Nosocomial infections—a new approach towards preventive medicine using plasmas

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Abstract. A new, very efficient, large area scalable and robust electrode design for plasma production in air at atmosphere pressures has been developed and tested. This has made the development of a ‘plasma dispenser’ for hospital disinfection possible, which has certain advantages over current fluid disinfection systems. The properties of this device are presented, in particular the bactericidal and fungicidal efficiency, and the advantages are described. Such plasma dispensers could play an important role in the future fight against the alarming and growing threat posed by nosocomial (= hospital and community associated) bacterial infections.

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Disinfection and sterilization in hospitals (and also in other public areas) is of increasing concern. One reason is the growth of nosocomial (= hospital induced) infections, the second is the increase of antibiotically resistant bacteria and the third is the transmission of such bacteria, even via animals. The figures are sobering. In the US alone, about 200 000 patients per year suffer a bloodstream infection following catheter insertion [1]. About 100 000 new infections each year with multidrug-resistant Staphylococcus aureus (MRSA) are reported, resulting in 18 000 deaths annually [2]. The figures for Europe, taken from a recent EU study show 37 000 annual deaths because of hospital-induced infections [3]. Community-associated (Ca-MRSA) infections are increasing—in Canada and the US they already account for 8–20% of all MRSA infections. Animals are also affected (45% of farms in Canada) and transmission from animals to humans is possible (the USA 300 strain) [4]. New resistant bacteria, e.g. Clostridium difficile (C. difficile), an intestinal agent causing profuse diarrhea which spreads readily, are causing a sharply rising threat, and it is believed that other resistant bacteria will emerge. The reason for the increased bacterial resistance lies in the limited number of antibiotic classes. In a recent study, it was concluded that ‘of 340 antibiotic patents and 370 pipeline candidates, 75% are based on the four classes against which bacterial resistance is developing rapidly.’

The most effective method of containment is disinfection—of instruments and especially hospital staff and visitors [5]. Again some numbers are instructive: there are about 21 million surgical interventions annually, worldwide. The surgeons’ disinfection procedure—hand rubbing (3 min) or hand scrubbing (5 min)—has to be repeated many times a day, with

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2 (According to the US FDA ‘70% of bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used to treat infections’.) Other bacteria developing resistance are vaccinycin-resistant enterococci (VRE), penicillin-resistant streptococcus pneumoniae (PRSP) and coagulese-negative staphylococcus (CONS).

3 See http://www.researchandmarkets.com/reports/c80028.
a number of negative side effects arising from the mechanical irritation, chemical and, possibly, allergic stress for the skin [6], quite apart from the time required. For the hospital staff, the issue of the hand disinfection is equally daunting. On a typical working day some 60–100 disinfections are (in principle) necessary—each requiring 3 min—i.e. a total of 3–5 h! A similar issue prevails in all medical practices to a greater or lesser extent. A new, more efficient, more benign and especially faster disinfection method is urgently needed.

Plasma sterilization (of equipment) is a well-established technology in medicine. It works at the atomic/molecular level and therefore is able to reach all surfaces, including the interior of hollow needle injections and other regions not accessible to fluid disinfectants. A more recent development is the ‘design’ of biocompatible low-temperature atmospheric pressure plasmas, which can be of use for in vivo applications [7]. Most developments employ one form or other of ‘plasma jets’ or ‘dielectric barrier discharges’. The former are generally too small for large area sterilization (such as hands) while the latter require the close proximity (of the order mm) of the skin to the plasma device—the skin is in fact the second (grounded) electrode.

In this report, we describe the development of a new atmospheric plasma dispenser which is specifically designed for large area (biocompatible, e.g. hands) disinfection.

2. The plasma dispenser

The design constraints for the hand plasma dispenser are power consumption \( \leq 0.5 \text{ W cm}^{-2} \), temperature increase during operation \( \leq 5 \degree C \) above ambient (air) temperature, large area sterilization capability (>100 cm\(^2\)), scalable, more than five decades of bacterial decrease in \( \leq 10\text{s} \) (for gram-positive and gram-negative bacteria, including specifically MRSA), UV level \( \leq 1/10\text{th of the limits advocated by the WHO (ICNIRP) guidelines}, \) toxic gas production levels \( \leq 1/10\text{th WHO safe limits}, \) electrical currents \( \leq 1/10\text{th of the WHO (ICNIRP) safety levels} \) and finally, full compatibility with the CE electrical and emission standards.

