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Research Article

Disinfection with the Low Power Laser of the Material Proposed to Manufacture the Prototype to Support the Limb with Exposed Fractures

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Abstract

Disinfection of materials is one of the ways to prevent infection, chemical disinfectants are toxic and can cause respiratory problems during handling. The material proposed to manufacture the prototype was steel. This work aimed to disinfect steel samples, containing biofilm with *S. aureus*, using Photodynamic Therapy, Prior to the biofilm culture, the steel sample received electropolishing treatment to increase microbial adhesion. The results showed an increase in biofilm of 6.2 $\log_{10}$ CFU/mL, after electropolishing performed on the steel surface and a total reduction of biofilm with *S. aureus*, after application of PDT with AM and urea within 10 minutes, this reduction can be compared to intermediate level disinfection. We conclude that it is possible to use PDT for disinfection, cautiously.

Keywords: *Level of Disinfection; Photodynamic Therapy; Biofilm; Steel*

Introduction

Disinfection is one of the ways to prevent infection, characterized by the elimination of all microorganisms from the materials, instruments and surfaces of objects and furniture used in health care [1,2].

Chemical disinfectants include: peracetic acid, aldehydes, chlorinated solutions and alcohol. They are classified into: low level, intermediate level and high level, according to the contact with the tissues during use [1,3-5].

The low-level disinfectant penetrates only through the membranes of vegetative bacteria and destroys lipid viruses and some non-lipid viruses, they are indicated for non-critical materials that come into contact with the whole tissue or surface of objects belonging to patients or the hospital.

The intermediate level disinfectant penetrates the cell membrane of *Mycobacteria* with a high content of lipid material, vegetative bacteria, fungi, lipid and non-lipid viruses, it is indicated for non-critical materials, which come into contact with the patient's skin and surfaces of objects belonging to the hospital or the patient.

The high-level disinfectant crosses the wall of bacterial spores, *Mycobacteria* and vegetative bacteria, they are used for semi-critical materials that come into contact with non- integral tissue, membrane and mucosa.

The biofilm can form through poorly washed instruments, and it can spread infection to the patient. It is important to wash before performing sterilization or disinfection, to guarantee the effectiveness of the process [1].

The prototype to support the exposed fracture limb is used during the washing of the exposed wound, which constitutes one of the initial treatments of the exposed fracture, which is classified as critical material for coming in contact with the mucosa, subepithelial tissue and the vascular system [6-8].

AISI 304 steel is the material indicated for the manufacture of the limb support prototype, due to its characteristics such as: corrosion resistance, mechanical resistance, easy cleaning and recycling [9].

In this study, laser was used through Photodynamic Therapy (PDT), which is presented as a technique that leads to death by microorganisms. The cytotoxic effect is achieved using a light source and the dye. This technique has been used for the disinfection of blood and dental materials [10].

The action of PDT occurs through the absorption of the photon of light by the photosensitizer molecule, which raises the energy level, going from the So level to the level I, in this phase, it can return to the So level, emitting fluorescence, or it can go to the triplet state, in this state the photosensitizer molecule may return to So emitting phosphorescence or type I and type II reactions occur. In the type I reaction, the photosensitizer molecule can react with substrate and produce reactive oxygen species, such as superoxide (O_2^-) or by reducing oxygen giving hydrogen peroxide (H_2O_2), which can suffer a reduction and produce more energetic hydroxy radicals (OH^-) [11].

In the type II reaction, the formation of single oxygen occurs through the transfer of energy from the excited photosensitizer to an oxygen molecule, forming either singlet oxygen. As type I and type II act on the biomolecules of viruses, bacteria, fungi and parasites, leading to oxidation and consequently cell death [11].

The photosensitizer used was Methylene Blue, because it is efficient against microorganisms, has a positive charge and during photoactivation it binds to the cell membrane, which is negatively charged, inactivating viruses, fungi, bacteria and cancer cells, through reactions I and PDT II [12-14].

The *Staphylococcus aureus* bacterium is classified as a gram positive, prokaryotic bacterium, constituted by a cell wall, it is present in numerous infections, in hospital environment, such as: infection in catheter, in wound, in orthopedic implant, can cause endocarditis and pneumonia [15].

Aim of the Study

This work aims to describe the use of PDT, for disinfecting the surface of AISI 304 steel; material used to manufacture the limb support, containing the biofilm with *Staphylococcus aureus*.

Material and Method

A range of bacteria from *S. aureus* ATCC 25923 was grown in TSA (Trypticase Soy Agar) for 24 hours at 37°C. After this period, a range of *S. aureus* in saline was used. The saline was added to a concentration of 10^8 CFU / mL, on the 0.5 scale of Mac Farland, which corresponds to 2 x 10^8 CFU/mL, and was vortexed for 15 seconds; after the reading was made on the spectrophotometer at a wavelength of 625 nm at an absorbance value of 0.08.

