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NEW METHODS OF INACTIVATION OF SOME PATHOGENIC AGENTS IN DENTAL CLINICS. REVIEW

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Abstract:

The interior space of a cabinet of this type is composed of the air in the rooms, the contact surfaces (walls, ceiling, lighting fixtures, floor, doors, windows, furniture, equipment), instruments and dental materials being exposed to various pathogens that must be kept under strict control through various accredited decontamination methods. Most dental treatments are aerosol-generating procedures that produce a mixture of sprays, droplets, and aerosols containing saliva, blood, water, and viable microorganisms (including bacteria, fungi, and viruses). Chemical agents - antiseptic biocidal products and chemical disinfectants used in sanitary facilities must have a bactericidal, fungicidal, mycobactericidal, virucidal and sporicidal effect, depending on the purpose of use. The interaction between the chemical compounds of disinfectants and microbial cells can cause a serious reaction to public health and sanitary security. The risk factor intensifies the probability of selection and dissemination of multidrug resistance among pathogenic bacteria. The use of a UVC air decontamination device that is safe for human exposure could provide the desired antimicrobial benefits without accompanying human health concerns raised by conventional UVGI germicidal lamps.

Keywords: *pathogenic agents, dental clinics, decontamination methods, antiseptic biocidal, physical agents.*

1. Introduction

Given that, according to the Ministry of Health, the space in which a dental office operates is an interior space, a closed environment, prone to contamination through the influx of patients but also of medical personnel, as well as the doctoral research topic through which I propose an interdisciplinary approach for conducting experimental research on the effect of some decontamination agents including UVC on some pathogenic species frequently found on surfaces and aerial microflora in this space and testing the effectiveness of new ways of decontamination, this report includes data from the literature on decontamination methods and agents used for this type of space.

The use of *physical agents* involves the inactivation of pathogens by high dry or wet temperature and high pressure but also exposure to UV-UVC radiation.

An aeromicroflora decontamination device that uses UVC light radiation with LED technology is clearly superior to the conventional tube system, which provides assurance of the device's effectiveness. LED technology provides a stable and self-adjusting frequency and intensity for maximum efficiency at the 254 nm wavelength.

A UVC irradiation in a closed, UVC-resistant controlled environment

allows human presence during device use and thus ensures continuity of activity without the need for breaks for decontamination.

Chemical agents - antiseptic biocidal products and chemical disinfectants used in sanitary facilities must have a bactericidal, fungicidal, mycobactericidal, virucidal and sporicidal effect, depending on the purpose of use.

Most dental treatments are aerosol-generating procedures that produce a mixture of sprays, droplets, and aerosols containing saliva, blood, water, and viable microorganisms (including bacteria, fungi, and viruses) [1]. Frequently used dental instruments, rotating handpieces and ultrasound equipment, generate a high potential of contaminated dispersion particles that pose a high risk to professionals and patients [2,3].

These microparticles are invisible to the eye, therefore their spatial distribution within the clinical environment is neglected, therefore the development of better ways to mitigate the risk of disease transmission is of great importance.

Contaminated dispersion particles generated during appointments can stay in the air for less time (droplets, 5–100μm) or longer (aerosols, 5μm) and they fall on the surfaces of the environment under the influence of gravity, following a ballistic trajectory from the point of origin.

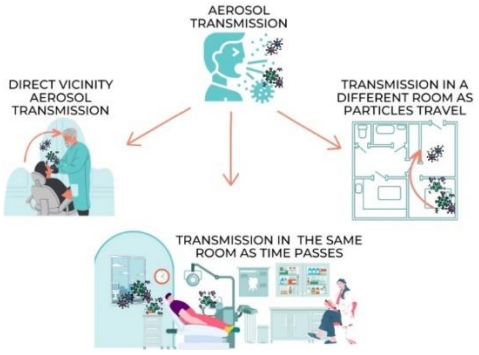

Fig. 1. Potentially contaminated dispersion particles in dental office.

Studies show that droplets can remain suspended in the air until the water evaporates, and aerosols can remain suspended for several hours and disperse over several meters from the source of origin [4, 5].

