Application Note

Effect of piezoelectric direct discharge plasma on microorganisms

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Abstract. We show qualitative data on the effect of a novel source of cold discharge plasma on typical model system microorganisms. First interaction models are discussed.

Keywords: cold plasma, sterilization, piezoelectric discharge

1. Introduction

Cold atmospheric pressure plasmas are now being widely used for many types of surface disinfection and sterilization [1–4]. Despite the wide area of applications and high demand for new effective treatments, the understanding of interactions of plasmas with living cells and tissue remains rather limited.

The following paper will introduce a novel plasma system that, due to its variability and simplicity, is very suited to conduct basic studies in biological and medical research. Plasma is defined as a quasineutral ionized gas. It is constituted by particles including photons, electrons, positive and negative ions, atoms, free radicals, and excited or nonexcited molecules in permanent interaction. Some kinds of plasma may have a general oxidative effect on the cell surface layers of microorganisms, inhibiting growth. Of special interest is the use of plasmas as a rapid, additive-free disinfectant in a medical setting where plasma is applied directly to living tissue without damaging it.

Two major types of plasma treatment have been discussed in connection with wound disinfection. One is indirect treatment using gas flow through plasma, which brings mostly neutral active species in contact with living tissue. The other is direct application of non-thermal plasma to living tissues [5]. Indirect treatment permits to decouple plasma system design from constraints related to applying the treatment to living tissues. For example, it permits to employ thermal plasma for generations of active species including substantial quantities of NO [7-10]. Indirect plasma treatment of skin and wounds has been investigated over the last several years, demonstrating a significant effect [6]. On the other hand, applying non-thermal plasma directly to living tissue makes it possible to employ charges and short living neutral species [7, 8]. It has been demonstrated that bacterial inactivation on surfaces of agar and even skin can be achieved significantly faster with direct exposure to non-thermal plasma such as the Dielectric Barrier Discharge (DBD) [5]. No significant toxicity was observed during relatively low power and duration plasma exposure, which is typically sufficient to achieve significant reduction in the bacterial load [6, 9, 8, 10]

Only recently has it been shown that non-thermal plasma can be applied to cells in sub-lethal doses to elicit specific biological effects, including gene transfection [11-13], cell detachment [14-17], cell proliferation [18], and wound healing [19, 20–23]. Non-thermal plasma can even have selective effects. In recent studies on plasma-initiated blood coagulation [19, 22], skin sterilization [19, 24], and tissue toxicity

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after plasma treatment [25, 26], plasma did not demonstrate measurable toxicity in the surrounding living tissue.
Even direct application of cold plasma to open wounds has been demonstrated using the rat model representing a surgical treatment situation [27].
Air plasmas are good sources of reactive oxygen-based species, ROS (O, O_2, O_3, and OH) and nitrogen-based active species RNS (NO, NO_2, NO_x). If humidity is present the equilibrium is shifted towards peroxide species such as H_2O_2. These reactive species affect the outer structure of cells by methods of oxidation and disturb its function, leading directly or indirectly to the death of the cell. It can be assumed that non-thermal plasma primarily produces ROS and RNS extracellular, in contrary to ionizing radiation or photodynamic therapy, which primarily produce intracellular ROS and RNS.

2. Practical relevance

2.1. Dental medicine

Periodontitis is an inflammation of the periodontium, i.e., the tissues that support the teeth. In the early stages, periodontitis has very few symptoms; and in many individuals the disease has progressed significantly before they seek treatment.
Conventional methods of controlling and treating periodontal diseases target pathogens using mechanical procedures (scaling and root debridement) and chemotherapeutic agents (systemically or locally delivered antibiotics and antimicrobial agents). These interventions often fail to achieve long-term control of periodontal pathogens.
Cold discharge atmospheric pressure plasma has been discussed as a novel approach to inactivate P. gingivalis, a pathogen highly associated with chronic periodontal disease, localized aggressive periodontitis, and periodontal abscesses. P. gingivalis is a gram-negative, non-spore-forming, anaerobic bacteria. An aggressive periodontal pathogen, P. gingivalis can invade soft tissues. One of the more practical aspects is that an efficient treatment needs to bring the active species closely to the inflammation source, often localized in small inaccessible gaps.