To confirm the bacterial load of the inoculum, serial dilution of the inoculum was made up to 10^{-7} CFU/mL; and 10 µL was pipetted into a TSA plate in triplicate. The counting was done and the value of 0.08 corresponds to 2 x 10^8 CFU/mL.

Preparation of the surface of AISI 304 steel for microbiological testing

Twenty-seven steel samples were obtained, measuring 0.6 cm x 0.6 cm, divided into 3 groups: control group, irradiated group 5 minutes and irradiated group 10 minutes. All samples were subjected to electropolishing to improve the adhesion of the biofilm with *S. aureus*, through the electropolished rough surface [16]. The electropolishing was performed using the DC POWER SUPPLY device, the steel

samples were immersed in 500 mL of water with 30 grams of NaCl, using a voltage of 7 Volts, with a current of 0, 44 Amps, for 20 seconds, and then washed in deionized water. Subsequently, they were sent for sterilization in moist steam at 121°C, for 30 minutes [16]. The density (J) of the current (q) of the electropolishing, calculated over the area of the steel, was 2.50J.

Biofilm formation with *S. aureus*

The biofilm formation was carried out according to Wu, 2018 with some modifications. The material samples were incubated in 24-well plates, with 999 µL of TSB, plus 0.25g of glucose (KASVI) and 0.01 mL of bacterial suspension prepared at a concentration of 10^8 CFU/mL. After 24 hours, the medium was changed.

Application of TFD with 100 mM A.M. and urea on the biofilm with cultured *S. aureus*

After culturing the biofilm with *S. aureus*, on the steel surface, the samples were irradiated by TFD, using the times of 5 and 10 minutes, power density of 111 mW/cm^2, energy density of 66.66 J/cm^2. The steel samples were immersed in 500 µl of urea and 500 µl of 100 mM methylene blue for 5 minutes, individually. After the sample received irradiation for 5 minutes and 10 minutes. The laser tip coincided with the sample (6 x 6 cm x 1 cm), 0.9 cm in diameter in the 24-well plate.

Preparation of samples of materials containing the biofilm with *S. aureus* for counting UFC

The preparation of the samples of the materials of the control group and of the samples irradiated with PDT with AM and urea, followed the following preparations: the samples were washed individually with 1 mL 0.9% saline, once, on the plate itself of culture, discarded the solution and resuspended in 1 mL of 0.9% saline. Then, each sample was scraped with the pipette tip for 8 times [17] with modification. Afterwards, the solution containing the material sample was left in the eppendorf and placed on the Cristofoli 60Hz ultrasound for 525 seconds. After the samples were stirred, for 2 minutes, on the Vortex Nova instruments NI 1069, for 3 times [18].

Afterwards, serial dilution was performed, with the sample containing the material, 10 µL was pipetted in the form of a drop drained into the TSA (Trypticase soy agar) plate, in columns, from -3 to -7, as shown in figure 18. Plating was done in triplicate to better characterize the result.

Results and Discussions

For statistical analysis, the test of normality and homogeneity of variances was first performed, using the Levene and Shapiro-Wilk tests. In cases of normality and homogeneity of variances, the analysis of variance test (ANOVA) was performed, followed by Tukey's post hoc test, using the GraphPad Prisma 7.04 statistical program and the Microsoft Excel version 2010 program.

Cellular adhesion on the surface of AISI 304 steel was made possible by electropolishing performed using the time of 20 seconds, which increased the biofilm adhesion by 117% for steel. According to the study by [16], who reported the difficulty of cell adhesion on smooth surfaces, demonstrated that the smooth electropolished surface facilitates the formation of microcolonies of this *Staphylococcus aureus* bacterium. In this same study, the authors used electropolishing with a 20-second time on the steel surface and obtained an increase from 3.1 x 10^2 CFU/mL to 9.3 x 10^3 CFU/mL, as shown in figure 1.