Much more attention has been placed on dental aerosol generation procedures due to Covid-19 [6] especially when people are close to each other, since it has been proven that Covid-19 spreads through airborne transmission. [7-9]

Wang et al. draw attention to microorganisms that are potentially contagious for dentists operating near the face and oral cavity, especially when potentially contaminated dispersion particles are generated, [10-12] hepatitis B and C virus, HIV (human immunodeficiency virus), as well as SARS-CoV-2. [8,9,13]

The latter can remain infectious in aerosols for long periods, even when the water evaporates, on surfaces they can remain infectious for up to 72 hours.[14,15]

It is of considerable interest to have methods to reduce the dispersion of sprays/droplets/aerosols during procedures.

Montalli et al. tested the Individual Biosafety Barrier in Dentistry, a biosafety device, used to reduce the dispersion of droplets and aerosols generated during working hours, reducing the number of colony-forming units by 95% [16], the number of bacterial colonies [17] and other fluorescent markers to show the distribution of ejected material in general. [18,19]

While medical environments are regularly cleaned and disinfected using manual techniques (detergents, alcohols, etc.), evidence suggests that adequate cleaning often does not ensure effective decontamination, especially when the focus is only on surfaces perceived as high risk, as they are frequently reached.[20]

Inadequate cleaning using manual techniques has led to the development of touch-free systems that can decontaminate objects and surfaces in the patient's

environment, [21] among these technologies are found those that use ultraviolet (UV) light. [22,23]

Automatic UV disinfection devices that continuously emit UV-C in the 254 nm gamma wavelength range have been used in medical environments for the purpose of decontaminating. Some of these systems can reduce the microbial load of the environment by up to 4 log.[24] However, there are no established standards of effectiveness for UV devices.

UV-C disinfection technology can be used to supplement manual cleaning and has recently become an acceptable non-touch decontamination method in medical facilities and is now routinely used.[25]

2. Possibilities of aeromicroflora decontamination.

The mandatory steps for an activity aimed at inactivating pathogens are represented by:

- cleaning, the mandatory, permanent and systematic preliminary step in any procedure for removing organic and inorganic matter from surfaces or objects, through mechanical or manual operations, using physical or chemical agents;

- disinfection, the procedure for destroying the majority of pathogenic or non-pathogenic microorganisms on any surfaces, using physical or chemical agents;

Chemical agents - antiseptic biocidal products and chemical disinfectants used in sanitary facilities must have a bactericidal, fungicidal, mycobactericidal, virucidal and sporicidal effect, depending on the purpose of use. Chemicals commonly used to decontaminate premises include alcohol, formaldehyde, chlorine, hydrogen peroxide and phenols. The use of a decontaminating agent should depend on the target and the device used to spread/spray it. Correct application and use of the appropriate method increase effectiveness and reduce the risk of contamination of staff and patients.

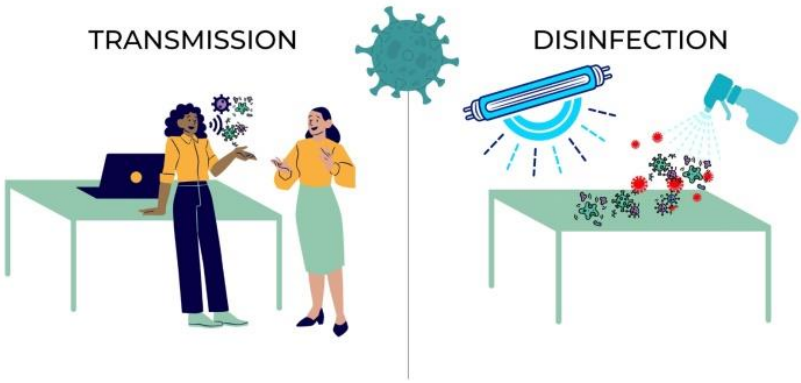

Fig.2. Possibilities of aeromicroflora decontamination in Dental Clinics.

As documented in the scientific literature, inadequate asepsis of medical offices can lead to an increase in infections and diseases.[26]

