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Investigation of Microbiological Effects of Atmospheric Pressure Cold Plasma on Hand Disinfection

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Abstract

Aim: Atmospheric pressure cold plasma is the fourth state of matter obtained at low temperature with atmospheric pressure, which has antimicrobial, anti-inflammatory, sterilization, disinfection and surface modification enhancing properties. The aim of this study is to investigate the effectiveness of plasma activated medium (PAM) obtained from atmospheric pressure cold plasma on hand disinfection.

Methods and Materials: The study was carried out on 15 individuals aged between 18 and 65 with healthy hand structures (no wounds, no pathology). Of the 15 individuals, 5 were selected as cleaning staff, 5 as office stuff, and 5 as academics. Samples were first taken from individuals using swap for bacterial identification. Afterwards, they were asked to wash their hands for 5 minutes in 250 ml PAM. At the end of the period, samples were taken from the individuals in the same way for bacterial identification using swap. The effectiveness of PAM in terms of hand disinfection was determined by comparing these two samples.

Results: As a result of our study, it was found that bacterial colonies decreased in 3 of the samples taken from the hands of 5 cleaning personnel, remained stable in 2, a decrease in bacterial colonies in 4 of 5 office stuff, remained constant in 1, a decrease in bacterial colonies in 3 of 5 academicians, and remained stable in 2 of them. *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus megaterium, Staphylococcus capitis, Streptococcus mitis, Staphylococcus warneri* bacteria were identified on the hands of individuals.

Conclusion: As a result of our study, it was found that PAM, which has no side effects in the literature, could be an effective tool for hand disinfection.

Keywords: Plasma, Hand disinfection, Microbiological Efficacy, Plasma activated environment.

Introduction

Materials in the universe are found in four states: solid, liquid, gas, and plasma. The history of plasma dates back to the 300s BC. At that time, Aristotle proposed that there were three states in the universe (solid, liquid, and gas) but that fire (plasma) was a different state. In 1929, Nobel laureate Irving Langmuir first named fire (plasma) as plasma. Its current meaning was first used by British chemist Sir William Crooke [1–4].

Plasma is the fourth state of matter that contains electrons, ultraviolet photons, positive and negative ions, neutral atoms, and neutral particles. Plasma is a state of matter that we frequently encounter in

nature, industry, and healthcare. Lightning, flames, fluorescent lamps, welding machines, and plasma pens in beauty centers are some of the best examples. Plasma is formed when gas is passed through direct or alternating current. During formation, depending on the type of gas, reactive species and free radicals emerge [1, 3–6].

Plasma has a wide range of medical applications. There are two main methods of obtaining plasma in medical use. The first is dielectric barrier discharge, which was first introduced by Siemens in 1857. It is obtained by passing gas between the high-voltage electrode made of dielectric material and the ground electrode. The second method is the plasma jet obtained by passing gas between electrodes. If the plasma jet is obtained under atmospheric pressure and the gas temperature is lower than 1000 K, it is called atmospheric pressure cold plasma jet. [6–10].

Plasma jet can be used directly or indirectly. Indirectly, the substance obtained by applying plasma to water, 0.9% NaCl solution, phosphate-buffered saline, and organic matter solutions is called a plasma-activated media (PAM) [11, 12].

In the medical field, plasma's anti-cancer, anti-inflammatory, antimicrobial, sterilization, and disinfection effectiveness is a heavily researched topic. Plasma exhibits antimicrobial activity by damaging the DNA and membrane of microorganisms. This feature was first discovered by Laroussi et al. in 1996. In later years, it was shown to affect many microorganisms such as bacteria, viruses, and fungi [4, 5, 13–16].

The aim of this study is to investigate the microbiological effectiveness of PAM produced from atmospheric pressure cold plasma jet, which has no side effects, on hand disinfection.

Materials and methods

Population sample and Ethical permissions

The study was conducted on 15 healthy individuals aged between 18–65 years with no injuries about hand, surgical interventions or pathologies. The individuals were divided into three separate groups according to their occupations. The first group consisted of academicians (n=5), the second group consisted of office stuff (n=5) and the third group consisted of cleaning staff (n=5). The study was carried out with the decision of the local ethics committee of İzmir Bakırçay University dated 28.09.2022 and numbered 718. The study was carried out in accordance with the Declaration of Helsinki.

Statistical analysis

Visualization of the data used in the study and obtaining the percentages were made using Microsoft Office 365 Excel and Powerpoint (2023, USA) programs.

Study design

Firstly, a system was created for the plasma jet. The system was obtained using an oscilloscope, high voltage power supply, computer, optical emission spectrometry, ground electrode, high voltage electrode, plasma pen, flowmeter, argon gas, argon gas cylinder, and flow control valve. The obtained plasma jet was applied to distilled water in a petri dish for 15 minutes and PAM was obtained (Figure 1). To perform the optical emission analysis of cold argon plasma obtained at atmospheric pressure, Thunder Optics brand SMA-E model and optical emission spectrometer with a wavelength measurement range of 350–920 nm were used. In order to obtain high resolution results during plasma formation, the fiber optic cable was placed at a distance of 1 cm to see the center of the plasma jet. After converting the results from the spectrometer device into a graphic form, the emission line graph was obtained. NIST Atomic Spectra Database was used to define emission lines.

