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The Effect of Plasma Jet on Bacterial Species and Correlation with Physiotherapy

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Abstract

Introduction: As representatives of prokaryotic beings, bacteria were selected in nature for their very high capacity to adapt to the environment. The human body comprises a vast population of bacteria needed for health maintenance; skin lesions represent a major public health problem involving the nutritional, hospital and illness of the patient. **Objective:** The objective of this study was to verify the effect of the plasma jet on bacterial species and to correlate it with clinical physiotherapy. **Method:** This is an *in vitro* experimental study carried out at FSG University Center. Performed through cultures of bacteria *Sthaphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* under the effect of plasma jet. **Results:** As observed all strains were resistant to penicillin, creating no inhibition halo in any of them. In the plates of the bacteria *Staphylococcus aureus*, in the area where the Plasma Jet was applied there was no microbial growth. In *Klebsiella pneumoniae* strains microbial growth was achieved in 90 and 120 seconds. Finally, *Pseudomonas aeruginosa* plates in the 90 seconds were microbial growth compared to the 120 and 180 seconds. **Conclusion:** It can be concluded that the time of application of the plasma jet has an important role in the antimicrobial action, when compared to Physiotherapy, a new method of treatment for skin lesions may be an option, with the aim of reducing infections and accelerating the process of tissue healing.

Keywords

Bacteria, Infection, Pressure Injury, Physiotherapy

1. Introduction

As representatives of prokaryotic beings, bacteria were selected in nature for their high ability to adapt to the environment around them due to their high multiplication rate, as well as their metabolic rate. These organisms are still used in studies, and used in several areas of microbiology [1].

The human body comprises a vast population of bacteria necessary for maintaining health [2]. The ordering of microorganisms depends on several factors, such as humidity, acidity, temperature, and availability of nutrients. Such organisms influence the immune system, resistance to pathogens, and the use of food. The human organism provides distinct favorable environmental conditions that provide the growth and distribution of microbial populations in response to external and physiological factors of the host such as age, diet, hormonal status, health and personal hygiene [3].

Bacteria cause two types of infections: one is called pyogenic, being responsible for the production of pus; the other is granulomatous, where the defense cells clump, which serve to isolate insoluble bacteria or foreign substances that the organism was unable to expel [4].

However, the bacteria end up infecting the skin lesions, which result in a major public health problem involving the nutritional and hospital aspects and causing sickness of the patient, in addition to social and personal care issues [5]. In Brazil, although there are few studies on the incidence and prevalence of bedsores, a study carried out in a general university hospital showed an incidence of 39.81% [6].

Pressure injury occurs due to some factors that are triggered during hospitalization, since it is an environment where patients are exposed to risk factors such as hemodynamic instability, impaired physical mobility, compromised general health, and nutritional condition [7]. Pressure injuries can also be developed in the home and in long-term care facilities for the elderly, when related to household items due to prolonged contact, such as to a wheelchair and bed [8].

Infections in pressure injuries end up generating high costs to healthcare facilities, influencing the increase in the hospitalization period with direct repercussions on the discomfort and dysfunction that are caused to patients affected by this type of wound [9].

All wounds are colonized by microorganisms, among them, the most common found in skin lesions are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Acinetobacter spp. and *Candida albicans* that form a colony in the wound, however, not all are infected. Infections can present themselves in different ways, usually with local signs of soft tissue involvement such as heat, erythema, edema, purulent secretion and the presence of a foul odor [10] [11].

The treatment of pressure lesion infections is based on the severity of the infection and the resistance profile of the bacteria [10]. There are several laboratory methods that can be used to predict the *in vitro* sensitivity of bacteria to antimicrobial agents. Countless laboratories use the disk diffusion method, which aims to test common fast-growing pathogens and certain troublesome bacteria [12]. The author further describes that disk diffusion tests are based only on the presence or absence of an inhibition halo; regardless of the size of the halo, the tests are not acceptable.

Penicillin was discovered in mid-1928 by Alexandre Fleming, considered as a fluke in medicine, being used until today as a treatment for diseases such as Syphilis and other pathologies [13].