Table 1. The main decontamination agents used in medical practices

Decontaminant	Advantages	Disadvantages
Hydrogen peroxide / Oxygenated water	• No activation required • Increases the efficiency of organic matter removal • Can be easily removed from the surface • Odourless, does not irritate the skin • Bactericidal, virucidal and fungicidal	• Requires stabilization (chemical or with plasma) • Possible reaction with surfaces coated with brass, zinc, copper and silver • Irritates the eyes

	properties • Scientifically proven effectiveness	
Gas - ozone	• Bactericidal, virucidal and fungicidal properties • Strong disinfectant effect at a concentration of 13 μg/dm^3 • Breaks down into oxygen (O_2)	• Damages plastic and rubber • Bad smell • Ozone irritation can cause coughing, sore throat, drowsiness and headache
Chlorine compounds	• Bactericidal, virucidal and fungicidal properties	• Damages plastic and rubber • Causes metal corrosion • Unstable, its distribution accelerates light and heat • Antagonistic effects with detergents and formaldehyde • Irritates the skin, conjunctiva and respiratory tract
Acid peroxide 0.2%	• Ecological by-products (acetic acid, O_2 and H_2O) • May improve removal of organic matter • Easy to remove from the surface • Strong fungicidal effect	• May damage/stain aluminium surfaces • Possibility of serious damage to the eyes and skin through contact with the undiluted solution
Ortho-phthalaldehyde 0.55%	• Fast disinfection effect • No activation required • Slight smell • Can be easily removed from the surface	• Leaves stains on skin, mucous membranes, clothes and surfaces • Big price • Eye irritation • Slow fungicidal action
Glutaraldehyde > 2%	• Scientifically proven action • Low price • Bactericidal, virucidal and fungicidal properties	• Irritates the respiratory tract • Pungent, irritating smell • Adheres to the surface • Allergic contact dermatitis
Phenolic compounds 1.5-5%	• Bactericidal and virucidal properties	• Weak fungicidal effect • May damage porous surfaces • Irritating to skin and eyes

Many studies have emphasized the validity of decontamination of room surfaces and air using various devices/methods, including ventilation systems, ultraviolet lamps, high volume exhaust, automatic decontamination systems with hydrogen peroxide vapor (fumigation). [27-29]

The air ozonizer is a device, in which ozone is formed under the influence of electric current, which has a strong decontaminating effect. Ozonators completely eradicate bacteria, viruses, fungi and their spores.

However, the disadvantage of air ozonizers, which are made of ceramic plates, is that they form nitrogen oxides causing a destruction and discoloration effect on plastic and rubber elements. On the other hand, the advantage of using ozonation results in a strong decontamination of the air, the removal of inhalation allergens and unpleasant odors.

Ozone generators cannot be used in conjunction with other decontamination devices such as UV lamps. Choosing the appropriate ozone dose for the size of the room is particularly crucial, as disproportionate ozone saturation can oxidize medical devices and equipment, leading to their damage.

The average use of room decontamination with the ozonizer takes approximately 4 to 5 hours, with a waiting time after ozonation of at least 2 hours before re-entering the room. It is also recommended to ventilate the office after ozonation. [30-32]

Table 2. Decontaminate and disinfection with ozone therapy.

Author, Year	**Study**	**Result**
Moat et al.[33] 2009	The effectiveness of the ozone gas approach was evaluated for room sanitization	Applying the process to a 30 m^3 room shows similar reductions in viable counts for Clostridium difficile, Escherichia coli and methicillin-resistant Staphylococcus aureus spores
Hudson et al. [34] 2009	Develops a practical method of using the known antiviral properties of ozone in a mobile device that could be used to decontaminate rooms in health facilities	All 12 viruses tested, on various hard and porous surfaces and in the presence of biological fluids, could be inactivated by at least 3 log10, in laboratory and simulated field tests
Rowen,[35] 2019	Ozone therapy, the most studied and least expensive to perform, is itself a germicide, not an antibiotic, and improves several physiological parameters essential for defense against infections	Very favorable responses to both bacterial and viral diseases including Ebola. Despite the lack of commercial profitability (it is not patentable), the drug would do well to review its preantibiotic, the basis of oxidation therapy, especially ozone in the current crisis
Hudson et al. [36] 2007	The ability of ozone gas to inactivate norovirus and its animal surrogate, feline calicivirus (FCV) in dry samples placed at different locations in a hotel room, a cruise cabin and an office	QRT-PCR tests indicated similar decreases in both viral RNAs. Samples containing dried virus on hard surfaces (plastic, steel and glass) and soft surfaces such as textile, cotton and carpet were equally vulnerable to treatment
Miller et al. [37] 2018	Acute inhalation of ozone induces DNA methylation of apelin in the lung and whether a change in expression is related to altered DNA methylation in the lung	Ozone exposure reduced DNA cytosine-5-methyltransferase activity and Dnmt3a/b gene expression. Epigenetic changes accompanied the ozone-induced reduction of apelin expression and the development of pulmonary edema
Ding et al. [38] 2019	Disinfection with chlorine-resistant ozone of bacteria in drinking water	Ozone resistance of Aeromonas jandaei bacteria < Vogesella perlucida < Pelomonas < Bacillus cereus < Aeromonas sobria was smaller than that of Bacillus alvei spores < Lysinibacillus fusiformis < Bacillus cereus at an ozone concentration of 1.5 mg/L. More than 99.9% of Bacillus cereus spores were inactivated by increasing ozone concentration and treatment duration

