedure or during healing, also due to anatomical and/or technical problems, such as the formation of niches of microorganisms between the dental roots or implant grooves. Therefore, it is extremely important to use a substance to inhibit bacterial growth in these phases.

GOALS

The objective of this study is to evaluate and compare the antimicrobial action of 0.12% chlorhexidine solutions and iodoform paste on the surface of dental implant healing.

MATERIAL AND METHODS

MATERIAL

The samples consisted of patients with missing teeth who were submitted to the placement of dental implants of the Conectação brand, duly authorized by Anvisa, at the clinic of the specialization course in Implantology at Universidade Cidade de São Paulo.

Aspects that could influence the maintenance of the patient's systemic health were observed through the study of the patient's chart, that is, through anamnesis, it was evaluated whether the patient had previous and current diseases of the cardiovascular, respiratory, nervous, endocrine, urinary and gastrointestinal; infectious diseases; hereditary diseases already registered in the family, consumption of drugs (especially tobacco and alcohol), allopathic medicines; allergies to synthetic substances and materials and possible habits and addictions.

Thus, the sample exclusion criteria were patients with uncontrolled local or systemic disorders that compromise tissue healing or regenerative capacity and with the presence of periodontal disease. The inclusion criteria were adult patients, of both sexes, who present an area to which they will receive at least 3 implants (upper or lower), absence of relevant systemic alterations, non-smokers and who do not use alcohol.

CLINICAL METHODS

First clinical stage

The first step of the methodology of this research consisted in the sterilization of the healing devices to be used, that is, these accessories were previously autoclaved 24 hours before the clinical procedures, in the sterilization center of the Universidade Cidade de São Paulo, in order to promote the absence of full of microorganisms.

Second clinical stage

After the period of osseointegration of dental implants, that is, 6 months for the upper arch and 4 months for the lower arch, Professor Ms. Cláudio Braz Haro (CROSP 56801) carried out the procedure to reopen the implants, in the which the "coverscrew" were removed.

This clinical procedure consisted of a minor surgery, called opening surgery, with the purpose of exposing the dental implant head and, consequently, the covering screw. Initially, the patient received infiltrative anesthesia (2% Mepivacaine HCI + 1:100,000 Epinephrine) at the site to be incised, and this anesthetic substance was chosen for this procedure.

Then, with the aid of a scalpel (blade 15), an incision was made on the ridge in the places

7. Von Blücher AG. Chlorhexidine slow release devices for peri-implantitis prevention, Rio de Janeiro: Instituto Militar de Engenharia, p-16, 2007.

where the implant is located, which exposed the cover screw, which was then removed with a wrench with a diameter 09, hexagonal, digital implant prosthesis.

In the same patient, a minimum of 3 healing devices (Connection brand) were placed, duly authorized by Anvisa, and one implant did not receive surface treatment (control group), a second implant was soaked in 0.12% chlorhexidine solution (group 1) and in the third, iodoform paste was applied (group 2). Group 1 healers were left in the solution for a period of 60 seconds prior to placement, according to the manufacturer's instructions.

Iodoform paste (PROHEAL®) was applied at the time of placement of the healer.

There were no harmful consequences for the participants who did not receive the surface treatment (control group), as the routine practice of the clinic is not to apply any surface treatment to the healers.

The guidelines for research participants, after application of the substances, were disciplined daily brushing, flossing, not smoking or drinking alcohol and taking analgesics in case of postoperative pain.

After a period of 15 days for gingival tissue repair (healing), the healing materials were removed from the oral cavity of the sample patients and subjected to inspection for the presence of bacteria, that is, a smear was performed with the material collected around these accessories. in the microbiology laboratory of Universidade Cidade de São Paulo, authorized by the person in charge Nelson Alves Pazzim (CRBio 020852/01-D).

MATERIAL

· Ready-made culture medium AGAR BHI (BRAIN HEART INFUSION - derived from brain and heart nutrients, peptone protease, sodium chloride, sodium phosphate, glucose and agar).
· Test tubes with thread
· BHI broth (a medium derived from brain and heart nutrients, peptone and dextrose).
· Drigalski handles
· Petri dishes
· Greenhouse incubator (QUIMIS)
· Alcohol 70°
· Bunsen burner: used as a heat source for buckling
· Sterile metal forceps
· 6.5mm sterile filter paper discs

LABORATORY METHODS

Method 1

After 15 days, the healers were collected from the oral cavity of the patients, then deposited in liquid culture medium in screw test tubes containing BHI Broth, later they were placed in an incubator (QUIMIS) at 37°C for 48 hours to observe the growth, which will mean the development of bacteria. This process can be seen with the naked eye in the form of turbidity.