However, clinical physiotherapy works with the multidisciplinary team to prevent the development of skin lesions through patient assessment. It acts mainly in the promotion of early mobilization, in active and passive exercises that favor the increase of circulation and cellular nutrition, in the observation of the general condition of the patient, identifying associated factors as well as the physical integrity of the skin, accelerating the healing process through various techniques and conducts that correspond to the specialties of the physical therapist [14].

Technology over time encompasses several areas, so it has been incredibly assisting the work that is done by the physiotherapist. In this study, a technology called plasma jet will be approached, which can be used as another physiotherapeutic resource for the treatment of wounds due to its bactericidal action. In addition, the three most common bacteria that may be present in these lesions will be analyzed, namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The antimicrobial action of penicillin and their resistance to this antibiotic will be studied. Thus, the aim of the present study was to monitor the effect of the plasma jet on bacterial species and to correlate it with clinical Physiotherapy.

2. Method

2.1. Study Design

This *in vitro* experimental study was carried out at Faculdade da Serra Gaúcha.

The study was conducted using cultures of bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* in a Petri dish, under the effect of the plasma jet.

2.2. Plasma Jet

Plasma is popularly known as the fourth state of matter and can be defined as a gas with a low degree of ionization, consisting of charged particles that have multiple interactions, being macroscopically neutral [15].

Described as a gaseous substance produced from helium, argon, among other possibilities where part of its particles are ionized, the plasma can be obtained with different compositions. It consists of several active components including particles charged with ions, electrons and neutrons, radicals, highly Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), ultraviolet, among others [16]. Plasma is used in several applications, namely: sterilization of medical equipment, food industry, blood clotting, wound treatment, surface material modification, among others [16].

The interaction of the constituent components of plasma can give rise to different actions such as the chemical effect that is characterized by the disruption of the cell membrane, which can lead to cell death by oxidative stress. In the physical effect, ions cause an increase in the potential at the surface of the microorganisms, creating an electrostatic tension between the different layers of the cell wall and, finally, the neutralizing effect of the plasma neutralizes the cell wall, which, by nature, is negatively charged, triggering cell death. The effects mentioned can occur both in healthy non-cancer cells and in cancer cells [16].

Argon is a noble gas, the largest amount of which is found in the gaseous mixture of atmospheric air and about 0.93% of the volume of air we breathe consists of it. The plasma jet with argon gas has a wide indication in the healthcare area and makes it an important antimicrobial resource, aiming at improving the patient's quality of life, allowing the physiotherapist new therapeutic possibilities for the rehabilitation of tissue repair of pressure injuries [17].

2.3. Microbiological Culture and Plasma Jet Procedures

This study included reference strains *Klebsiella pneumoniae* (ATCC 49168), *Pseudomonas aeruginosa* (ATCC 49027, and *Staphylococcus aureus* ATCC 49029; all strains were KWIK-STIK). The inoculants that were used in the tests were obtained from culture of these microorganisms on MacConkey agar for the bacteria *K. pneumoniae* and *P. aeruginosa*, and blood agar for the bacteria *S. aureus*, in a Petri dish.

The cultures of these bacteria were sown using the method of depletion in the culture media already mentioned and were incubated in an oven at 36˚C ± 2˚C for 24 hours. After this period, the bacteria were diluted in 0.9% sodium chloride (saline) according to the McFarland scale 0.5 to perform the antibiogram in the Mueller-Hinton culture medium.

The antibiogram was performed in the Mueller-Hinton culture medium, in which the bacteria diluted in saline were spread throughout the culture medium for homogeneous growth. This method was used to check whether the antibiotic and the plasma jet are effective in eliminating the bacteria. Each antibiogram culture of each bacterium was performed in triplicate.

To check for bacterial death, the plasma jet was used and to compare the efficiency, the antibiotic penicillin was used. To check the efficiency of the plasma jet, an area where the jet passed was delimited and used, then evaluated by direct and directed observation of the inhibition halo generated by the effect of the plasma jet. Penicillin was used in a diffusion disk, an area was delimited where the jet has been used, and penicillin was used in that disk.

The exposure of the plasma jet, together with a noble gas, called argon in the culture medium with the bacterium, was of 90, 120, and 180 seconds with a distance of 2 mm from the plate, programmed continuously, density of 40% with an amplitude of 100%. After exposure in triplicate at each time, the plates were incubated for 24 hours in an oven at 36˚C ± 2˚C and observed the next day (**Figure 1**, **Figure 2**). The parameters of time, distance and energy used in the present study were defined from a pilot test, in which parameters were tested within limits that would not damage the environment.