Fumigation or steam equipment can use different substances for spraying, such as hydrogen peroxide, chlorine dioxide, and a mixture of peracetic acid and hydrogen peroxide. Hydrogen peroxide generators are widely used for medical environment decontamination. Hydrogen peroxide can be applied as aerosols or steam to inactivate Staphylococcus aureus and Pseudomonas aeruginosa.[39]

Aerosol hydrogen peroxide generators typically use 3-7% concentration of hydrogen peroxide with or without silver ions. They contain H_2O_2 particles with variable sizes from 2 to 12 μm, which show a great bactericidal and virucidal property that inactivates Francisella tularensis. [40]

In turn, hydrogen peroxide generators in a kind of steam use hydrogen peroxide in the so-called "dry form" (a hand placed on the nozzle of the generator at 1 m should remain dry during operation). Different concentrations of H_2O_2 are applied, for example 30% hydrogen peroxide, because it can eliminate several pathogens, such as: Mycobacterium, tuberculosis, Mycoplasma, Acinetobacter,

Clostridium difficile, Bacillus anthracis, viruses but also prions.

According to the studies of Meszaros et al., they were proved to have a very high bactericidal and virucidal effect. [41] Short asepsis procedure of about 10 minutes on average and short ventilation time after fumigation of about 30 minutes (time depends on fumigant concentration), strong decontamination effect, safe for medical and electronic devices.[42-44]

Low concentrations of hypochlorous acid (HOCl) have been shown to eradicate both the influenza virus and antibacterial activity. HOCl has a temporary and mild chlorine odour that dissipates immediately. HOCl is also used to kill rhinovirus,[45] human norovirus (HRV) but also Avian Influenza (H5N1).[46]

The UV germicidal lamp is the result of low pressure mercury discharge (germicidal radiation). During the use of these devices, a UV electromagnetic wave is generated with a length between 250 and 270 nm. The bactericidal mechanism of UV lamps through DNA damage is evoked. UV radiation eliminates or limits the ability of organisms to reproduce. The maximum of bactericidal effectiveness for UV is around 265 nm. Disinfection lamps should be selected according to the size of a dental office, for example, for 15-18 m^2, two 30 W lamps should be used.

In addition to using the classic bactericide, UVC lamps recently available on the market are used, in which the contaminated air is sucked in by a fan and prefiltered, the air is transferred to a decontamination chamber where it is subjected to irradiation [47].

UVC light radiation used in public locations can represent a safe and effective methodology for limiting the transmission and spread of airborne and surface-mediated microbial diseases. In fact, the potential use of ultraviolet light for aerial disinfection is by no means new and was demonstrated more than 80 years ago.

A UVC irradiation in a closed, UVC-resistant controlled environment allows human presence during device use and thus ensures continuity of activity without the need for breaks for decontamination.

Air filtration systems with ULPA and HEPA filters capture particles from the air stream, but HEPA filters initially retain larger amounts of bacteria. However, after a while, it is enough for a single bacterium to be released for it to start multiplying. The ULPA filter with much smaller orifice sizes requires more pressure and energy to run the system. HEPA filtration is often used in combination with other technologies. By retaining pathogens (particles larger than 0.3 μm) a localized biological hazard is created, which is why they must be replaced regularly. [48]

Electrostatic filtration uses an electric current to transfer a positive or negative charge to solid particles and microorganisms in the air. Afterwards, they are passed through an electrostatic filter with opposite charge. [49,50]

While ionizers can remove various contaminants from the air, some of them remain in the room. Impurities, when attached to a negative particle, settle on the walls and floor. Over time, ionizers can cause the so-called black wall effect, that is, they change the color of walls and furniture to gray. Particles combined as a result of the action of the ionizer can remain on the surface of the ventilation ducts.[51]

In a study, Grinshpun et al. tested five different ionic indoor air purifiers and evaluated their ability to reduce indoor aerosol exposure.