After hand antisepsis and bench asepsis with 70° alcohol, we collected the material from the test tubes. When opening the tube immediately, with the aid of a Bunsen burner, we flambé the mouth of the tube, for a sterilizing action. Then we used the inoculation loop, which was also flamed (sterilizing action: carbonization of microorganisms) and we sowed the contents of the tubes of each group in front of a solid culture medium in ready-made petri dishes Agar BHI. The plates remained on a flat surface and were incubated for 72 hours.

Methods for evaluating the antimicrobial efficacy of in vitro products.

Method 2

After 15 days, the healers were collected from the oral cavity of the patients, and then deposited in liquid culture medium in screw test tubes containing BHI Broth, later they were placed in an incubator (QUIMIS)

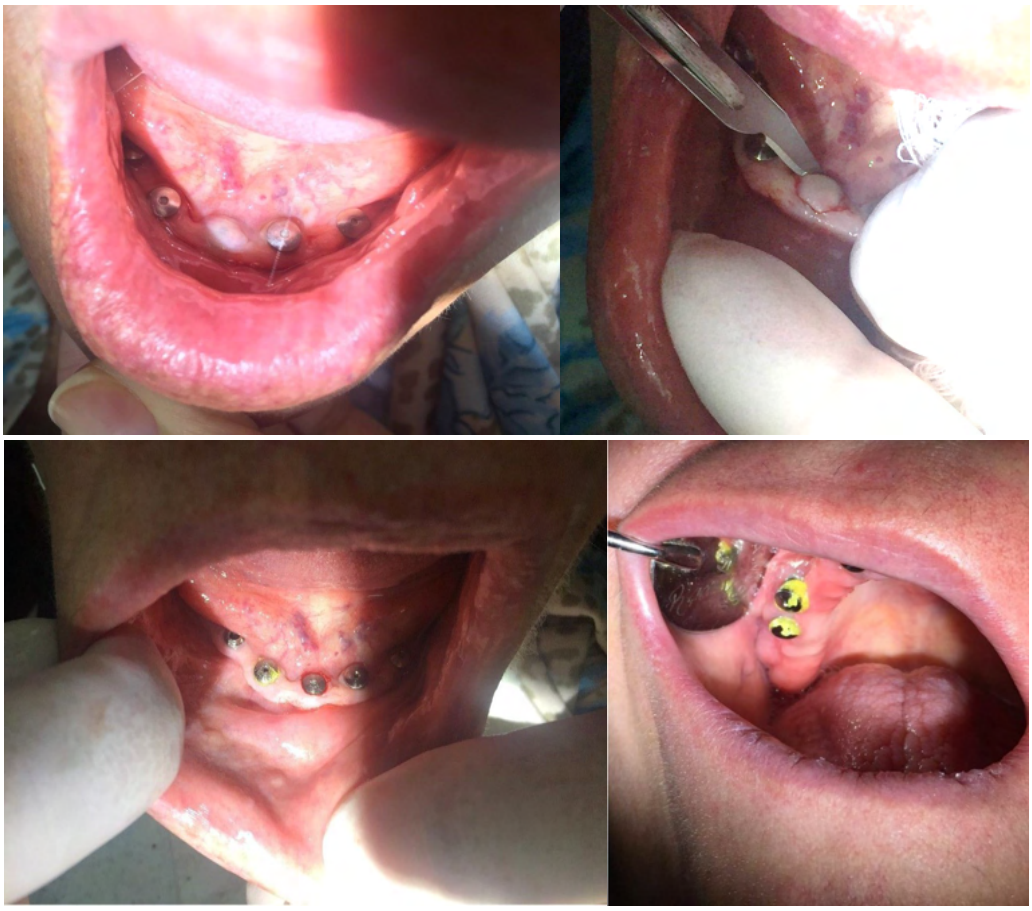

Figure 1: Implant covered by gingiva (A); Incision to expose the "cover screw" (B); Installation of healing agents with the substances to be evaluated (C).