All procedures were performed inside a laminar flow hood to avoid external contamination (**Figure 3**). Data analysis will be done in a qualitative way, through the visual comparison of bacterial growth.

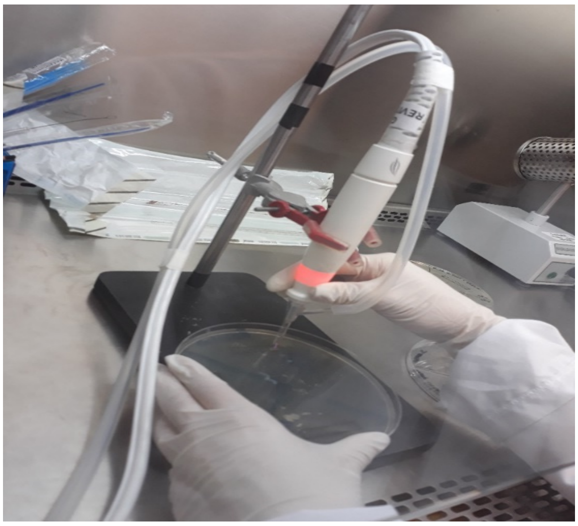

Figure 1. Plasma jet application on strains inside the Petri dish.

Figure 2. Parameters of the equipment used in the experiment.

Figure 3. Procedures being performed inside the laminar flow hood.

3. Results

The strains used in the study were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. As observed, all strains were resistant to penicillin, creating no inhibition halo in any of them (**Figure 4**).

As shown, in the *Staphylococcus aureus* bacterium plates, there was no microbial growth in the area where the Plasma Jet was applied. Thus, the Jet was considered efficient with an antimicrobial action at all times (**Figures 5-7**).

In the strains of *Klebsiella pneumoniae*, microbial growth was obtained in 90 and 120 seconds. Thus, the time of application of the Plasma Jet can influence the inhibition of its growth. The jet was only efficient in its antimicrobial action after 180 seconds, which, according to observation, was the time that had the lowest growth rate (**Figures 8-10**).

Finally, microbial growth was observed at 90 seconds in the *Pseudomonas aeruginosa* plates, when compared to the times of 120 and 180 seconds. Thus, the time of application of the Plasma Jet will influence the inhibition of microbial growth, considered efficient after 120 seconds (**Figures 11-13**).

4. Discussion

Neurological, cardiac, respiratory, infectious, and neoplastic diseases represent more than 85% of hospitalizations, being frequent in critically ill patients resulting in hemodynamic instability and limited mobility [6]. With immobility, bedridden, hypotrophic patients, and particularly those with pressure injuries, need physical therapy resources that aim to accelerate the healing process, making it possible to rapidly improve the patient's clinical condition, reducing suffering and hospital costs [18]. Thus, the plasma jet comes as another physiotherapeutic resource to act in the healing of these wounds, as a bactericidal agent. In order to minimize, or even eradicate microbial biofilms in health devices, many studies have been focused on this area. In this sense, the technology of atmospheric plasmas has been gaining prominence. The exact mechanism of inactivation is still not well understood. It is believed that the effectiveness of the plasma in this study is due to products from the plasma itself, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). These play an important role in vital physiological processes. In low doses, ROS and RNS act to promote cell survival, proliferation and migration. Excessive ROS concentration, on the other hand, causes oxidative stress, related to cell aging and the initiation and execution of apoptosis [19] [20].

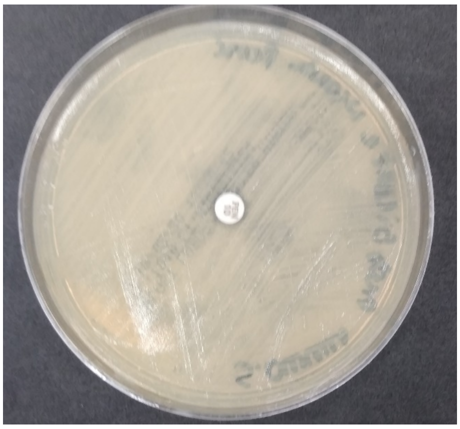

Figure 4. Penicillin application on diffusion disc.