The authors reported that "unipolar ionic air purifiers effectively reduce aerosol exposure in the breathing zone when used in confined spaces (car interior, airplane seats, bathrooms, offices, small living spaces).

Grabarczyk's study concluded that this technology should be considered for use in small enclosed spaces and is not suitable for larger spaces.[52]

A closed space differs as a medium of use but mostly by the volume of air it occupies. An aeromicroflora decontamination device that can be configured capacitively on the air volume of the targeted closed environment has certain effectiveness.

Once it has been calibrated and tested for effectiveness by aeromicroflora samples before and after different periods of time and operation, in different infectious medical environments, 53-56] it can decontaminate with certainty.

3. The impact of some frequently used decontamination agents on microorganisms, bacteria, viruses and spores.

Although microbiologists have been working for more than a century on problems associated with disinfection, the understanding of the mode of action of the active molecule remains vague with many hypotheses.

The cell wall, cytoplasmic membrane and cytoplasm are the regions through which the bacterial cell interacts with the biocide, access being concentration-related through bacteriostatic or bactericidal effect and specific physiological or biochemical changes.

The biocide, decontaminant, complex formulation of active molecules, sometimes also containing co-solvents, chelating agents, acid or alkaline agents or surface-active or anti-corrosive products, can act on microorganisms in two different ways: by inhibiting growth (bacteriostasis, fungistasis) or a lethal action (bactericidal, fungicidal or virucidal effects).

The antimicrobial action of alcohol is achieved by denaturing proteins, destroying dehydrogenases in *Escherichia coli* and increasing the lag phase of *Enterobacter aerogenes*.

Chlorine in low concentrations has a biocidal effect on mycoplasma and vegetative bacteria and in high concentrations it can inactivate *M. tuberculosis, Clostridium difficile spores and B. atrophaeus spores*.

Exposure of strains of *Escherichia coli, Pseudomonas spp., and Staphylococcus spp*. to lethal doses of hypochlorous acid causes a decrease in ATP production.

Formaldehyde in aqueous solutions inactivates a wide range of microorganisms, poliovirus, *M. tuberculosis Salmonella Typhi* implicitly spores of *B. anthracis*, by alkylating the amino and sulfhydric groups of proteins and nitrogen atoms of the purine base cycle.

Glutaraldehyde in aqueous solutions inactivates vegetative bacteria, *M. tuberculosis*, fungi, and viruses and spores of Bacillus and Clostridium species by alkylating sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis. Quaternary ammonium compounds (QACs) bind irreversibly to phospholipids and membrane proteins, thereby affecting permeability.

They have antimicrobial activity on Gram-positive and Gram-negative bacterial strains, inhibitory effect on *Pseudomonas spp.*, *Bacillus spp*. (due to the presence of lipoproteins and liposaccharides). Benzalkonium chloride makes the cell more permeable, a phenomenon observed in Enterobacter cloacae.

Hydrogen peroxide is active against a wide range of microorganisms including bacteria, yeasts, fungi, viruses and spores by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA and other essential cellular components. [12,56]

Iodophors are tuberculocidal, fungicidal, bactericidal, mycobactericidal and virucidal by disrupting protein and nucleic acid structure and synthesis.

The decontamination of tools and workstation surfaces against microbial contamination and the ineffectiveness of environmental decontamination could be risk factors for cross-infection.

Asepsis of surfaces is a method of reducing the risk of coming into contact with viruses and interrupting their spread.[57,58]

4. Conclusions

1. Many studies have emphasized the validity of decontamination of room surfaces and air using various devices/methods, including ventilation systems, ultraviolet lamps, high volume exhaust, automatic decontamination systems with hydrogen peroxide vapor (fumigation)
2. Chemical agents - antiseptic biocidal products and chemical disinfectants used in sanitary facilities must have a bactericidal, fungicidal, mycobactericidal, virucidal and sporicidal effect, depending on the purpose of use.
3. The use of a UVC air decontamination device that is safe for human exposure could provide the desired antimicrobial benefits without accompanying human health concerns raised by conventional UVGI germicidal lamps.
4. LED technology offers a series of advantages such as: low electricity consumption, a longer lifespan, the advantage of self-adjustment of the operating frequency, but also the guarantee of effectiveness during the entire operating period.

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