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Photoeradication of Pathogens Through the Irradiation of Superimposed Wavelengths of the Visible Spectrum

Kinetics of Photodynamic Eradication with Visible Light of Pathogenic Microorganisms and Guidelines for the Safe Control of Microbial Proliferation in Human Frequented Environments

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Abstract

It is proven that visible light (VIS) produces a photodynamic microbicidal effect that determines the eradication of bacteria and viruses, in particular with peak wavelengths in the band of the absorption spectrum of the porphyrin molecules found within the microbial cells and which, by exciting these endogenous porphyrins, generate Reactive Oxygen Species (ROS) for photolithotrophic and photoorganotrophic reactions, or Reactive Species of Metals (RMS) for autotrophy, causing oxidative damage to the microbial cell membrane and, therefore, its death.
However, while studies have always focused on single wavelengths of the visible spectrum and their action on bacteria, particularly at 405nm, a recent technique that uses LED irradiation with a combination of multiple interfering waves of the visible spectrum and with main peak in the 400-420nm range, has shown wide efficacy on both bacteria and viruses, including, for the first time, its ability to inactivate SARS-CoV-2 [70].
In this work, we examine what reactions may occur in microorganisms when they are irradiated with a flux of photons consisting of multiple combined wavelengths. We do so by investigating some selected mechanisms that, through the irradiation of visible light, can contribute to the eradication process of bacteria and viruses.

Keywords

Photoeradication, Bacteria, Viruses, SARS-CoV-2, Visible Spectrum, LED.

Cite

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1 Preface

The sanitization of environments has the purpose of reducing the amount of microorganisms present in them. Within, surfaces, objects and materials, as well as clothes, utensils and furnishing accessories, are the main objective of sanitation practices since they act as a reservoir for microorganisms [97] and, therefore, they increase the risk of cross contamination through direct and indirect contacts.

However, the common cleaning practices do not guarantee the necessary sanitary safety, as these practices are based exclusively on the use of chemical disinfectants, which have a series of disadvantages in addition to an environmental impact no longer sustainable [98]. And, in addition, they are not able to effectively counteract the phenomenon of recontamination.

Furthermore, the effectiveness of a chemical biocide is linked to several factors: the type of microorganism to be eliminated, the concentration of the biocide used, the contact time which can vary between 5 and 180 minutes [95, 96], the temperature environment, the type of material being treated and its pH, the possible presence of organic matter on the surface. And, in any case, the effectiveness of chemical disinfection can last a maximum of 30 minutes [98], after which the microbial load begins to grow back.

Finally, considering the continuous increase of bacterial species resistant to both disinfectants and antibiotics, and with the acceleration imposed by the ongoing SARS-CoV-2 pandemic, a paradigm shift is necessary for the sanitization of confined environments, with the use of modern, safe, and eco-sustainable technologies which can guarantee continuous microbiological safety.

With the use of visible light radiations, especially between the 400-420 nm range, that has amply demonstrated its validity thanks to countless scientific studies and tests conducted by universities and research centers against many microbial species [70, 93, 94], it is possible to obtain this result. And with the possibility of using a common element of everyday use, visible light, we can be able to realize a new paradigm: continuous sanitization of indoor environments.

This type of sanitization can be carried out in the presence of living beings and, unlike chemical disinfectants, it does not damage surfaces, has no environmental impact, does not generate resistance in microorganisms [92], avoids the phenomenon of recontamination and, above all, maintains control of the microbial load without reaching unsafe levels of complete environmental sterility. On the contrary, it favors the mechanisms of competitive antagonism between microorganisms and allows to maintain the resilience of the immune system thanks to the possibility of preserving a continuous interaction between living beings and sub-infectious doses of microorganisms [71].

2 Bacteria and Biofilm

Bacteria are single-celled organisms that occur naturally in two distinct forms:

a. the first is the familiar suspended, or planktonic, form in which separate cells float or move independently in a fluid (air, water).

b. the second is the aggregate, or sessile, state in which the cells are tightly bound and firmly attached to each other and, usually, also to solid surfaces.