2.2. Implant modifications

Plasma-assisted modification of biorelevant materials is an established technique to optimize the biofunctionality of implants or to qualify polymer surfaces for cell culturing and tissue engineering. Especially in the case of just in time surface activation of objects that have to be implanted into living tissue, a combined method for sterilization, decontamination and surface engineering (e. g. roughening, activation, introduction of functional groups or coating) would be of huge practical relevance.

2.3. Surgical

Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is also called oxacillin-resistant Staphylococcus aureus (ORSA). MRSA is any strain of Staphylococcus aureus that has developed, through the process of natural selection, resistance to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. Strains unable to resist these antibiotics are classified as methicillin-sensitive Staphylococcus aureus, or MSSA. The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of S. aureus that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous.
In healthcare environments, MRSA can survive on surfaces and fabrics, including privacy curtains or garments worn by care providers. Complete surface sanitation is necessary to eliminate MRSA in areas where patients are recovering from invasive procedures. Testing patients for MRSA upon admission, isolating MRSA-positive patients, decolonization of MRSA-positive patients, and terminal cleaning of patients' rooms and all other clinical areas they occupy is the current best practice protocol for nosocomial MRSA.
Studies published from 2004-2007 reported hydrogen peroxide vapor could be used to decontaminate busy hospital rooms, although taking significantly longer than traditional cleaning. One study noted rapid re-contamination by MRSA following the hydrogen peroxide application [28, 29, 30, 31, 32].

Therefore any unspecific method to treat or to prevent infections would have significant impact.

2.4. Wound care and dermatology

Initial experiments confirm the fact that infectious agents can be killed without adverse reactions on surrounding healthy body cells. Furthermore, it is possible to stimulate physiological and biochemical processes in living tissues by plasma treatment under special conditions. This opens the possibility to use plasma to support wound healing as well as to treat several skin diseases. Therefore, application-oriented research is directed to developing an integrated concept of plasma-based wound treatment comprising both superficial wound cleaning and antiseptics and stimulation of tissue regeneration in deeper tissue layers. On a solid scientific basis, further therapeutic plasma applications, e.g. in dentistry or surgery, will be opened during the next years [33].

2.5. Food technology

Antifungal activities of plasma were observed using various gas species under plasma discharge and it was reported that cold plasma was able to reduce spore germination for in vitro and in vivo test for Aspergillus niger, Aspergillus flavus, Penicillium sp. and Rhizopus sp., a few typical spores affecting food quality and known to produce strong toxins.
However, these species have shown to be much more stable against reactive oxygen species than bactericides [34].

3. Description of the plasma source

The choice of plasma source plays a tremendous role for the interaction channels that can be used to induce effects in biological systems.
A straightforward comparison of the physical properties of one plasma source to another (even if both are based on similar design principles) is very difficult and involves a lot of analytical efforts.
The situation is even worse in medicine, since clear monocausal correlations can be proven only in the absolute minority of cases and decision-making can often only be based on conclusion by analogy.
However, we think that a purely empirical approach can yield some quick results if a novel plasma source is screened in the field of microbiology, but it will also be very helpful to classify any novel design of plasma source according to its basic physical properties.
The plasma source that will be described in more detail in this report comes closest to dielectric barrier discharge (DBD) plasma generators, which generate a “cold” no equilibrium plasma [35]. The major difference to conventional DBD sources is that no external high voltage power supply is needed and the electric discharge is directly induced at the tip of a piezoceramic transformer (PZT).

3.1. Piezoelectric direct discharge: PDD®

relyon plasma has developed the PDD® technology (Piezoelectric Direct Discharge), which is based on direct electrical discharge of a piezoelectric transformer (PT) into a working gas.