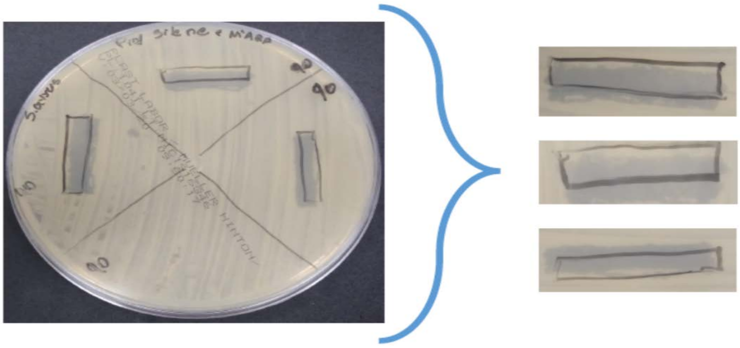

Figure 5. Plasma Jet application in 90 seconds on *Staphylococcus aureus*.

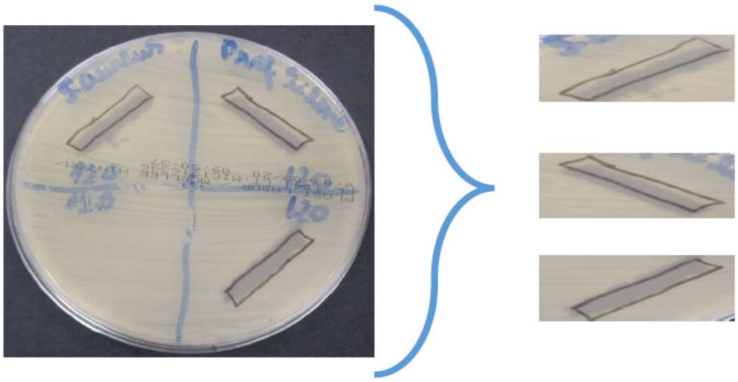

Figure 6. Plasma Jet application in 120 seconds on *Staphylococcus aureus*.

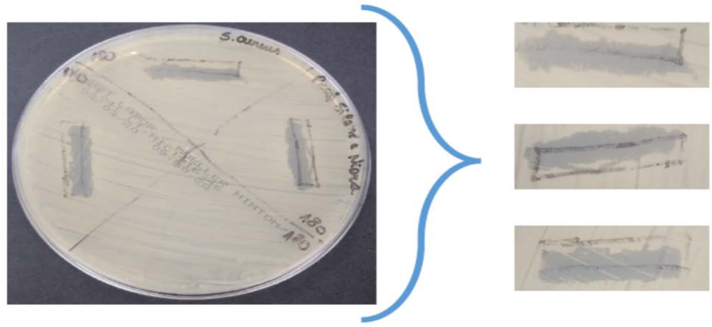

Figure 7. Plasma Jet application in 180 seconds on *Staphylococcus aureus*.

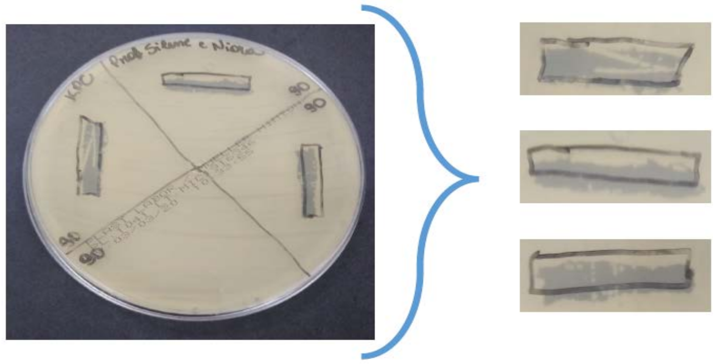

Figure 8. Plasma Jet application in 90 seconds on *Klebsiella pneumoniae*.