To date, most in vitro studies focus on the analysis of planktonic cells of single species grown in laboratory. However, this experimental situation does not compare to the ordinary conditions in which bacteria live. Most bacteria survive, in fact, in aggregates, often multi-species, which exist in colonies firmly linked to surfaces and that promote bacterial survival within the environment not only by producing a protective exopolysaccharide matrix from cells, but also by coordinating group behavior, improving metabolic interactions, improving genes transfer, and increasing their resistance to external microbicidal substances like antibiotics; in fact, behaving like an ordered multicellular organism. Hence, when bacteria aggregate on surfaces, they organize themselves into functional groups that contribute to the development and survival of the community by forming a microbial ecosystem, known as a biofilm. [60, 61, 62, 63, 64]

Biofilms are gelatinous-like structures, formed for the most part of water and proteins, which adhere with tenacity to the surfaces on which they form and that not only represent the main cause for microbial resistance – which contributes decisively to the spread of infections that are particularly difficult to eradicate even with latest generation antibiotic therapies – but they represent the biological substance known today that is most difficult to penetrate by chemical biocides; and that, by allowing the spread of resistant microorganisms, it also causes problems in sectors other than strictly health care, such as those of industry, both by colonizing equipment and by contaminating products [59].

And it is precisely within the biofilm that the phenomenon of the so-called *bacterial resistome* is amplified, through the exchange of pieces of genetic material bearing the information necessary to make the microorganisms resistant; and which is made openly available to all colonizers.

The formation of a biofilm begins with the adhesion to a surface of freely floating microorganisms. The first "colonists" adhere to the surface and, if not immediately separated from it, can anchor more firmly using cell adhesion molecules, such as pili.

These early colonizers facilitate the arrival of other cells by making available different cell adhesion sites and begin to build the matrix that allows the integrity of the biofilm. Some species are not able to autonomously attach themselves to a surface, so they often manage to anchor themselves to the matrix or to previous colonizers; and, once colonization begins, the biofilm grows through cell division and integration of external bacteria, including other species.

At this point, within the biofilm, the microorganisms begin to cooperate by exchanging information and coordinating with each other, transforming, in fact, into a truly unique living being.

While bacteria in planktonic form do not normally pose a real risk for the transmission of infections, bacteria living in a biofilm usually have significantly different properties from free-roaming bacteria of the same species, thanks to the protected environment that allows them to cooperate and interact in various ways. Numerous studies conducted on biofilm have shown that microorganisms develop a state of "physiological resistance" in this environment, increasing tolerance to chemical detergents, disinfectants and antibiotics. In some cases, the antibiotic resistance of bacteria in a biofilm can increase by 1000 times compared to planktonic forms [54].

For these reasons, biofilm is considered the most dangerous vehicle for the transmission of infections as well as the main cause of antibiotic resistance and hospital-acquired infections. According to the CDC (Centers for Disease Control and Prevention) in Atlanta, up to 80% of bacterial infections transmitted in Western countries are caused by polymicrobial biofilms; and, therefore, many of the recurrent infections are polymicrobial syndromes characterized by a significant increase in aerobic, anaerobic and fungal bacterial load, with a possible pathogenic dominant strain.

The microbial resistance of biofilms is not genotypic (i.e. carried by plasmids, transposons or linked to mutational events) but is, rather, due to multicellular strategies and / or the ability of single cells within the biofilm to differentiate into a phenotypic state that tolerates the antimicrobial action of antibiotics and antiseptics.

The need to prevent and control the spread of infectious diseases is, therefore, the real health challenge of our time, due to the continuous emergence of a series of extremely aggressive pathogens, inside and outside the hospital context, so much so as to induce the World Organization Healthcare considers antibiotic resistance to be the "21st century epidemic".

Antibiotics were an absolute success story, but from an earlier era: they are no longer as effective today. For this reason, the medical, academic and industrial communities are united in the search for new and effective alternative countermeasures [44]. And the new contrast strategies, also based on the indications of the WHO, consider the concept of prevention as fundamental, limiting the use of antibiotics only to situations of extreme necessity [45].

3 Microbial photoeradication by EM waves and photochemical phenomena underlying their action

The great evolutionary success achieved by microorganisms suggests that their presence plays a fundamental role in the evolution of life; and thinking of eliminating them indiscriminately appears to be an imprudent choice to say the least.

Unfortunately, every time we use disinfectants in an uncontrolled way, we also destroy those populations of microorganisms that form the *environmental microbiota*, thus favoring the processes of *environmental dysbiosis*; that is a condition which, even if it does not present immediately diagnosable signs, as are the symptoms of a disease in the case, for example, of human beings, leaves enormous spaces for colonization in favor of pathogenic microorganisms.

The phenomenon of environmental dysbiosis represents a serious problem for living beings, since the integrity of our immune system, or resilience, is due to continuous interactions with microorganisms, both pathogenic and environmental.

Even in humans, the imbalance between the microorganisms residing in the intestinal lumen causes a similar phenomenon: intestinal dysbiosis; a condition that can be the origin of gastrointestinal symptoms and food intolerances.