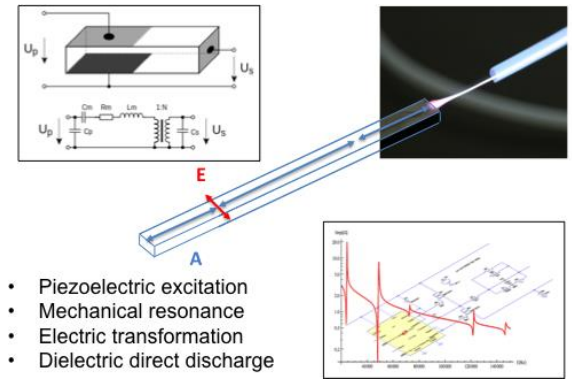


Fig. 1. Working principle of a PDD cold plasma generator

With maximum efficiency, very high alternating electrical fields are created and the ambient process gas, typically air, is dissociated and ionized. In the case of PDD®, the gas temperature in the plasma volume remains near ambient temperature. Electron densities of approx. 10^{14} to 10^{16} m^{-3} are achieved. Similar to dielectric barrier discharges (DBD), PDD® produces a typical "cold" non-equilibrium plasma.

3.2. PZ2 handheld device

One of the major advantages of this novel approach is that no external HV supply is needed and the voltage boost is done within only one integral ceramic component. PDD® technology has been integrated into a simple and robust handheld device with internal air feed (axial fan) and on-board control electronics that drives the piezoelectric transformer,

tracks the system resonance frequency and monitors process parameters and status.

Originally, the PZ2 handheld device had been designed for manual processes on industrial materials such as surface activation in precision engineering, micromechanics, optics, assembly technology, electronics, micro fluid technology, printing and labelling, inkjet, pad printing, coding and micro filling. But it soon turned out that the device has additional variable properties in food technology, medical technology, and pharmaceutical packaging, germ reduction and microbiology.

Fig. 2. PZ2 cold plasma device with transport case power supply and nozzle set.

Although the standard handheld device is operated with air and is delivered with only a small variety of nozzles, it can be quickly adapted to work with different process gases, gas mixtures at variable gas flow. So far the PDD® principle has been confirmed to work with air, nitrogen, argon, helium and gas mixtures such as Ar/O_2, N_2/H_2 and CO_2/N_2. Currently a huge variety of nozzles, ranging from micro jet or capillary to high diameter is under investigation. Easily the functional plasma module (driver board and PT) can be mounted into specific assemblies or even arranged into arrays to tap new applications.

Fig. 3. PZ2 igniting an intense glow discharge in a glas cavity purged with Ar/O_2.

3.3. Plasma composition (air operation)

The gas phase of the plasma is constituted of many more or less instable components, thus yielding a variable downstream chemical activity.
The typical time constant of a secondary jet directed to the substrate is a number of milliseconds (1ms to 100ms) from the excitation volume to the treated surface. If the treated surface is used as a counter electrode, short living species can reach the interface as well.
The gas phase dynamic and the equilibrium state depend strongly on the gas compositions and physical parameters such as pressure and temperature.
The lifetime of excited atomic oxygen species is only nanoseconds under ambient pressure in air.

Positively charged particles	N^+, N_2^+, N_3^+, N_4^+, O^+; O_2^+, O_4^+,
	NO^+, N_2O^+, NO_2^+,
	H^+, H_2^+, H_3^+; OH^+, H_2O^+, H_3O^+
Negatively charged particles	e, O^-, O_2^-, O_3^-; O_4^-,
	NO^-, N_2O^-, NO_2^-
	NO_3^-, H^-, OH^-
Neutral species	$N(^2D)$, $N_2(A^3\Sigma)$,
	$N_2(B^3II)$, $O(^1D)$, H
	N, O, O_2, $(\alpha^1\Delta)$, O_3, NO,
	N_2O, NO_2, NO_3, N_2O_3,
	N_2O_4, N_2O_5, H_2, OH, HO_2,
	H_2O_2, HNO, HNO_2, HNO_3, N_2, O_2, H_2O

Table 1: Main chemical components of cold plasma ignited in humid air (Sakiyama et al. 2012)

More information on the gas phase dynamics can be found in the good overview from U. Kogelschatz et al. [36]

4. Experimental setup

4.1. Configuration

In order to define the interaction more precisely and to gain a satisfactory degree of reproducibility the handheld device (Type PZ2 PDD® device from relyon plasma) was mounted into a desktop style experimental setup (see figure 4).
Using this simple arrangement permits to control independently such parameters as dynamic xyz position, working distance, the flow rate of the process gas ionized in the discharge nozzle. The possibility to shield the sample from direct plasma flow but still allowing the exposure to UV radiation was crucial to discuss the contribution of the latter in our experiment.
Likewise the small chamber was designed to be flooded with some purge gas to show the effect of the surrounding atmosphere.