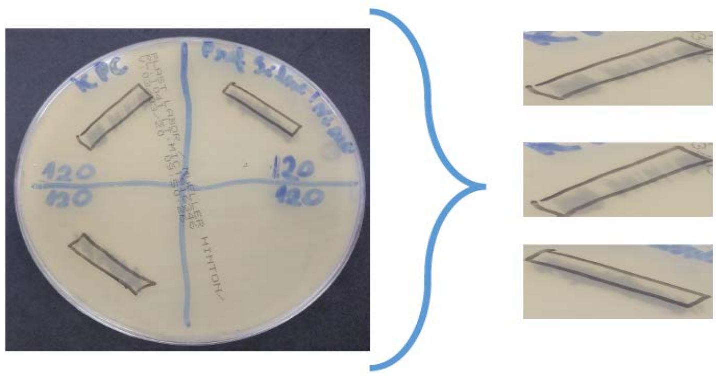

Figure 9. Plasma Jet application in 120 seconds on *Klebsiella pneumoniae*.

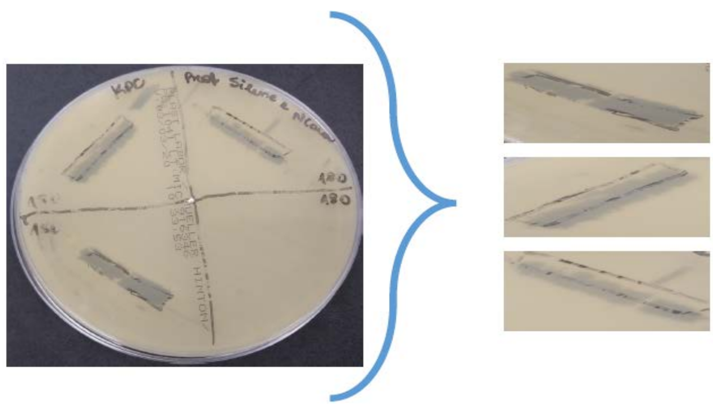

Figure 10. Plasma Jet application in 120 seconds on *Klebsiella pneumoniae*.

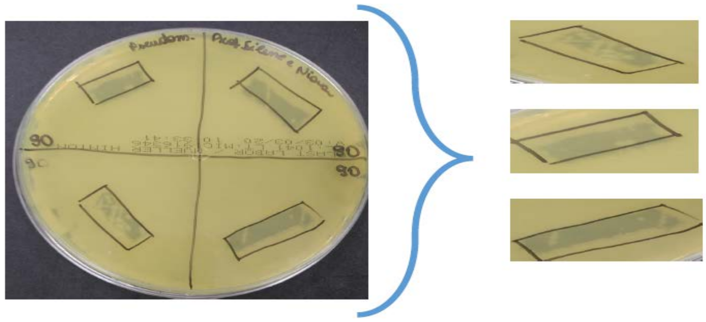

Figure 11. Plasma Jet application in 90 seconds on *Pseudomonas aeruginosa*.

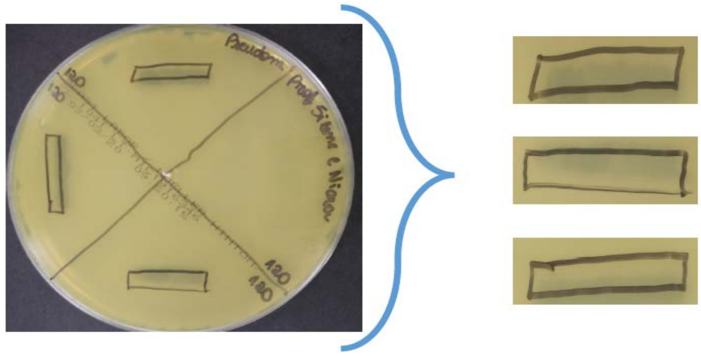

Figure 12. Plasma Jet application in 120 seconds on *Pseudomonas aeruginosa*.

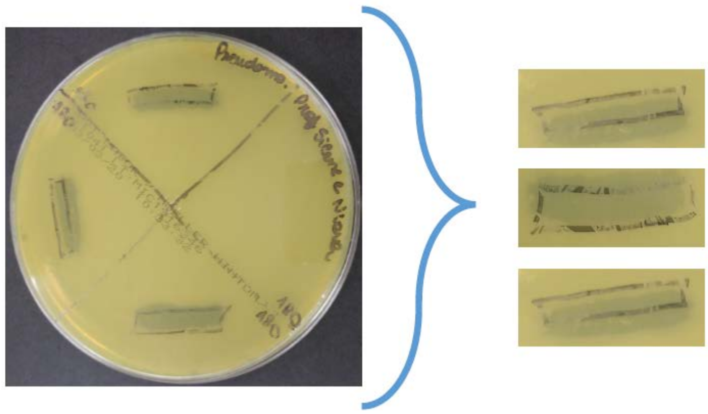

Figure 13. Plasma Jet application in 180 seconds on *Pseudomonas aeruginosa*.