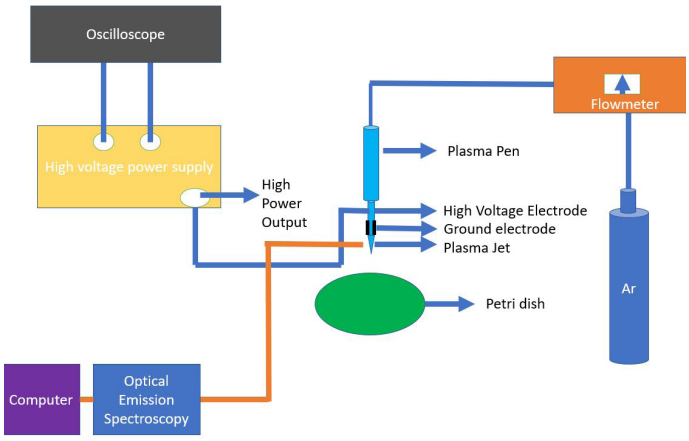

Figure 1 – Plasma assembly

After obtaining PAM, samples were taken from individuals using a swab for microbiological identity. Later, individuals were asked to wash their hands in a container for 5 minutes. After washing, samples were taken again from individuals' hands for bacterial identification.

Samples were inoculated onto 10 µL of 5% sheep blood agar, Eosin Methylene Blue (EMB) agar, and chocolate agar using a loop, and incubated at 37°C for 24–48 hours. Conventional methods and the Phoenix™ fully automated system (Becton Diagnostics, USA) were used for the identification of microorganisms that grew on the culture media.

Results

Staphylococcus epidermidis, Staphylococcus warneri, Staphylococcus capitis bacteria were identified in the group of academicians involved in the study. *Staphylococcus epidermidis, Staphylococcus capitis, Streptococcus mitis, Staphylococcus warneri* were identified in the tong office stuff group. *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus megaterium bacteria* were identified in the cleaning staff group. It was found that the bacterial colonies decreased in 3 of the samples taken from the 5 cleaning personnel in the study, remained stable in 2 of them, bacterial colonies decreased in 4 of the 5 office stuff, remained stable in 1, a decrease in the bacterial colonies in 3 of the 5 academicians, and remained stable in 2 of them (Figure 2).

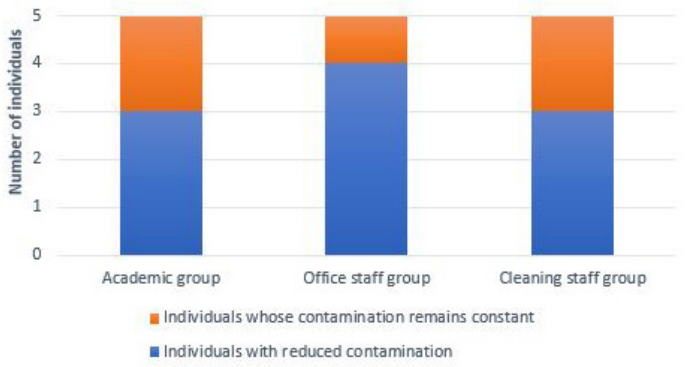

Figure 2 – Contamination rate

The bacterial colony counts obtained from the first and second samplings are shown in Table 1. It was observed that the colony count was higher in the cleaning staff group and the decrease due to PAM was the highest there.

Table 1 Number of bacterial colonies obtained as a result of the first and second sampling

Samples	Reproducing Bacteria	Number of Colonies
CS 1-1	Staphylococcus aureus	50
CS 1-2	Staphylococcus aureus	50
CS 2-1	Staphylococcus epidermidis	1.000
CS 2-2	Staphylococcus epidermidis	35
CS 3-1	Bacillus megaterium	10.000
CS 3-2	Bacillus megaterium	1.000
CS 4-1	Staphylococcus epidermidis	50.000
CS 4-2	Staphylococcus epidermidis	200
CS 5-1	Bacillus megaterium	10.000
CS 5-2	Bacillus megaterium	10.000
OS 1-1	Staphylococcus epidermidis	300
OS 1-2	Staphylococcus epidermidis	50
OS 2-1	Staphylococcus capitis	20
OS 2-2	Staphylococcus capitis	20
OS 3-1	Staphylococcus epidermidis	20
	Streptococcus mitis	20
OS 3-2	Staphylococcus epidermidis	4
	Streptococcus mitis	1
OS 4-1	Staphylococcus warneri	60
OS 4-2	Staphylococcus warneri	6
OS 5-1	Staphylococcus epidermidis	10
OS 5-2	No reproduction	0
A 1-1	Staphylococcus epidermidis	100
A 1-2	Staphylococcus epidermidis	100
A 2-1	Staphylococcus warneri	15
A 2-2	Staphylococcus warneri	15
A 3-1	Staphylococcus capitis	15
A 3-2	Staphylococcus capitis	3
A 4-1	Staphylococcus epidermidis	200
A 4-2	Staphylococcus epidermidis	100
A 5-1	Staphylococcus epidermidis	10
A 5-2	Staphylococcus epidermidis	2

CS: Cleaning staff, OS: Office staff, A: Academician

The maximum decrease in colony count was observed in the individual of group CS 4-1 (the colony count was found to be 50,000 before PAM and 200 after PAM) (Figure 3).