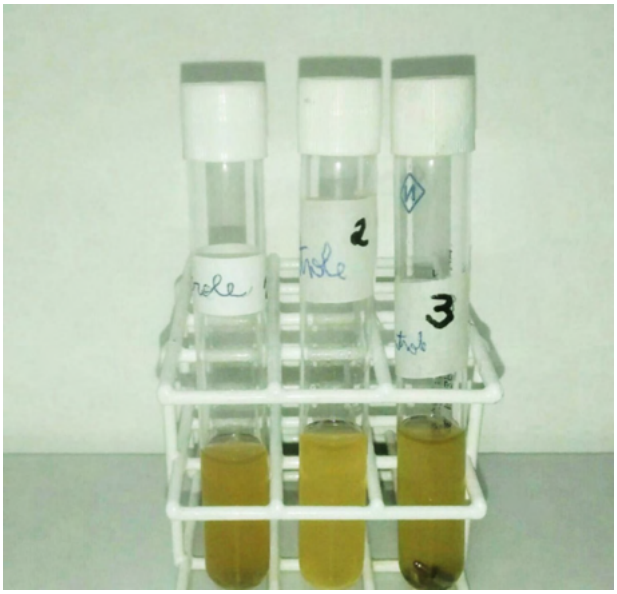

Figure 2: Test tubes with thread, showing turbidity.

at 37°C for 48 hours to observe the growth, which will mean the development of bacteria. This process can be seen with the naked eye in the form of turbidity.

After hand antisepsis and bench asepsis with 70° alcohol, we collected the material from the test tubes. When opening the tube immediately, with the aid of a Bunsen burner, we flambé the mouth of the tube, for a sterilizing action. Subsequently, 1.0 ml of material was collected from the tubes of each control group and then deposited in solid culture media, ready-made petri dishes Agar BHI. With the aid of the Drigalski loop, after being flamed in a Bunsen burner (sterilizing action: carbonization of microorganisms) we spread the substances to be analyzed on each plate, chlorhexidine (group 1), and on another plate, with another loop, iodoform (group 2). The plates remained on a flat surface and were placed in an incubator (QUIMIS) at 37°C for 120 hours (five days).

Method 3

After hand antisepsis and bench asepsis with 70° alcohol, we collected the material from the test tubes. When opening the tube immediately, with the aid of a Bunsen burner, we flambé the mouth of the tube, for a sterilizing action. Subsequently, 1.0 ml of material was collected from the tubes of each control group and then deposited in solid culture media, ready-made petri dishes Agar BHI. With the aid of the Drigalski loop, after being flamed in a Bunsen burner (sterilizing action: carbonization of microorganisms) we sowed this content from the control group on the plates. We then manipulated sterile metal tweezers, which after being buckled, we used to pick up the filter paper discs and insert them into the plates. For each control group, two discs impregnated with the solutions to be analyzed, chlorhexidine (group 1), and iodoform (group 2) were inserted. The plates remained on a flat surface and were placed in an incubator (QUIMIS) at 37°C for 78 hours to analyze the formed halos.

RESULTS

The results obtained in method 1, evaluating the effectiveness of chlorhexidine and iodoform solutions during the 15 days of healing in the patient's oral cavity, we could observe in the laboratory that the solutions were not able to reduce the formation of colonies, not promoting bacteriostatic effect.

However, when we performed method 2, the sample of the chlorhexidine solution placed in a contaminated culture medium (control group) showed strong antimicrobial activity, not allowing the formation of colonies. The iodoform paste proved to be ineffective against the test microorganisms.

To determine the antimicrobial efficacy of chlorhexidine and iodoform solutions, we also used method 3, Disc-diffusion. We could then observe the formation of a halo when we used chlorhexidine, proving its bacteriostatic effect.

However, the iodoform paste, once again, did not show antimicrobial efficacy, as it did not reproduce halos, demonstrating microbial resistance.

DISCUSSION

There was a difference in bacterial growth for all methods chosen. In method 1, when we evaluated the effectiveness of the substances during the 15 days of healing, the results obtained were not as expected, as there was no action or decrease in antimicrobial activity, when we performed the in vitro tests. As a result, we obtained a similar growth of bacterial colonies, both in healing without surface treatment, and in those that received the substances to be evaluated.