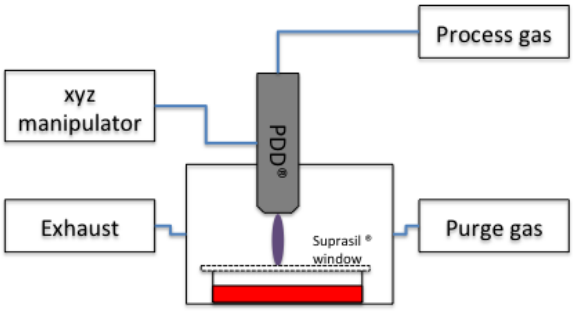


Fig. 4. Experimental setup a intense glow discharge in a glass cavity purged with Ar/O_2.

4.2. Sample preparation

All samples have been prepared on 60mm petri dishes that have been seeded with known spore dilutions. The dilution series have been checked with calibrated optical density measurements.
The processed samples together with the reference plates have been incubated at 37°C for 15 hours. Only treated and untreated samples of the same batch have been compared.

5. Results

The goal of this report is not to go into detail for each individual species and to show a full DoE on the variable parameters. The complexity of the problem and the quantity of the data would overburden the basic conclusion and go beyond the scope of this report. We therefore take out some typical examples and content ourselves with a qualitative interpretation if a quantitative analysis seems to be insufficiently consolidated.

Fig. 5. Two similar time series of dry air plasma treated S.A. culture at 50mm distance with 6L/min airflow and exposures of 1min, 2min, 6min against the reference first row.

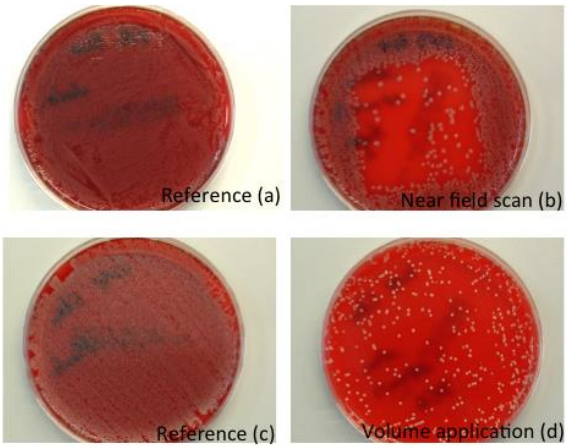


Fig. 6. E.C. treated wit a near field scan of the PZ2 kept at 1mm to the surface and scanned with a meander pattern in 3 minutes (a,b). E.C. treated with PZ2 at a working distance of 50mm using 6L/min dry air for 3 min (c,d).

6. Discussion

6.1. Cause-and-effect chain

The interaction of plasma with surfaces is a multiphysics process that involves excited neutral and charged gas phase species, energetic photons with up to several eV of energy, electric field gradients on the surface and thermal energy. All these effects can induce secondary effects such as surface heating, surface drying, surface charging, surface sputtering, surface photochemistry and changement of the PH value at aqueous interfaces. It is well known that even a chemically rather inert thermoplastic polymer such as polypropylene (PP) will change its surface properties under the action of plasma exposure. New surface functional groups are found, the polymer is partly oxidized and the topology roughened. Wettability can be changed from hydrophobic to hydrophilic. It seems therefore obvious that living cells will show some sensitive reaction on plasma exposure as well.
However it is not fully understood how the conditions of plasma exposure have to be tuned and which predominant physical or chemical effects contribute the major share in this treatment.
Although different types of microorganisms have adapted to totally different environments and conditions it is possible to rank the different interactions and to execute some simple experiments to confirm these statements.