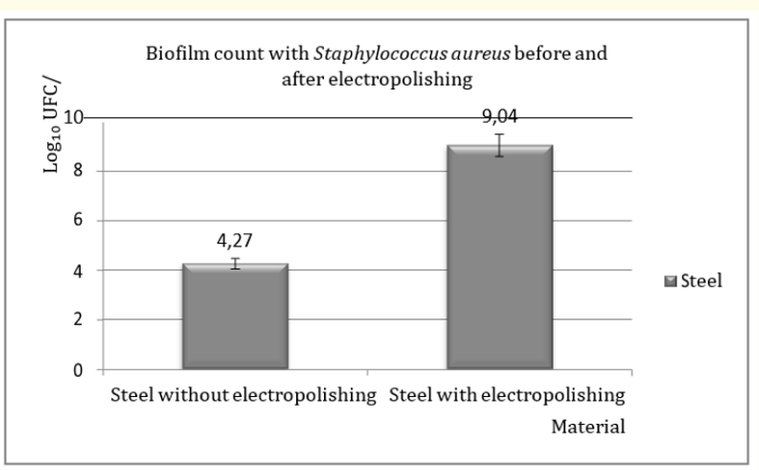

Figure 1: *Shows the increase in biofilm with staphylococcus aureus grown on the steel surface before and after electropolishing.*

The type of surface of the material and the type of bacteria influence the adhesion of microorganisms [19]. The hydrophobic surface facilitates their adhesion [20-22]. There are controversies about cell adhesion on rough surfaces [23,24], while [25] report that cell adhesion on rough surfaces is easy. It is important to note that materials that present a surface with difficulty in bacterial adhesion are excellent for manufacturing devices for hospital use, as they indirectly contribute to the reduction of infection transmission during use [21].

Figure 2 shows the average value of the amount of bacteria (in Log_{10} UFC/mL) present in the AISI 304 steel samples, before and after going through the PDT using the A.M. photosensitizer and urea. It is possible to notice a reduction from 9.04 log_{10} CFU/mL to 3 log_{10} CFU/mL after 5 minutes of irradiation and a total reduction within 10 minutes. The reductions presented for both samples showed statistically significant differences (with $p < 0.001$). The total reduction of the biofilm with *Staphylococcus aureus*, after the use of PDT with A.M. and urea, demonstrated efficacy.

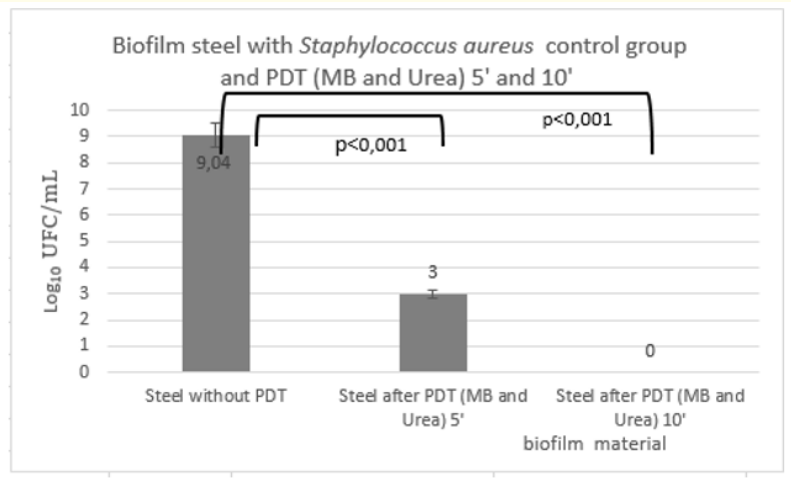

Figure 2: *Average values of the amount of bacteria (in Log_{10} UFC/mL) from AISI 304 steel containing biofilm with S. aureus without irradiation and after PDT (A.M. + urea) irradiated for 5 and 10 minutes. The bars indicate standard deviation.*

According to the American National Standard Institute (ANSI), 2011, the level of sterilization considered safe for materials for hospital use classified as critical is 10^{-6}, a value equivalent to the survival of 1 in 1000,000 microorganisms. Comparing the effect of PDT with AM and urea, on samples of materials containing the biofilm with *S. aureus*, PDT demonstrated a total reduction using the time of 10 minutes but for validation as a sterilizing agent, for use in medical equipment, there is a need to prove efficiency on other types of microorganisms such as viruses, fungi and protozoa.

According to the RDC n.35 of August 2010, of the Health Surveillance, for the disinfection of intermediate level, it is necessary to destroy all microorganisms, except for the high number of bacterial spores. The total reduction of biofilm after PDT with A.M. and urea can be considered as intermediate level disinfection. It can be indicated, according to Rutala's descriptions; Weber, 2013 and the Center Disease Control (CDC, 2009) disinfection guide, for use in non-critical materials; that is, they come into contact with the skin integrates. Therefore, TFD with A.M. and urea cannot be used for the member support tray, as it is classified as critical material.

Conclusion

The technique of disinfection by chemical agents produces toxicity during manipulation, which can cause respiratory problems, which does not occur with the PDT disinfection technique, it is fast, efficient, does not generate heat, in this study this technique demonstrated

the total reduction of biofilm with *Staphylococcus aureus* on the steel sample, therefore, it can be used for the disinfection of materials, it is necessary to be careful during use, because where the light does not reach, the disinfection process does not occur.

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