But in an environment there are no symptoms that can warn us of the imbalances in progress, exposing visitors to the risk of the establishment of populations of pathogenic microorganisms that are difficult to detect if not due to repeated episodes of infection in living beings.

In addition to keeping the risk of environmental dysbiosis under control, the need to develop a system of continuous sanitation of closed environments, which can also be used in the presence of living beings, is therefore dictated by the fact that no infectious pathology manifests itself immediately after contact. with the pathogenic organism that causes it: since the incubation times are very variable (from 24-48 hours up to 15-20 days and beyond), the consequence is that the expression of a possible infection does not appear until after some time, making the possible spread of infections promoted by apparently healthy carriers very high.

Studies on the ability of EM waves to eradicate microorganisms date back to the late 1800s and early 1900s, but initially focused on ultraviolet (UV) wavelengths. Only more recent studies have focused on the wavelengths of the visible spectrum (VIS), demonstrating their greater effectiveness and easier usability. This is an area of growing interest for scientific research, which today identifies photoinactivation as a primary method of prevention also thanks to the increasingly numerous demonstrations of efficacy not only in clinical applications but also in the extra-hospital setting.

3.1 Wavelengths in the UV (ultraviolet) spectrum

The best known and most used antimicrobial irradiation model so far uses wavelengths included in the spectrum of UV rays, in particular in the range between 240 and 260 nm (UV-C); traditionally used for surface disinfection [3].

However, it has been shown that the absorption of UV rays does not cause the destruction of a microorganism but causes its inactivation, damaging the nucleic acids, that is: the microorganism, although active, is in fact unable to carry out its pathogenic function and to replicate within a guest. And, to date, this has been found to be sufficient for UV disinfection to be effective.

In fact, inactivation is caused by the action of UV frequencies which, when they pass through an organism, are absorbed by numerous cellular components. But, in relation to the wavelength, each of these components absorbs different amounts of radiation. And it has been observed that only proteins and nucleotides, which make up DNA and RNA, absorb sufficient doses of UV radiation.

The photochemistry of RNA and, above all, of DNA, the basic elements of life, was tackled by analyzing the photochemical behavior of the "building blocks" that make up the DNA double helix. The four building blocks are known to be Adenine (A), Cytosine (C), Guanine (G) and Thymine (T), and DNA

inactivation occurs specifically due to the ability of UVs to create a bridge (dimer) between two adjacent T bases. The formation of thymine dimers (and other similar dimers) thus alters the structure of the genetic material, inactivating microorganisms and, therefore, preventing their replication.

However, microorganisms, especially bacteria, have mechanisms that, during the cell replication process, can repair or bypass the thymine dimers within DNA. And some viruses can also reactivate by harnessing specific host cell enzymes.

These reactivation mechanisms are divided into reactivation in the dark and in the presence of light, where:

a. the reactivation in the dark takes place by replacing the thymine dimers with a new synthesis of the two thymine molecules which restores the original DNA situation, reactivating the microorganism. While, in other cases, during DNA replication, the areas not damaged by UV rays are used to replace the thymine dimers, obtaining reactivation;

b. the reactivation in the presence of light takes place, on the other hand, by exploiting the energy of the photons to break the bond of the dimer and restore, with a photochemical reaction, the original sequence.

Furthermore, beyond their partial effectiveness, side effects are what make the use of UV wavelengths less advisable.

In fact, UV rays are harmful to humans and other forms of life. Therefore, UV devices require the implementation of important safety measures. Such as, for example, the use of explicit warnings (e.g., danger signs, signaling of equipment in operation, information on risks) and, if necessary, the use of personal protective equipment (e.g., goggles, gloves). For these reasons, the lamps that adopt UV technology are either shielded or closed in places that limit their exposure and are often equipped with locking devices that automatically stop their operation if the environment or the system is opened to the outside access.

In fact, in humans, prolonged exposure to wavelengths of UV rays can cause burns and (in some cases) skin cancers. In addition, exposure of the eyes to UV rays can cause very painful inflammation of the cornea and can damage the retina, causing temporary or permanent vision problems and, in addition, leading to blindness.

Furthermore, a further potential danger is the generation of ozone, which is a toxic gas, produced when UV-C, which has wavelengths between 100 nm and 280 nm, interacts with diatomic oxygen (O_2) molecules. in the environment.