To determine the antimicrobial efficacy of chlorhexidine and iodoform solutions, we used

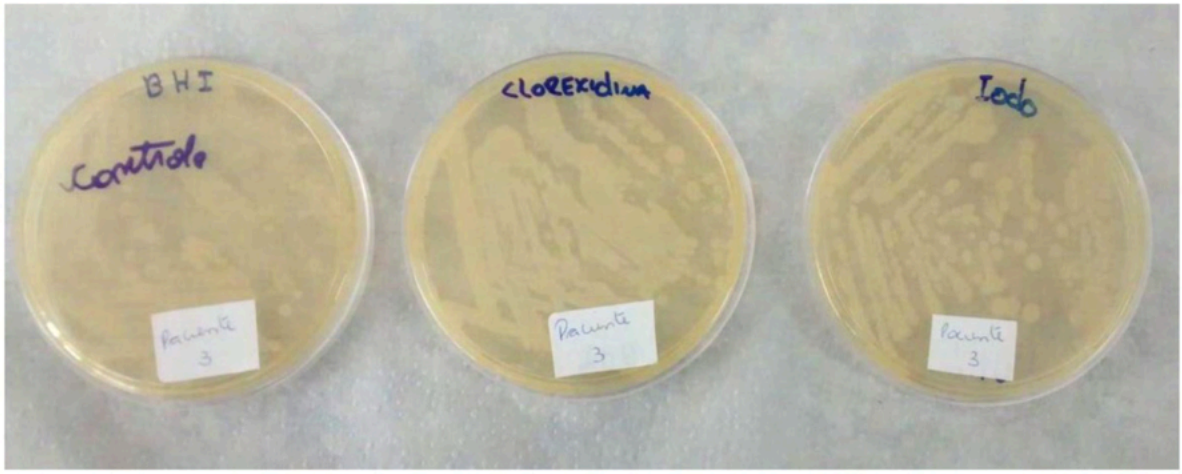

Figure 3: formation of bacterial colonies in all groups, demonstrating that there was no efficacy in the antimicrobial action of 0.12% chlorhexidine solutions and iodoform paste on the surface of dental implants healing in 15 days.

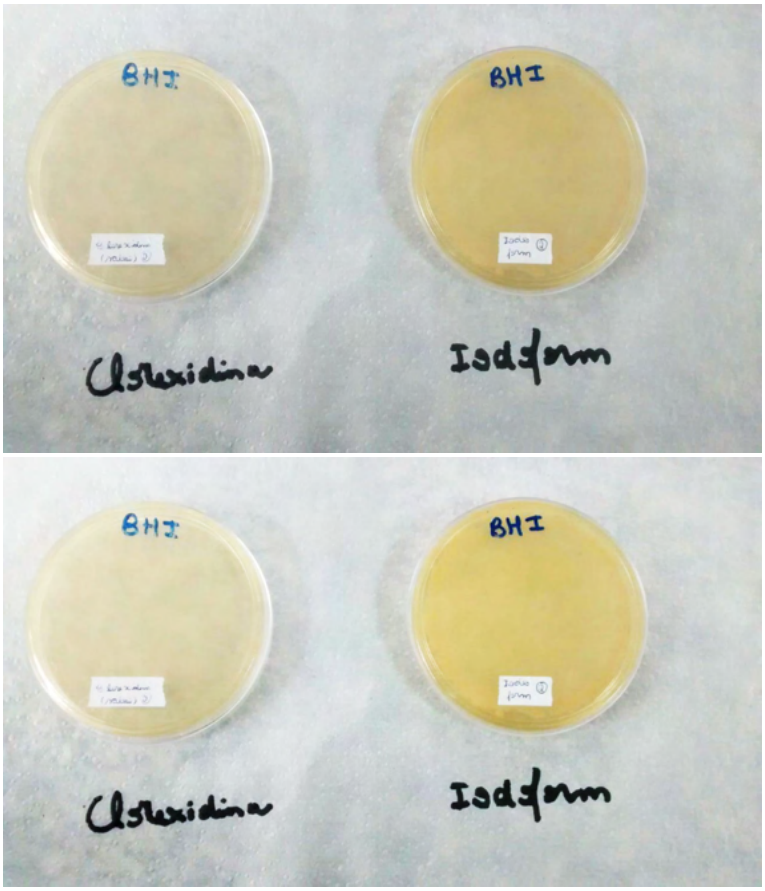

Figure 4: Method 2, demonstrating the effectiveness of the chlorhexidine solution when placed in a contaminated culture medium, as there was no bacterial colony growth. Iodoform paste, without any antimicrobial efficacy, as there was growth throughout the plate.