In the present study, it was possible to verify the resistance of bacteria to penicillin. In this perspective, resistance to antibiotics occurs due to biological adaptations by which microorganisms develop defense mechanisms against the action of the antibiotic, resulting in the development of new, more potent and more active drugs [21].

According to data obtained by BrCAST, the clinical cutoff points for the diameter of the halo with penicillin are: *Staphylococcus aureus* is sensitive when its inhibition zone is >26 mm and resistant <26 mm; *Pseudomonas aeruginosa* is sensitive >18 mm and resistant <18 mm, and finally, *Klebsiella pneumoniae* is sensitive >24 mm and resistant <24 mm. Therefore, there was no inhibition halo in any of the bacteria, which makes them resistant to penicillin [22].

In the present study, it was observed that there was no microbial growth in the *Staphylococcus aureus* plates, in the area where the plasma jet was applied continuously. Thus, the jet was considered efficient with an antimicrobial action at 90, 120, and 180 seconds. However, in the *Klebsiella pneumoniae* strains, microbial growth was observed between 90 and 120 seconds. Therefore, the plasma jet application time factor is considered important to inhibit growth, since it was only efficient in its antimicrobial action after 180 seconds, which, according to observation, was the time that had the least development, being necessary the application in longer times to obtain a satisfactory result.

On the other hand, microbial growth was observed at 90 seconds in the plates of *Pseudomonas aeruginosa* when compared to the times of 120 and 180 seconds. Therefore, the time of application of the plasma jet will influence the inhibition of microbial growth, considering it more efficient when applied after 120 seconds.

In one study, it can be analyzed that the closer to the agar it is and the longer the exposure time, the greater the diameter of the halo, showing that the increase in the application distance may hinder the effect of the ion molecules generated by the jet plasma, which are responsible for antimicrobial activity [23]. The plasma jet fed with argon gas continuously reaches a greater depth, when compared to the pulse that ends up penetrating more superficially over the lesions [24].

To evaluate the clinical application of argon plasma jet coagulation in the ablation of Barret's esophagus, a group of 30 patients was selected, 25 of whom were male, aged between 12 and 72 years old and 5 of them were female, aged between 45 and 60 years, who underwent antireflux surgery and later referred for treatment with argon plasma coagulation. Patients were submitted to sessions at 30-day intervals until the lesion completely disappeared on endoscopy; a success rate with a total ablation of 93.4% was observed, with no death data during the study period, supporting our study in the safe use of argon gas for therapeutic use in skin lesions [25].

The use of plasma sterilization technique offers advantages over other methods used, as it is very effective in reducing microbial load, in addition to developing at low temperatures and not using toxic gases, consisting of exposing reactive materials generated by the ionization of a gas, using electromagnetic fields; therefore, it becomes efficient in the process of sterilizing microorganisms [26].

The healing of skin wounds is a complicated process that involves several cells and cytokines, divided into inflammatory, proliferative, and remodeling phases. Due to multiple healing phases, the lesions are affected by internal and external disorders that can lead to chronic wounds, such as pressure injuries, diabetic foot, cancerous ulcers, and post-operative wound infections [27].

However, the plasma jet, when applied to wounds, reduces the bacterial load, providing an improvement in vascularization at the wound site, in addition to the oxygen supply and supply of nutrients, thus promoting healing. According to a literature review, the plasma jet, in addition to exerting bactericidal effects, acts on tumor cells inhibiting cell metastasis, inducing DNA damage, causing death by proliferating and malignant cell apoptosis. The author also mentions that a clinical study of patients with head and neck carcinoma showed an improvement in cancerous ulcerations and a reduction in tumor proliferation after plasma application [27].