Recently, there have also been attempts to introduce the use of UV radiation with a wavelength of 222 nm in the presence of humans. However, the European Commission has already established, with a definitive opinion, the danger of these radiations for humans, which lead to the formation of pyrimidine cyclobutane (CPD) dimers at the basal layer of the epidermis with doses lower than those required to reach the bacteriostatic effect claimed [99].

Last, but not least, the effect of UV-C radiation on materials, which can alter chemical bonds, leading to a rapid deterioration of the structural characteristics of paints, insulators and plastic gaskets (note that the materials plastics sold as UV resistant are only tested for UV-B, as UV-C do not normally reach the earth's surface).

So, in practice, since they cannot be used in the presence of living beings - that is, when the real risk of environmental contamination and contagion is greater - UV rays do not guarantee any form of real protection.

3.2 The wavelengths in the VIS (visible) spectrum.

There are also other non-harmful forms of radiance: in fact, the photodynamic inactivation of microorganisms with the use of the light energy emitted in the visible spectrum region (VIS) has recently been validated as a powerful means of contrasting the development of bacterial species, including multidrug resistant species [44].

The efficacy of certain wavelengths of visible light with respect to the replicative capacity of microorganisms has been known since the early 1900s. The first studies examined the efficacy of the 405 nm wavelength and all studies agree on the fact that this frequency is effective on different species of bacteria, both Gram-positive and Gram-negative, applying different powers and with variable action times. Several laboratory studies have shown that light at 405 nm, and longer lengths of violet light, have a broad spectrum of activity, including the ability to inactivate a wide range of microorganisms, including antibiotic-resistant bacterial strains such as *Staphylococcus aureus* resistant to methicillin (*MRSA*). The bacterial species that have shown the greatest susceptibility to these wavelengths include microorganisms that cause health care associated infections (HAI) including *S.aureus*, *C.difficile*, *A.baumannii*, *E.coli*, *S.epidermidis*, *P.aeruginosa*, *K.pneumoniae*, *S.pyogenes*, and *Mycobacterium spp*.

Typically, photodynamic inactivation requires the exposure of microbes to a light energy source that emits wavelengths in some monochromatic peaks of the visible spectrum, causing the excitation of endogenous photosensitizing chromophores (porphyrins) and resulting in the production of oxygen singlet and other reactive species (RS) which, by reacting with the intracellular components, damage the cytoplasmic structures and the membrane, causing the disappearance of the microorganism (eradication).

Inside the bacterial cell, membrane lipids are the class of molecules most susceptible to attack by RS (enveloped viruses acquire lipid membranes as their outer coat through interactions with cell membranes, during internal morphogenesis and upon exiting infected cells) [46]. Oxidation occurs on fatty acids present in cell membranes, or in lipoproteins; and as the number of double bonds present in the molecule increases, their susceptibility to oxidation increases. The peroxidation reaction leads to the formation of secondary products, such as aldehydes and ketones, universally recognized as toxic substances for microorganisms. Following the oxidation by free radicals of some amino acids such as lysine and arginine, and the release of iron resulting from the degradation of porphyrin rings (caused by the formation of the H2O2 molecule), proteins lose their physiological structure and functionality.

> *"Many organic molecules of biological origin can act as photosensitizers as they exhibit a good quantum yield of triplet formation and an excited state with a relatively long duration (up to hundreds of microseconds). In fact, the absorbed light, only when coupled to a suitable wavelength and in the*

presence of oxygen, can define the passage of the photosensitizer to its triple photoactive state, producing cytotoxic radicals (type 1 reaction) or cytotoxic singlet oxygen and free radicals. (type 2 reaction). The cytotoxic molecules produced can ultimately induce cell death.
The requirements for an optimal photosensitizer are manifold; it should be non-toxic and show local toxicity only after light activation and should have a highly selective accumulation and a high quantum yield of singlet oxygen production [48, 49].
Among the first molecules used as photosensitizing agents of living organisms with visible light, there were some agents of natural origin, such as porphyrins [47]. Porphyrins, like all chromophores, undergo electronic excitation following the absorption of a quantum of light, passing from the fundamental electronic state to a higher level, the excited singlet [50]. The absorption spectrum of these molecules varies from 400 to 700 nm. For this reason, porphyrins are optimal photosensitizers for applications in this field [51] ". [52]

Even in higher living beings (humans and animals) porphyrins play a fundamental role in metabolic functioning, but since the frequencies required for microbial photoinactivation are unable to overcome the epidermal barrier (the fat stored in adipose and subcutaneous tissue), they have no effect on them.