Figure 3 – Blood agar image of maximum colony reduction

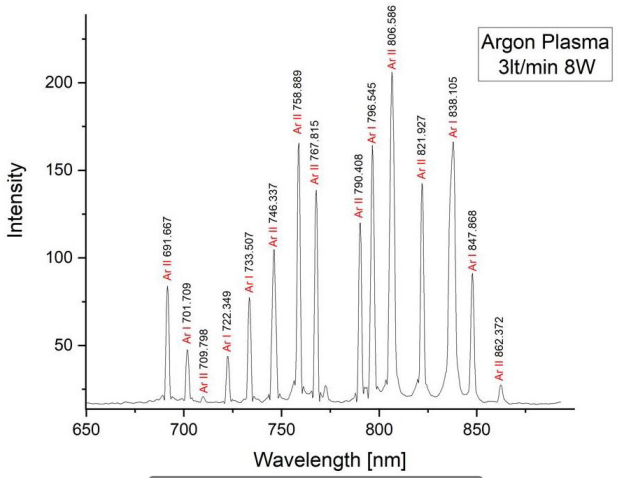

Figure 4 – Atmospheric pressure cold plasma jet spectrometer values

The spectrometer values obtained as a result of atmospheric pressure cold plasma produced from argon gas are shown in Figure 3. In the figure, the emission lines of argon at different wavelengths are shown and Argon I and II. degrees of ionization were determined.

Discussion

In this study investigating the effectiveness of PAM obtained from cold plasma on hand disinfection, it was found that microbial contamination associated with PAM decreased in the hands of 3 out of 5 cleaning personnel, 4 out of 5 desk officers, and 3 out of 5 academics.

In this study, the effectiveness of PAM obtained from cold plasma on hand disinfection was investigated. Reactive species, UV radiation, free radicals, ionized gases in cold plasma at atmospheric pressure cause damage to the DNA and cytoplasm of bacteria, viruses and fungi, resulting in antimicrobial activity [5, 15]. It has been reported in the literature that atmospheric pressure cold plasma exhibits antimicrobial, anticancer, and anti-inflammatory activity, while having minimal effect on healthy tissues in individuals, and this effect can be ignored. In short, there are no side effects of atmospheric pressure cold plasma [15, 17–19].

Daeschlein et al. [20] reported that cold plasma showed antimicrobial activity against Escherichia coli, Candida albicans, Pseudomonas aeruginosa, Lancefield, Klebsiella, Staphylacoccus aureus, Proteus, Acinetobacter spp., Stenotrophomonas spp., Enterococcus faecalis, and Staphylococcus epidermidis. The study also revealed that cold plasma could be an alternative to antiseptics. In another study, Daeschlein et al. [21] reported that antifungal activity was observed without resistance against Candida albicans, Trichophyton interdigitale, Microsporum canis, and Trichophyton rubrum. Isbary et al. [22] reported a high clinical improvement in patients with herpes zoster and varicella zoster viruses within 2 days after plasma treatment in their study.

Hand hygiene is critical for preventing the transmission of microorganisms and disease. Studies on hand hygiene date back to 1822. In 1822, a French pharmacist suggested that a solution of chlorinated lime could be used for disinfection. In 1843, Holmes stated that the hands of healthcare workers had a significant effect on the spread of puerperal fever. Three years later, Semmelweis stated that handwashing of healthcare workers could reduce sepsis and mortality and prevent puerperal fever.

Different solutions and substances are used for hand hygiene, such as soap, hexachlorophene, alcohol, and chlorhexidine [23–25].

Hand hygiene is critical in preventing hospital-acquired infections, fecal-oral transmission, reducing microorganism contact, and preventing infections [26]. Hospital infections have emerged as an important health problem especially in the last 30 years [27]. The use of antiseptic solutions for hand hygiene dates back to the 19th century. From the 19th century to the present, different antiseptic solutions have been preferred for hand hygiene. Chlorinated lime solutions, alcohol-based solutions, chlorhexidine-containing solutions, phenol-containing solutions, and iodine-containing solutions have been used as antiseptic solutions [28]. However, these solutions have different disadvantages as well as advantages [23–25].

The antimicrobial activity of atmospheric pressure cold plasma has been tested in the literature using jets. It was found that PAM, which had no side effects, showed antimicrobial activity. We believe that this study, which is original in this respect, will guide the future studies.

Limitations

Is there a solution for hand hygiene that has no side effects? This study, which started with the hypothesis, was planned with a small sample; Due to the high cost in the production process of PAM, the number of samples was limited, which is the limitation of the study.

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Informed Consent: A voluntary consent form was obtained.

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