Ion impact
Ion sputtering is a well-introduced technology to remove surface atoms at rates of some nm per minute [37]. However only under low pressure conditions or extreme electric fields charged species can be accelerated to kinetic energies high enough (above 10eV) to exceed the surface etching threshold. Under atmospheric conditions the mean free path between two collisions is too short to accumulate sufficient energy and even a sensitive surface will exhibit no damage. Therefore high-energy ion impact can be excluded to contribute under atmospheric conditions.

Surface charging
It has been reported that microorganisms are sensitive to surface charging [38]. Although dielectric barrier discharge type plasma sources are known to supply free electrical charge carriers (electrons an ions) at densities above 10^{13} m^{-3} plasma sources are inefficient charge sources compared to electron or ion beam devices. The sensitivity to charge transfer effects can be verified by applying an electrical bias to the treated sample. As expected biasing the sample with low voltages yields no substantial variation of the microbiological efficiency. Also using a process gas with low ionization threshold and long ion lifetime such as Argon (Ar^+) which should lead to more efficient surface charging leads to a reduction of the microbiological damage on all tested species. Generally the humid and slightly conducting environment of living cells contributes to the robustness against charging. In contrary to cells scattered on an insulating surface, charge is quickly redistributed in natural environments where the cells are embedded in some kind of electrolyte.
Only at very low distances from the dielectric field source (at the tip of the piezoceramic) charge transfer with high net current occurs through arcing. Under these operating conditions local thermal effects and high ionic current densities in the liquid phase come into play. This regime, where unspecific burn pattern appear, is not applicable to sensitive tissue treatment.
It can be concluded that under normal conditions surface charge effects do not seem to be relevant in natural environments.

Electric field effects
In food industry high inhomogeneous electric fields are known to have germicidal effects [39]. The electric fields needed to impact biology on a short timescale are in the order of magnitude of about one Volt/10 micron equivalent to 10^5V/m. In High inten-

sity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20 - 80 kV/cm) to foods placed between 2 electrodes.
The typical dielectric barrier or piezoelectric plasma sources yield peak to peak voltages of up to 20kVpp. Due to the working principal the gap between the treated interface and the field source is filled with a gas with typical permissivities of $\varepsilon = 1$, whereas the liquid interface has a permissivity of $\varepsilon >> 1$ ($\varepsilon_{water}=80$). Hence most of the field drops in the gap between the dielectric tip of the plasma generator and the treated surface. Field intensity at the dielectric interface drops rapidly with the working distance. In addition the resistive loss in the treated sample is damping the field intensity. Therefore the coupling of the electric field into the micro active layer is very inefficient. Field effects would only play a role in direct contact mode where no gas phase or plasma effects are involved.

Drying
In most of the described flow type plasma treatments the process gas blows over the sample surface. If the gas is expanded from high pressure to ambient (compressed gas supply) it retains only a very low relative humidity. This very dry gas stream will desiccate the treated interface and could deteriorate the conditions for microorganisms. We therefore checked how much effect this drying effect has on the cell vitality. All experiments have shown that the tested species were quite tolerant to water loss on the timescale of several minutes and vitality was not affected if water balance was established again. We conclude that drying out plays only a minor role in the context of this report.

Temperature
The PDD® plasma source only increases the equilibrium temperature of the working gas by less than 20K. Even extended treatment of the tested species with streams of warm air showed no effect on vitality. Some species would even survive much higher temperatures up to 90°C during many minutes. Temperature measurements directly on the sample surface have shown that even at low working distances and maximum power settings of the plasma source the surface warm up was too low to cause significant harm to the cell cultures. Thermal effects are therefore ruled out.