4 Operating principles of white light photoeradication: emission spectrum and advantages

Some studies [30, 31] that verified the possible microbicidal effect of white light on two bacterial strains (*H.pylori*, *P.mirabilis*, *P.aeruginosa*), required a combination of remarkable power densities (180 J/cm^2) and photosensitizing compounds (methylene blue). On the contrary, the use of specific frequency peaks in the 400-420 nm blue-violet region of the Soret band not only demonstrated microbicidal efficacy at 5 times lower energy values (36 J/cm^2) even in the absence of concomitant photosensitizing factors. but, above all, the efficacy on a wide range of bacterial species, both Gram-positive (*S.aureus*, *MRSA*, *S.epidermidis*, *S.pyogenes*, *E.faecalis*, *C.perfringens*) and Gram-negative (*A.baumannii*, *P.aeruginosa*, *E.coli*, *P.vulgaris*, *K.pneumoniae*) [32].

Since the most effective wavelengths for the photodynamic eradication of microorganisms are emitted in the region of the visible blue-violet spectrum, the availability of a sanitizing system that associates white light with the microbicidal frequencies of blue-violet light can ensure use continuous photo-inactivation during normal daily activities, thus creating a special lighting device capable of replacing the standard lighting systems in each room but allowing the application of these devices also in specialized environments, such as operating theaters or dental units, in this way, simultaneously lighting and sanitizing the surgical field.

And by adopting a precise combination of frequencies (400-420 nm, 430-460 nm, 500-780 nm) of the visible light spectrum (Fig. 1), in particular very narrow peaks, equal to or less than 5 nm, in the range 400-420 nm, it is possible to target the different types of porphyrins and flavins [57], resulting in both a high efficacy on pathogenic microbial species (both bacteria and viruses) and a shift in microbial competition in favor of the more useful groups, such as lactobacilli and saccharomyces. Furthermore, by emitting white light with the desired color temperature, this technology can replace any existing lighting device; thus creating a Multifrequency Interferent-waves for Microbial Eradication (MIME) System that provides continuous sanitization services through artificial lighting.

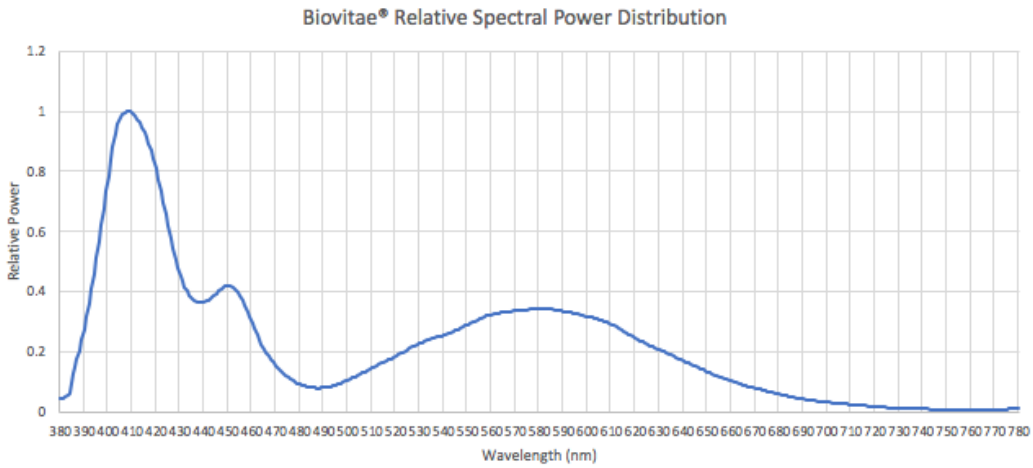

Fig. 1 – *Relative distribution of the spectral power of the MIME wavelengths.*

5 The risks of overlooking the competition among microorganisms

Bacterial colonies are usually subject to continuous attacks that come not only from disinfectants or antibiotics, but also from other microorganisms (e.g., viruses). In addition to this, different species coexist in the same biofilm in a kind of "armistice", in which they do not face the same stiff competition as an unprotected environment, since all they want is to get everything they need to live, in addition to receive protection from external attacks.

However, bacteria growing in multispecies communities employ "high-risk, high-reward" strategies, which are reflected in their specific metabolic adaptation. This concept arises from bacterial growth patterns: bacteria that undergo constant growth (i.e., insensitive to nutrients) will always outperform their neighbors whose growth rate depends on the external environment (i.e., sensitive to nutrients) [58].

Most of the in vitro studies have focused on demonstrating the efficacy of the 405nm wavelength on Gram-positive bacteria. But, as we have discussed, when different species share the same stage in a real-life environment, any decrease in one group sees an increase in the others.