The antimicrobial use of plasma in dermatology is of great importance because it is used at low temperatures and allows a direct action on pathogens present in the skin. In a study carried out with pig skin, because it is equivalent to human skin, it was reported that treatment with plasma led to the decolonization of the tested bacteria *Staphylococcus aureus* and *Escherichia coli* without harming the skin of the pig sample [28].

A study carried out in diabetic rats with skin wounds was submitted to the application of the plasma jet, where the healing process would be analyzed, divided into two groups, one with the application of the plasma jet and the other not. The results were positive where it was possible to verify that the wounds were practically closed around the 14th day, the author also reports that the fastest healing happened in the group treated with the plasma jet with an important reduction in the inflammatory process [17].

So this anti-inflammatory action occurs due to the increase in tissue temperature during plasma treatment in conjunction with ultraviolet radiation and gas ionization, resulting in a reduction in the microbial load [29]. Therefore, it is suggested that the plasma jet may be an excellent resource for physiotherapy to assist in the healing process of pressure injuries, reducing the inflammatory and bacterial process, in addition to providing the patient with an improvement in their quality of life and reduction of hospital costs.

5. Conclusions

From the results obtained, it can be concluded that the time of application of the plasma jet has an importance in the antimicrobial action, since in the strains of *Staphylococcus aureus*, growth was inhibited at all times. When correlated with Physiotherapy, one can think of a new treatment method for skin lesions, in order to decrease infections and accelerate the tissue healing process, promoting an improvement in the patient's quality of life, pain relief, besides reducing hospital expenses.

In light of the difficulty of locating plasma jet studies and because it is a technology still under development, additional studies are recommended for further technical-scientific deepening on the subject as well as for a direction for clinical trials followed by a systematic monitoring of at least 6 months of all patients involved.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Moreira, B.L.J., Carvalho, D.M.B.C. and Frota, C.C. (2015) Visualização bacteriana e colorações. UFC, Fortaleza.

[2] Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2010) Microbiologia de Brock. 12 Edition, Artmed, Porto Alegre.

[3] Gonçalves, M.A.P. (2014) Microbiota—Implicações na imunidade e no metabolismo. Ph.D. Thesis, Universidade Fernando Pessoa, Porto. http://bdigital.ufp.pt/bitstream/10284/4516/1/PPG_21951.pdf

[4] Levinson, W. (2016) Microbiologia Medica e Imunologia. AMGH, Porto Alegre.

[5] Brito, K.K.G., Soares, N.J.G.O. and Silva, M.A. (2014) Cuidado de enfermagem nas ações preventivas nas úlceras de pressão. *Revista Brasileira de Ciências da Saúde*, **12**, 56-61. https://doi.org/10.13037/rbcs.vol12n40.2159

[6] Rogenski, N.M.B. and Santos, V.L.C.G. (2005) Estudo sobre a incidência de úlceras por pressão em um hospital universitário. *Revista Latino-Americana de Enfermagem*, **13**, 474-480. https://doi.org/10.1590/S0104-11692005000400003

[7] Araujo, T.M., *et al.* (2011) Diagnósticos de enfermagem para pacientes em risco de desenvolver úlcera por pressão. *Revista Brasileira de Enfermagem*, **64**, 671-676. https://doi.org/10.1590/S0034-71672011000400007

[8] National Pressure Ulcer Advisory Panel (2016) NPUAP Pressure Injury Stages. Washington DC. http://www.npuap.org/resources/educational-and-clinical-resources/npuap-pressure-injury-stages/

[9] Brasil Ministério da Saúde and Agência Nacional de Vigilância Sanitária (2013) Annex 02: Protocolo para Prevenção de Úlcera por Pressão. Brasília.

[10] TelessaúdeRS-UFRGS (2017) Núcleo de Telessaúde da Universidade Federal do Rio Grande do Sul. Porto Alegre. https://www.ufrgs.br/telessauders/ https://doi.org/10.22456/0104-6594.69185

[11] Lim, K., Yoon, C. and Lee, J. (2017) Antimicrobial Effect of Skin for Allograft and Management in Burn Wound. *Open Journal of Organ Transplant Surgery*, **7**, 1-11. https://doi.org/10.4236/ojots.2017.71001

[12] NCCLS (2003) Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eighth Edition. NCCLS Document M2-A8.

[13] Kennedy, M. (2004) A Brief History of Disease, Science and Medicine: From the Ice Age to the Genome Project. Asklepiad Press, California.