UV
Depending on the process gas a broad spectrum of UV photons can be created during the discharge process. Using rare gases even VUV down to 130 nm can be generated. Generally speaking light emission from the gas plume indicates that there are highly reactive components that emit light during recombination and transition into the ground states. UV effects and gas phase effects will therefore go hand in hand in plasma surface interaction.
Ultraviolet germicidal irradiation (UVGI) is a disinfection method that uses ultraviolet (UV) light at sufficiently short wavelength to kill microorganisms [40]. By 1903, it was discovered that wavelengths around 250 nm (DNA damage band) were most effective for inactivation of bacteria [41]. Typical doses range from 2000 $\mu W \cdot s/cm^2$ to 10.000 $\mu W \cdot s/cm^2$.
Full daytime sunlight reaches intensities of 10^5 $\mu W/cm^2$ ($1000 W/m^2$) over the full integrated terrestrial spectrum. Below 400 nm (UV range) less than 5% of the total intensity is found. Below 300nm only less than 5ppm of the intensity reaches the terrestrial surface. Therefore microorganisms are not adapted to this short wavelength spectral range. Below 200nm water absorption gets dominant and microorganisms in liquid environment are shielded from deep UV, however indirect effects of photo induced chemistry in water will come into play.
The described PDD® plasma source emits UV especially when operated with monoatomic gases such as helium or argon. However the integrated spectral output has been measured to be below $1000 \mu W/cm^2$ at a working distance of 20mm using 6L/min argon and below $200 \mu W/cm^2$ using air.
To be sure to discriminate the chemical from the optical effects, a high quality quartz window (suprasil) with good transmission was used to protect the sample from reactive gas species. The window transmits light down to 190 nm so that the treated surface is exposed to UV of the efficient range without substantial intensity loss. This simple experiment with the PZ2 using air showed that a cell culture that is exposed to the given UV radiation but protected from the direct plasma gas stream is hardly affected on the timescale of 5 minutes treatment. This only demonstrates that there are much more efficient UV sources than cold atmospheric plasma jets. Using Argon showed a weak but substantial effect on the cell cultures. The UV emission using argon is more intense.
It can be concluded that using noble gases will yield some additional effect by UV light, but if air is used the effect of UV light can be neglected. Since the PZ2 will perfectly work with both gas regimes, se-

quential treatments with changing gas composition can be applied easily.

PH value

Qualitative measurements with humid test stripes have shown that the acidity quickly drops below PH=5 in the near surface layers. Quick changes in acidity can yield to partial denaturation of protein structures held within the bilayer of the phospholipid membrane. This can lead to the point of membrane pin holing and cell leakage. If the plasma jet is operated with air the PH value can change via solvation of nitric oxides to form nitrous or nitric acid ($2\ NO_2 + H_2O \rightarrow HNO_3 + HNO_2$).

If humidity is present either in the gas phase or at the treated interface several path leading to the formation of hydrogen peroxide and OH radicals are possible.

Hydrogen peroxide is a weak acid but a strong oxidizer and is used as a bleaching agent and disinfectant.

Although PH value is only a qualitative indicator, dynamic decrease of this value indicate a high efficiency of the treatment process.

Hydroxide radical

The hydroxyl radical, •HO, is the neutral form of the hydroxide ion (HO–). Hydroxyl radicals are highly reactive and consequently short-lived. Most notably hydroxyl radicals are produced by the reaction of excited atomic oxygen with water. Direct gas phase reaction in humid air excited by electric discharges can also induce hydroxyl radical formation. However due to the low stability of the hydroxyl radical, formation directly at the wet interface seems more probable.

The hydroxyl radical can damage virtually all types of macromolecules: carbohydrates, nucleic acids, lipids and amino acids. The hydroxyl radical has a very short in vivo half-life of approximately 10 nanoseconds and a high reactivity [42]. This makes it a very dangerous compound to the organism [43, 44]. Since the dynamic build-up of hydroxyl radicals is strongly related to the interactions of reactive oxygen species, the isolated effect of hydroxyl radicals cannot be weighted precisely.

Reactive Nitrogen Species

Reactive nitrogen species (RNS) are a family of antimicrobial molecules derived from nitric oxide. Depending on the temperature of the discharge regime in air the equilibrium is shifted towards ozone formation trough different ROS intermediates at low temperatures. At high temperatures such as produced in high current atmospheric arcs, ozone concentration drops in favor of nitroxyde components such as NO and NO_2.

The PDD® is not suited to produce high concentration of NO_x. Also using pure N_2 in the absence of oxygen has led to a much lower biological activity than using dry air.