Therefore, by favoring the drastic reduction of Gram-positive bacteria, space is left for the uncontrolled growth of all other species (especially Gram-negative ones), stimulating a shift in the equilibrium of the microbiological ecosystem in favor of more resistant microorganisms.

MIME technology is therefore also able to protect the so-called good microorganisms, such as probiotic bacteria, saccharomyces fungi and bacteriophage viruses, preserving the microbial ecosystem as much as possible. In fact, the energy required to damage these "beneficial" species is very high and requires exceptionally long exposure times to observe any measurable reduction of these species.

In bacteria, porphyrins absorb the wavelengths emitted by MIME technology and produce free radicals (such as singlet oxygen $^1O_2$, superoxide anion O_2^-, hydrogen peroxide H_2O_2); and, in the presence of free transition metals (especially iron and copper), they also give rise to the hydroxyl radical (•OH), which is particularly toxic.

But while the species of pathogenic bacteria have different absorption spectra, due to the presence in such microorganisms of metal-free tetrapyrroles that react at different wavelengths, such as coproporphyrin (390-425 nm), protoporphyrin (300-450 nm), uroporphyrin (380-430 nm), deuteroporphyrin (375-425 nm), which are organic compounds capable of absorbing visible light and generating reactive species (RS), probiotic bacteria and saccharomyces fungi have an absorption band in the deep red region of the visible spectrum and show ability to scavenge RS due to their ability to synthesize extracellular polysaccharides (EPS), a class of biomolecules characterized by the ability to remove reactive oxygen species (ROS) formed by various metabolic reactions [81]. Hence, they exhibit antioxidant activities that protect them from the action of MIME.

The metabolic conversion of photoenergy is not the only way to produce RS. Pathogenic viruses have a pericapsid consisting of phospholipids and proteins. The phospholipids in turn are composed of glycerol, an organic compound in whose structure there are three hydroxyl groups -OH (or triol, 3 -OH). The energy transferred through the wavelengths of MIME technology to the molecules that make up these shells generates a photolysis process of glycerol which releases hydroxyl radicals (• OH), which is one of the most powerful oxidizing agents capable of interacting in a way non-selective and instantaneous with the surrounding molecules and to cause irreversible damage both to the genetic material and to the external envelope of microorganisms.

But bacteriophages, which are not pathogenic viruses for humans, lack lipid membranes (since they do not possess intrinsic mechanisms for lipid biosynthesis), which are acquired by the host cytoplasmic membrane during virion assembly. Therefore, the lipid composition of a phage is minimal and reflects only that of its host bacterium, at least to some extent [82]. Therefore, any photolysis processes on phages, which may be made possible by the presence of lipids acquired by the host bacterium, will not have the same efficacy found on pathogens.

Also, during colonization, phages slow down the metabolic rate of host bacteria without killing the cell. For example, when infected with phages, E. coli is able to dedicate only 10% of the total energy to activities other than the direct synthesis of the phage components of the progeny [83].

Furthermore, abortive infection (Abi) promoted by the bacterial community prohibits the release of functional phage particles along with the Toxin-Antitoxin (TA) systems, which reduce the level of metabolism without killing the bacteria. In this case, the phage progeny is reduced due to the lower metabolic activity of the host [84].

It means that phage-infected host bacterial colonies will not be able to show the effects of MIME-activated RS production-induced apoptosis simply because the metabolism of the bacteria is severely reduced. But being infected with phages is still a condition that means that any bacteria do not pose a threat to humans.

6 The photoeradication mechanisms triggered by MIME technology

The sensitivity of microorganisms to MIME technology tends to depend on the species. For example, it appears to work faster on viruses than bacteria (e.g., in culture media) [1], while Gram-negative bacteria tend to be more susceptible to inactivation than Gram-positive species, possibly due to their higher replication rate induced by a faster metabolism, with a higher production of RS.

It is also recognized that the microbial density affects the dose-log ratio, which can be explained by the fact that the energy delivery is influenced by phenomena such as the absorption and scattering of light, which depend on the cell concentration and the size of the vessel. of culture [70, 76].

Therefore, while remaining an open topic, the identification of all the phenomena that, through the irradiation of visible light, allow MIME technology to be extremely effective in killing microorganisms has been the subject of in-depth investigations. Consequently, it was possible to identify several possible mechanisms involved in addition to RS production, and which allow to better explain the MIME technology compared to other systems based on the emission of single wavelengths of visible light.