[14] Andrade, R.F. and Folchini, N.F. (1998) Úlcera de pressão [monografia]. Universidade Católica Dom Bosco—UCDB, Campo Grande.

[15] Oliveira, J.R. (2014) Biofísica para ciências Biomédicas. 2 Edition, ARTMED, Porto Alegre.

[16] Pinto, E.K.M. (2018) Desenvolvimento de um Dispositivo de Jato de Plasma para o tratamento de cancro da pele. Ph.D. Thesis, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Lisboa.

[17] Cheng, K., *et al.* (2018) Wound Healing in Streptozotocin-Induced Diabetic Rats

Using Atmospheric-Pressure Argon Plasma Jet. *Scientific Reports*, **8**, Article No. 12214. https://doi.org/10.1038/s41598-018-30597-1

[18] Bren, H. and Lyder, C. (2004) Protocol for the Successful Treatment of Pressure Ulcers. *American Journal of Surgery*, **188**, 9-17.

[19] Arjunan, K.P., Sharma, V.K. and Ptasinska, S. (2015) Effects of Atmospheric Pressure Plasmas on Isolated and Cellular DNA—A Review. *International Journal of Molecular Sciences*, **16**, 2971-3016. https://doi.org/10.3390/ijms16022971

[20] Kong, M.G., *et al.* (2009) Plasma Medicine: An Introductory Review. *New Journal of Physics*, **11**, Article ID: 115012. https://doi.org/10.1088/1367-2630/11/11/115012

[21] Calixto, C.M.F. and Cavalheiro, E.T.G. (2012) Penicilina: Efeito do acaso e momento histórico no desenvolvimento científico. *Química Nova na Escola*, **3**, 118-123.

[22] BrCAST/EUCAST (2019) Manual de Antibiograma 2019—Segundo BrCAST/EUCAST Laborclin Produtos para Laboratórios Ltda.

[23] Borges, A.C. (2016) Atividade antifúngica e citotoxicidade do jato de plasma frio sob pressão atmosférica. Ph.D. Thesis, Pós-graduação em Biopatologia Bucal—Universidade Estadual Paulista (UNESP), Instituto de Ciência e Tecnologia, São José dos Campos.

[24] Metelmann, H.R., von Woedtke, T., Bussiahn, R., Weltmann, K.D., Rieck, M., Khalili, R., Podmelle, F. and Waite, P.D. (2012) Experimental Recovery of CO_2-Laser Skin Lesions by Plasma Stimulation. *American Journal of Cosmetic Surgery*, **29**, 52-56. https://doi.org/10.5992/AJCS-D-11-00042.1

[25] Brasil, H.A. (2007) Avaliação da aplicação clínica da coagulação com plasma de argônio na ablação do esôfago de Barrett. Faculdade de Medicina, Universidade de são Paulo, São Paulo.

[26] Fricke, K.J., *et al.* (2012) Atmospheric Pressure Plasma: A High-Performance Tool for the Efficient Removal of Biofilms. *PLOS ONE*, **7**, e42539. https://doi.org/10.1371/journal.pone.0042539

[27] Gan, L., Zhang, S., Poorun, D., Liu, D., Lu, X., He, M., *et al.* (2017) Medical Applications of Nonthermal Atmospheric Pressure Plasma in Dermatology. *Journal der Deutschen Dermatologischen Gesellschaft*, **16**, 7-13. https://www.ncbi.nlm.nih.gov/pubmed/29211323

[28] Tiede, R., Hirschberg, J., Daeschlein, G., vonWoedtke, T., Vioel, W. and Emmert, S. (2013) Plasma Applications: A Dermatological View. *Contributions to Plasma Physics*, **54**, 118-130. https://onlinelibrary.wiley.com/doi/abs/10.1002/ctpp.201310061

[29] Landemann, O., Richter, H., Patzelt, A., Alborova, A., Humme, D., Weltmann, K.D., Hartmann, B., Hinz, P., Kramer, A. and Koch, S. (2010) Application of a Plasma-Jet for Skin Antisepsis: Analysis of the Thermal Action of the Plasma by Laser Scanning Microscopy. *Laser Physics Letters*, **7**, 458-462. https://doi.org/10.1002/lapl.200910158