We have therefore used a comparison with a high power gliding arc atmospheric plasma nozzle (PAA® pulsed atmospheric arc technology) with up to one kW of power. The qualitative comparison was done by cooling down the gas stream rich in NO_x to almost room temperature. Surprisingly the effect on two of the most responsive test species (SA and EC) was far below the expectation and much weaker than for the ROS rich stream of the low power PDD® device.

Prior investigations have shown that using humid air fed into a hot arc plasma nozzle will cause severe corrosion even on stainless steel components. But even the use of humidified air with the high power PAA® plasma jet did not lead to higher cell mortality. It therefore can be concluded that the efficiency of gas phase RNS on the tested microorganisms is much lower than expected and will not be efficient on a short timescale without additional influence of other effects such as UV, heat or ROS species.

Reactive oxygen species

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include oxygen ions and peroxides. High ROS levels may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. In air ROS are generated by exogenous sources such as ionizing radiation or electric discharge.

Harmful effects of reactive oxygen species on the cell are most often:

- damage of DNA
- oxidation of unsaturated fatty acids in lipids (lipid peroxidation)
- oxidation of amino acids in proteins
- inactivation of specific enzymes by oxidation of cofactors

Finally in the gas phase all oxygen intermediates lead to molecular oxygen or ozone O_3 that is rather stable for many minutes at moderate temperatures and low relative humidity.

However in the presence of water (gasphase or liquid interface) sequential formation of hydroxyl radicals (-OH), hydrogen peroxides (H_2O_2), superoxide radicals (O_2-) will occure. The hydroxyl radical is extremely reactive and immediately removes electrons

from any molecule in its path, turning that molecule into a free radical and so propagating a chain reaction. But hydrogen peroxide is actually more damaging to DNA than hydroxyl radical since the lower reactivity of hydrogen peroxide provides enough time for the molecule to travel into the nucleus of the cell, where it will damage macromolecules such as DNA.
The presence of ROS at the sample interface has the highest impact of all contributions for the described experimental plasma configuration. Dry air seems to be the best precursor gas used in PDD® plasmas to generate a highly germicidal gas phase. Although ozone is only one of the more stable products ozone sensors can be used to probe the process.
Using the setup as described in figure 4, we have used different precursor process gases such as nitrogen or argon. Even though Argon leads to a more intense plasma phenomenon and a higher degree of ionization, it will only show significant effect if oxygen species are added to the process or the purge gas contains oxygen, so that secondary gas phase reaction will lead to ROS. The same has been observed using nitrogen.
We conclude that the presence of ROS is crucial for the germicidal processes.

7. Summary

For the case of indirect exposure to an air feeded PDD® plasma source, a first very qualitative summary can be drawn. The biochemical effects on the target systems of the reactive oxygen species is dominant over all other effects. Therefore the highest leverage to optimize the process efficiency will be to tune the operating conditions so that a maximum ROS concentration will be localized as close as possible to the microorganisms to be attacked. Application concepts of integrated PDD plasma sources for laminar treatment of the human body have been proposed [45]. Transport phenomena of the active species at the gashase/liquid interface that are strongly depended on the flow conditions will have to be analyzed in the future.

ROS	high
NOx, RNS	medium
H_2O_2, OH*	medium
PH	medium
UV	low
Temperature	none
Drying	none
Electric field	none
Surface Charge	none
Ion impact	none

Table 2: Shows qualitatively the contribution of different mechanisms to the effects of cold atmospheric plasma on living cells.