	Organism	Initial Count	Irradiance (mW cm^{-2})	Total Dose (J cm^{-2})	Log | % reduction	Dose/Log (J cm^{-2})	Time/Log (min)
	BACTERIA						
1	S. aureus ATCC 6538	1.4E6 CFU	0.034 [1]	0.25	5.15 (99.9993)	0.048	23
2	P. aeruginosa ATCC 27853	9.6E6 CFU	0.034 [1]	0.25	7.98 (99.9999990)	0.031	15
3	E. coli ATCC 25922	2.8E7 CFU	0.034 [1]	0.25	8.45 (99.9999996)	0.029	14
	SPORES						
4	B. atrophaeus ATCC 9372	2.0E5 CFU	0.034 [1]	0.25	3.35 (99.955)	0.073	36
	VIRUSES						
5	YFV	2.0E5 PFU/ml	0.88 [1]	2.38	3.00 (99.900)	0.79	15
6	Sars-CoV-2 (5-22)	2.0E5 PFU/ml	0.88 [1]	3.17	2.70 (99.800)	1.17	22

[1] The irradiance values are calculated from the radiant flux measured with the integration sphere.

Tab. 1. Examples of multiple case studies performed in vitro to demonstrate germicidal efficiency for the eradication of bacterial and viral species from surfaces using the low radiant flux MIME technique.

[1] One of the possible explanations is that bacteria are equipped with a metabolism that, in favorable conditions, provides them with some protective capacity against oxidative insults, and which, in some cases (e.g., on bacteriophages) might also be protecting the parasite viruses.

6.1 Photolysis processes supported by low energy incident light

A ray of light, in the quantum representation, consists of an emission called a photon flux. This emission is described as the average number of photons traveling through a cross section of the flux per unit of time and calculated by dividing the energy of the flux by the energy that the individual photons contain for each wavelength.

The central tenet of bioenergetics is that photons energize electrons inside molecules, electrons push protons, and proton concentration gradients create chemical bonds. Understanding how it works – at the atomic level – is elusive, especially in organisms that live life on the verge of survival. But in van Wonderen et al. it is shown that an electron transport mechanism from heme to heme transmembrane allows electrons to escape through the bacterial cell envelope [80]. This escape mechanism can contribute to the monovalent reduction of molecular oxygen (O2) and, therefore, to the production of the superoxide radical (O2-), which is the first product by transferring an electron from electron carriers directly to oxygen [88]. The superoxide radical is biologically toxic and, produced in large quantities in phagocytes by the enzyme NADPH oxidase, is normally used by the immune system to kill pathogenic microorganisms [89].

Furthermore, bacteria and viruses share the type of structure of their outer envelope (the membrane for bacteria and the pericapsid for viruses), which is made up of phospholipids and proteins. The phospholipids in turn are composed of glycerol, an organic compound in whose structure there are three hydroxyl groups -OH (or triol, 3 -OH).

The energy transferred by the wavelengths of MIME technology to the molecules that make up these envelopes generates a process of photolysis, or the splitting of a molecular entity into its compounds. While the photolysis of glycerol frees hydroxyl radicals (•OH), the photolysis of water can free both dissolved hydroxides, which release the hydroxyl group (-OH) and a positive metal ion, with molecular oxygen (O_2) and hydrogen ions (hydrogen ion, H^+). Once autonomous, these atoms are free to recombine to form hydrogen peroxide (H_2O_2), ozone (O_3) and trioxidane (H_2O_3); that is, all highly unstable reactive species that cause damage to the outer envelope of microorganisms.

On coronaviruses, this course of action is also supported by a recent study [90], which concludes that "*the degradation and destabilization of phospholipid compounds of the viral envelope can be a significant pathway for the inactivation of coronaviruses*" [91].

Furthermore, while in bacteria the porphyrins absorb the wavelengths emitted by the MIME technology and produce free radicals (such as singlet oxygen $^1O_2$, superoxide anion O_2^-, hydrogen peroxide H_2O_2); and, in the presence of free transition metals (especially iron and copper), they also give rise to the hydroxyl radical (•OH), which is particularly toxic, in viruses the states of photo-excited triplet chromophores, linked to viral peptide models, lead to the up-conversion of triplet annihilation (TTA-UC), which is a photo-physical process that produces high-energy photons from low-energy incident light, hence fast-tracking the destruction of microorganisms [55, 56].

6.2 Stochastic resonance assisted response of weak signals

The stochastic resonance (SR) phenomenon was revealed in 1981 [79]. This is a phenomenon in which a signal, which is normally too weak, can be boosted by adding non-zero noise levels containing a broad spectrum of wavelengths to the signal [2].