The prototype, developed at MPE, is shown in figure 1. It has a total electrode area of 200 cm\(^2\) and can be scaled up (or down) to the required size. Two electrodes are placed with a separation of 4 cm. The electrode design is new—a ‘sandwich electrode’ consisting of three parts, shown schematically in figure 2—a copper sheet electrode of 1 mm in thickness, a Teflon plate of 1 mm in thickness and a stainless steel mesh electrode of 6 mesh inch\(^{-1}\). The wire diameter of the mesh electrode is 0.5 mm. The plasma is produced in the squares between the wires of the mesh electrode as shown in figure 3. The sinusoidal high voltage is applied to the copper electrode and the mesh electrode is grounded. The typical electrical parameters for this prototype model were \( V_{pp} = 18 \text{kV}_{pp}, \ f = 12.5 \text{ kHz}, \) power = 0.5 W cm\(^{-2}\) (the power was evaluated by the Lissajous figure method using 1 \( \mu \)F capacitor). The plasma is produced in many nano- and microdischarges, aided by the strong electric field produced around the wires of the mesh electrode by the potential difference with respect to the other (sheet) electrode. The operation of this electrode can best be described as a ‘BCD”—barrier corona discharge. Tests have shown that the grounded mesh electrode can be insulated or not—this does not alter the performance significantly.

The plasma produced by this device in air has a typical charge/neutral ratio of \( 10^{-9} \) to \( 10^{-10} \), the ions are at ambient (room) temperature and the electrons are at sub — ev energies. The nonequilibrium plasma chemistry is complicated, resulting in reactive oxygen and nitrogen species as well as atomic O,N and H\(_2\)O\(_2\) by interaction with water vapor. This has been verified using spectral measurements. Numerical simulations are in progress.
Figure 1. The plasma dispenser. The plasma was produced on both electrodes with a voltage of 18 kV\textsubscript{pp}.

Figure 2. Schematic of the sandwich electrode. It consists of a copper electrode, a Teflon plate and a mesh electrode. The Teflon plate is sandwiched by the copper and mesh electrodes.

In summary, the plasma dispenser is equipped with a robust, scalable, low-power electrode—a system that is easy to manufacture at reasonable cost. It operates in air at (essentially) room temperature, producing reactive (O,N) species capable of rapid bacterial disinfection.

2.1. Bacterial inactivation

It is important to ‘design’ a bactericidal (or inactivation) agent that does not impair or cause mutations in human (eukaryotic) cells. Plasmas appear to be ideally suited for this.

Firstly, there is an electrostatic effect—surfaces in contact with plasmas become charged negatively. The associated electric field depends on the size of the charged object, increasing roughly inversely with its diameter. This implies that the field (and hence the disruptive force) acting on bacteria of typical size 1 µm is much stronger than the corresponding force acting on tissue [8].

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Figure 3. The discharge produced in the squares of the mesh electrode. The black lines in the figure are the wires of the mesh electrode.

Secondly, and more important than this electromechanical disruption, is the action of reactive species (NO, OH, H$_2$O$_2$, etc) produced rapidly by non-equilibrium plasma–chemical reactions in air. These induce stress on proteins (through oxidation of sulphhydril groups, peptide fragmentation and protein damage), on lipids (e.g. through lipid peroxidation, aldehyde production), on membranes (through increased hydrophobicity and decreased membrane fluidity) and directly on DNA (by attacking base moieties, producing DNA breaks) since in bacteria the DNA is not protected by a cell nucleus as is the case for eukaryotic cells [9]).