Species	Description	Qualitative effect
Staphylococcus aureus	Staphylococcus aureus is a Gram-positive bacterium that is frequently found in the human respiratory tract and on the skin. Although S. aureus is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. The emergence of antibiotic-resistant forms of pathogenic S. aureus (e.g. MRSA) is a worldwide problem in clinical medicine.	• Up to log5 at 3 minutes exposure to 5L/min air ionized in PDD®-device • Less than log2 at 3 minutes exposed to 40L/min intercooled PAA®-device • Low effect of humidity • No effect of UV under present setup • No clear near field effect (Low sensitivity on working distance) Compare figure 5
Pseudomonas aeruginosa	Pseudomonas aeruginosa is a common bacterium that can cause disease in animals, including humans. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal.	Starting at a poulation of 1e6 Equivalent to SA 
Klebsiella pneumoniae	Klebsiella pneumoniae is a Gram-negative, nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic conditions. Although	Starting at a poulation of 1e5 Equivalent to SA

	found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated, specifically to the alveoli resulting in bloody sputum. In recent years, klebsiellae have become important pathogens in nosocomial infections.	
Enterococcus faecium	Enterococcus faecium is a Gram-positive, alpha-hemolytic or nonhemolytic bacterium in the genus Enterococcus. It can be a coexisting organism in the human intestine, but it may also be pathogenic, causing diseases such as neonatal meningitis or endocarditis.	Starting at a population of 1e5 
Candida albicans	Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans, and candidal onychomycosis, an infection of the nail plate. Systemic fungal infections (fungemias) including those by C. albicans have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). C. albicans biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by C. albicans have become a cause of major health concerns.	Starting at a population of 1e5 More resistant than EC and SA, even at high exposition times saturation at log3 
Escherichia coli	Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.	Starting at a population of 1e6 Equivalent to SA with about factor 5 less sensitivity 
Bifidobacterium (Bifidus or "Lactobacillus bifidus")	Bifidobacterium is a genus of Gram-positive, nonmotile, often branched anaerobic bacteria. They are ubiquitous, endosymbiotic inhabitants of the gastrointestinal tract, vagina and mouth of mammals, including humans. Bifidobacteria are one of the major genera of bacteria that make up the colon flora in mammals. Some bifidobacteria are used as probiotics.	Dtarting at a poulation of 1e5. More resistant than EC and SA, even at high exposition times saturation at log3 
Bacillus atropheus	Bacillus atrophaeus is a species of black-pigmented bacteria.	Equivalent to EC 
Enterococcus faecalis	Enterococcus faecalis – formerly classified as part of the Group D Streptococcus system – is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. E. faecalis can cause life-threatening infections in humans, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance	Starting with a total population of 1e6.

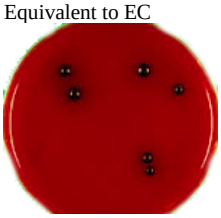
	found in E. faecalis contribute to its pathogenicity.	
Porphyromonas gingivalis	Porphyromonas gingivalis belongs to the phylum Bacteroidetes and is a nonmotile, Gram-negative, rod-shaped, anaerobic, pathogenic bacterium. It forms black colonies on blood agar. It is found in the oral cavity, where it is implicated in certain forms of periodontal disease, as well as the upper gastrointestinal tract, respiratory tract, and in the colon. Collagen degradation observed in chronic periodontal disease results in part from the collagenase enzymes of this species. It has been shown in an in vitro study that P. gingivalis can invade human gingival fibroblasts and can survive in them in the presence of considerable concentrations of antibiotics.	Equivalent to EC 

Table 3: Photographs of the petri dishes at the endpoint of plasma exposure using the PZ2 to excite dry air with a flow of 6L/min for 3 minutes.

8. Conclusion and Outlook

A simple piezo driven low-temperature plasma system allowing different operation modes and variations of gas composition was designed for a wide spectrum of biomedical applications. The described device is capable of generating stable plasma discharges of high intensity. For the interaction mechanisms with biological systems parallels can be drawn with DBD setups for which an extensive literature can be found.

As to start it seems that reactive oxygen species play the predominant role in the impact of the treatment on many of the typical microorganisms chosen in this investigation. A clear correlation wih acidity in the liquid phase was also identified. Depending on the process gas UV light contributes with a minor portion.

These findings open up the path for system optimization while using the same robust and simple PDD® technology.

The very simple device has shown in this qualitative study that a broad bandwith of microorganisms is affected with high impact. However since the dynamic mechanisms are far from beeing understood and so far data has only been generated with simplified “in vitro” model systems a lot of work has to be done. We have started to developp optimized PDD® systems for specific medical problems and to broaden both statistical evidence and fundamental understanding.

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