The wavelengths in the noise levels (which, in the case of MIME technology, is produced by white LEDs) that match the wavelengths of the original signal will then favor each other, amplifying the original signal without amplifying the rest of the noise white and consequently increasing the signal-to-noise ratio, which makes the original signal more prominent. This phenomenon of amplification of undetectable signals by resonating with added noise extends to many systems, be they electromagnetic, physical or biological.

The SR phenomenon occurs in bi-stable systems, that is, when a minor periodic force (eg sinusoidal) (the signal) is applied together with a stochastic (random) broadband force (the noise). The reaction of the system is thus driven by the competition / cooperation of the two forces that make the system alternate between the two stable states.

6.3 Quantum effects induced on nanoscopic systems

Since both microorganisms and light waves interact at the nanoscale, at these magnitude levels other mechanisms could be involved in the microbicidal process which include the introduction of quantum effects: a) microorganisms can be considered nanocavity systems, with the unique property to confine photons for a long time in a small volume, significantly improving the light-matter interaction [85]; furthermore, b) when light interacts with a nanocavity, the matter acts as a resonator, in which a wave spins in a ring (in a preferred direction, depending on the direction of the incoming light), still significantly improving the interaction of light with matter [86]; moreover c) when the quantum interference is generated by different transition pathways of the optical fields, the resonant waves are excited by the constructive interference and both the absorption and dispersion properties change strongly, which results in extended effects; such as Electromagnetically Induced Transparency (EIT), which is a phenomenon in which an electromagnetic field controls the optical response of a material: it makes a medium transparent within a narrow spectral range around an absorption line and leads a "slow light" (light delay) within these "windows of transparency" which, in essence, allow the propagation of light through an otherwise opaque atomic medium [69, 87], further extending the effects of light on the colonies adjacent.

[2] Although wavelength and frequency are often used as interchangeable terms, we wish to clarify that by wavelength we define the distance between two specific points of a wave (the total length of a complete wave), while by frequency we define the total number of wave oscillations in a given time (the number of complete wave cycles per second). However, the two units are related:

$$f \cdot \lambda = c$$

where: f = oscillation frequency, λ = wavelength, c = speed of light.

7 Conclusions: Practice safe environmental decontamination for the promotion of a resilient immune system

For a long time, sterilization was thought to be the only way to ensure safe environments. But the truth is that living in sterile or excessively clean environments can lead to major impacts on the immune system, caused by the lack of interaction with the microbial ecosystem.

Indeed, exposure to microbes can increase human host resistance to pathogens [72], as exposure to commensal and pathogenic microbes has a major impact on immune competence and overall health [73].

The distinctive feature of MIME technology is that of promoting continuous sanitization by maintaining a constantly low microbial load, without completely eradicating microorganisms.

Especially in recent times, particularly in light of the latest COVID-19 pandemic, this position has been supported and further developed by the authors; as, for example, in [71]:

> "*Starting from the observation that in several European countries the number of COVID-19 infections in the second and third pandemic waves has increased without a proportional increase in the severity and mortality of the disease, it is hypothesized the existence of a further factor influencing the dynamics of SARS-CoV-2. This factor consists of an immune defense against severe COVID-19, provided by SARS-CoV-2 specific T cells that develop progressively following natural exposure to low doses of viruses present in populated environments. As suggested by recent studies, low-dose viral particles entering the respiratory and intestinal tracts may be able to induce T cell memory in the absence of inflammation, potentially resulting in varying degrees of immunization. In this scenario, non-pharmaceutical interventions would play a dual role, one in the short term by reducing the harmful spread of SARS-CoV-2 particles, and one in the long term by allowing the development of a widespread (albeit heterogeneous and uncontrollable) form of immune protection.*"

8 References

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The MIME technique has been translated into BIOVITAE®, the first technology to have been experimentally shown to be able to eradicate the SARS-CoV-2 (COVID-19) virus through tests conducted at the military laboratories of the Scientific Department of the Celio Military Polyclinic in Rome. , Italy, of the Bundeswehr Institute of Microbiology, Viral and Intracellular Pathogens Section, Munich, Germany, and of the CBRN Security and Protection Department, Swedish Defense Research Agency (FOI), Umeå, Sweden; replicated both at the Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA), France, and at the International Center for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy, and at the Department of Public Health and Infectious Diseases of the La Sapienza University of Rome, Italy.

The MIME technique, whose unique combination of wavelengths of the visible spectrum is able to photoeradicate the pathogenic microorganisms subjected to its action, is patented worldwide with registrations WO2017179082A4, WO2018020527A1.

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