Thirdly, there is hard UV light (the UVC band). Normal healthy skin is protected through the stratum corneum; bacteria do not have such a protection. However, for unprotected eukaryotic cells UVC may cause DNA damage, possibly leading to unwanted mutations. For this reason, it is expedient to design the plasma in such a way that the UVC emission is at least an order of magnitude below the WHO thresholds.

3. Performance tests

The most interesting performance test is the bactericidal (or perhaps more precisely, the bacterial inactivation) quality of the new device. Gram-negative (*Escherichia coli*, DSM 1116) and gram-positive (*Enterococcus mundtii*, DSM 3269) bacteria were grown in a uniform layer on agar (8.6 cm in diameter) and exposed to the plasma for different lengths of time. The distance between the agar surface and the electrode was 6 mm. The treated dish was then incubated for 18 h at 35 ºC and the number of colony forming units (CFU) was counted. Comparison with a diluted sample (by a factor of $10^4$) is shown in figure 4. It is seen that more than five orders of magnitude reduction in bacterial load is obtained in 2 s irrespective of bacterial type.
Figure 4. Number of bacteria (CFUs) surviving as a function of treatment time. For comparison the level of a $10^4$ diluted sample is shown. (a) *E. coli*, (b) *E. mundtii*.

In separate experiments, the incubation period was increased up to 48 h in order to investigate whether some ‘inactive’ bacteria might develop ‘late’ activity again. This was not the case. In relation to the typical timescales between disinfections this implies that the plasma effect may be considered to be essentially ‘bactericidal’.

The compatibility tests with regard to the WHO safety regulations gave the following results.

4. UV

The emission spectrum of the plasma-generated UV was measured using the AvaSpec-2048 Fiber Optic Spectrometer (Avantes, USA) at a distance of 6 mm from the plasma. The UV light power density was measured in the 170–340 nm wavelength range using the HAMAMATSU UV-Power Meter C8026 (HAMAMATSU Photonics K.K., Japan). From these measurements, an effective light power was calculated. The effective light power integrated with the ICNIRP weighting function was $0.43 \mu W \text{cm}^{-2}$. This is a factor 75 below the maximum recommended dose of $30 \mu W \text{cm}^{-2}$. In figure 5, the measured spectrum with 500 ms exposure time is shown.

5. Current

There is essentially no current through the skin, since the resistivity of the skin is many orders of magnitude larger than that of the exposed wire mesh electrodes, which are grounded.

6. Temperature

During the period of 5 s required for disinfection, no measurable air (+plasma) temperature increase was obtained at a distance of 6 mm from the electrode (measurements were made...
7. Toxic gas emission

The US National Institute for Occupational Safety and Health (NIOSH) recommends a ‘permitted exposure limit’ for NO$_2$ of 5 ppm and NO of 25 ppm over an 8 h period. The maximum ozone level allowed in industrial working areas (8 h a day, 6 days) is 0.1 ppm (data from IOA). The NIOSH permissible exposure limit for H$_2$O$_2$ is 1 ppm (inhalation average over 8 h). Using a ‘global’ estimate for the production of toxic gases by the plasma in air (O$_3$, NO, NO$_2$, H$_2$O$_2$, etc) and typical reaction cross sections we arrive at an upper limit for the production rate of reactive oxygen (ROS) and nitrogen (RNS) species of $10^{14}$ W s$^{-1}$. (This can be compared with measurements from an Argon plasma, which produced $10^{16}$–$10^{17}$ ROS+RNS in 300 s at around 100 W total power—i.e. around $10^{12}$ W s$^{-1}$. The Argon plasma should be less efficient at producing ROS and RNS than air plasma.) Then, taking this level of $10^{14}$ W s$^{-1}$, the plasma dispenser produces $\leq 2 \times 10^{16}$ molecules of ROS and RNS during the 2 s it takes to sterilize. One cubic meter of air contains $3 \times 10^{25}$ molecules at standard ambient temperature and pressure (STP). To reach a concentration of 1 ppm would require typically 1000 disinfections—provided there is no air exchange. These numbers imply that the plasma device remains well below the currently approved safety limits for reactive species. In spite of this, in order to avoid build up of (in particular) Ozone to a possibly uncomfortable (but still safe) level, the plasma dispenser will be equipped with a fan and filters, which will extract the reactive gases from the treatment volume after each disinfection. The aim is to ensure that reactive species escaping into the ambient air can be maintained at less than 1% of the approved safety levels.
Figure 6. Photos of agar plates with *E. coli* after 18 h incubation at a temperature of 35 °C. The original cultures (with about 20 million bacteria) were treated by the convected (plasma air) flow from the plasma dispenser as described in the text. No bactericidal effect is seen after 5 and 15 s, respectively. After 30 s, a strong disinfection effect (about $10^5$ reduction) is observed, caused by the arrival and bactericidal action of the reactive species produced by the plasma.