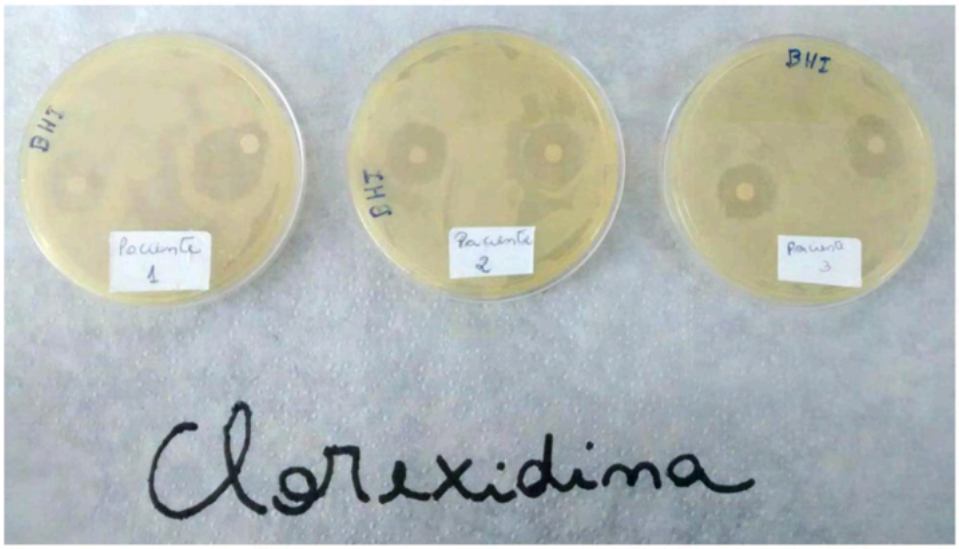

Figure 5: Formation of Halos, on the plates with the chlorhexidine solution. Demonstrating microbial sensitivity.

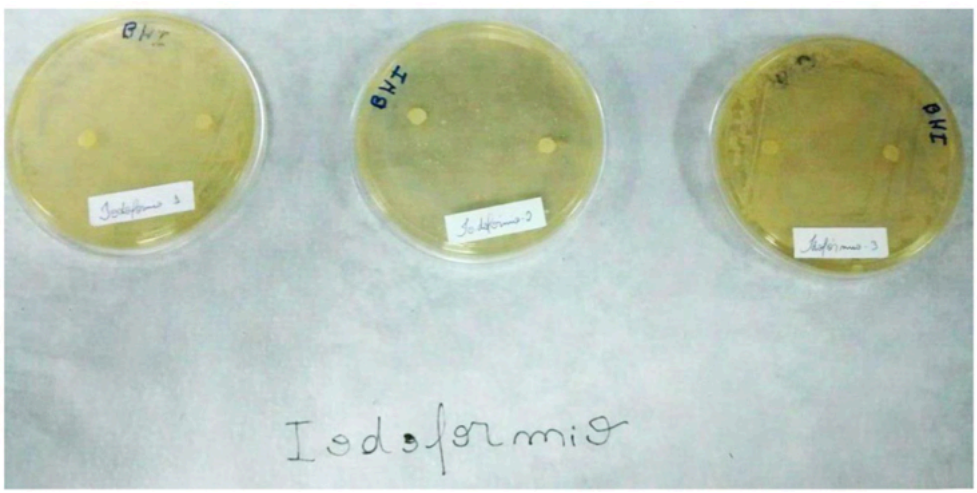

Figure 6: Resistant bacteria grow close to the disc, with no halo formation, proving the ineffectiveness of the iodoform paste on the plate contaminated by the control group

two different methods. In method two, the solutions were placed in contaminated culture medium (control group). The results obtained in this method were that chlorhexidine can exert a bacteriostatic effect on microorganisms, not allowing the formation of colonies, while the control group with iodoform shows that it has no antimicrobial efficacy against the test microorganisms.

In the third method, we used the Disc-diffusion method, after analyzing the results, it can be concluded that chlorhexidine showed strong antimicrobial activity, as an inhibition halo was formed around the disc in all plates. As for the plates with iodoform, there was no presence of halos, with microbial resistance. The absence of halo signifies resistance and the presence of halo implies the level of microbial sensitivity.

The realization of a work like this is extremely important so that it can serve as a starting point for many other studies and continue the analysis of the bacteriostatic effects of chlorhexidine and iodoform solutions. The present work evaluated the chemical substances in vitro, for a better, more detailed evaluation of the clinical antimicrobial efficacy of the solutions, a research and clinical tests would be indicated.

CONCLUSION

Based on the results obtained and in accordance with the conduction of the proposed experiment, it was found that: the substances to be evaluated 0.12% chlorhexidine and iodoform paste did not demonstrate antimicrobial efficacy in the 15-day healing protocol. However, when placed in contaminated culture medium (control group) to evaluate the effectiveness of the product, chlorhexidine showed strong antimicrobial activity, while iodoform remained ineffective in both methods.

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