In order to determine the ‘afterglow’ time for extracting surplus reactive species from the treatment (or plasma) volume, a fan (DC 12 V, 80 × 80 × 25.4 mm, 2900 RPM, 21.96CFM) was mounted at the back end of the plasma dispenser. The plasma was ignited and simultaneously the air was blown out the other side onto a Petri dish. Measurements were made for the time it took for the reactive species to arrive 15 mm from the opening simply by noting when bactericidal effects set in. At 15 s there was no effect, after 30 s about $10^5$ reduction in bacterial load was measured (originally there were $2 \times 10^7$ bacteria on one plate). This shows that the surplus reactive species can be extracted on time scales of about 20 s and defines the required filtering period following plasma disinfection (figure 6).

8. Bactericidal effect through textiles

To inactivate bacteria by plasma through (fibrous) textiles is an interesting topic because it can show many important parameters for developing the device, e.g. diffusion, shielding and absorption of the active components relevant for bactericidal effects. Moreover, this test is important for the practical use, e.g. treatment of athletes foot and fungi in general (see below). If plasma disinfection works even through fibrous textiles, then this would open the way for
very simple mass treatment options, where patients do not need to remove their socks. Figure 7 shows bactericidal effects with the *E. coli* inoculated on a ‘naked’ agar plate and that covered with a sock. The original number of bacteria on the plates was again \(\sim 10^7\). The photos show that the bactericidal effect is reduced by the textile (sock) material as expected, but the decrease is not dramatic. The bacterial load reduction on the uncovered plate with 2 s treatment was similar to that on the covered plate with 15 s treatment (\(\sim 10^5\) reduction). The result suggests that the active components responsible for the bactericidal property are predominantly molecular—they are not quenched significantly by the obstacle.

The plates originally contained \(\sim 10^7\) bacteria. After plasma treatment, the plates were incubated (35 \(^\circ\)C, 18 h) and the CFU’s were counted, the result for the uncovered specimen (left) is \(\sim 120\) CFUs, for the covered specimen (right) \(\sim 130\).

9. Treatment of fungi

It has been shown in previous experiments [10] that low-temperature atmospheric plasmas have a fungicidal effect of similar efficiency as the bactericidal effect. The new plasma dispenser designed for hospital disinfection (as well as other public places) is no exception. Experiments with *Candida albicans* (ATCC 90028) were carried out. There were about \(2 \times 10^6\) fungi on the agar initially—checked by the dilution technique. After 5 s of plasma treatment the fungi are reduced to about \(10^{-4}\) of the original level. This is in agreement with our earlier findings using other plasma sources.

10. Conclusion

We have succeeded in developing a very efficient large area scalable plasma dispenser that can be used for disinfection in hospitals and other public or private areas to reduce the spread of bacterial and fungal diseases. Plasma disinfection decreases the standard (rubbing or scrubbing) times for surgeons and assistants to a few seconds rather than the currently required several minutes for hand washing or scrubbing. Plasmas have the potential to provide a more thorough disinfection through their ‘molecular’ rather than fluid application (compare the use of plasmas in capillaries and our measurements through fibrous materials) and they should be less subject to causing skin irritations of all kinds. We have shown that the device operates far below the WHO
safety levels in all important respects (UV, toxicity, electromagnetics). It could become a major weapon in the fight against hospital (nosocomial) and community associated (Ca) bacterial infections and thus save many lives in future.

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