# Cold plasma for bacterial inactivation

R.S. Tipa[1], B. Boekema[2], E. Middelkoop[2], and G.M.W. Kroesen[1]

[1] *Eindhoven University of Technology, Eindhoven, The Netherlands*

[2] *Association of Dutch Burn Centres, Beverwijk, The Netherlands*

[3] *Plastische Reconstructieve en Handchirurgie VUMC Amsterdam, The Netherlands*

***Abstract:***

Non thermal plasmas had been used for many years for different applications. Plasma is a cocktail of reactive oxygen species, negative species, radicals etc. This mixture has a positive effect also in killing bacteria. This fact is highly important in applications like disinfection of burned wounds where large skin areas need to be cleaned before the treatment. In this work we studied in vitro the effects of cold plasma treatment in inactivation of bacteria. The inactivation mechanism was investigated using two types of bacteria: Gram Positive and Gram Negative. Bacteria were cultured in logarithmic concentrations in Petri dishes. Three types of plasmas were applied on the bacteria for different time points. After treatment, the plates were incubated overnight. The colonies formed were counted next day For the treatment, a cold atmospheric plasma needle (13.56 MHz micro-jet) was used. We analyzed the influence of cold plasma treatment in killing the bacteria. Three types of gases were used in our investigation. Under high doses bacteria suffered a damage which leaded in a decrease in the number of colonies.

***Keywords:*** cold plasma, bacterial inactivation

## 1. Introduction

Infected wounds represent a high research interest in wound management. Although some type of wounds are healing without leaving a scar. The main problem is the bacterial contamination of the wounded area which can lead to inflammatory reactions, delayed re-epithelialisation, impaired matrix remodeling. However not all the bacteria present in the wounded area behave the same. Due to the fact that mainly Pseudomonas Aeruginosa and Staphylococcus Aureus represent a clinical concern and are resistant to antibiotic therapy, a lot of interest was directed in finding new efficient therapies.

A biocompatible plasma is defined here as a plasma that is able to induce non lethal effects on cells or tissue. In general one expects from medical treatment that damage to the living organism should be avoided or at least minimized and that the final outcome of the treatment is beneficial to the organism in general. A plasma that complies with these demands is difficult to obtain. It has to be 1) non-thermal with a temperature preferably around 37°C, 2) at atmospheric pressure, 3) not extremely toxic and 4) electric fields cannot be too high.

Plasma possesses several properties which are detrimental to bacteria. Therefore the development of non thermal plasmas sparked the idea of using plasma for disinfection purposes. In the previous decade research was done to assess the disinfection power of these non thermal plasmas. [1, 2] Non thermal plasmas are very effective in killing bacteria. This makes these plasmas very useful for various biological and medical applications such as sterilization of medical instruments, decontamination in biological warfare and filtering of air or water.

Understanding of the working principles of plasma sterilization will give valuable insights in the effects of plasma on biological material. Research has shown that four inactivation factors are involved in the destruction of pathogens and chemical agents: heat, ultraviolet radiation, reactive neutral species and charged particles.

- Heat

It has long been known that heat has detrimental effects on living cells. Heat leads to the ultimate death of all living forms by destroying the cellular metabolic system, which includes enzymatic components. In heat-based conventional sterilization methods both moist heat and dry heat are used. In the case of moist heat, such as in an autoclave, a typical temperature of 121°C at a pressure of 15 psi is used. Dry heat sterilization, such as in an oven, requires temperatures close to 170°C and treatment times of about 1 hour. Wet-heat systems require water content sufficient to produce 100% relative humidity at the sterilization temperature.

- Ultraviolet radiation

Ultraviolet (UV) radiation is known to kill bacteria. Wavelengths in the 220-280 nm range and doses of several mJ/cm2 are known to have to optimum effect. However UV radiation is said to play a significant role in low pressure sterilization, but it is of less importance in atmospheric plasmas. In particular VUV photons (<180 nm) are strongly absorbed by air at atmospheric pressure, which prevents them from reaching the sample.

- Reactive neutral species

In high pressure non equilibrium plasma discharges, reactive species are generated through various collisional pathways, such as electron impact excitation and dissociation. Reactive species play an important role in all plasma-surface interactions. Many researchers concluded that reactive species play an important role in the sterilizing mechanism of plasma. [4, 5] Research has shown that discharges containing oxygen have a strong germicidal effect. This is due to the presence of reactive oxygen species (ROS).

- Charged particles

Charging of the cell membrane is believed to have effect on cells. Mendis suggested that charged particles might play a significant role in the rupture of the outer membrane of bacterial cells, although this is disputed. [3] They showed that the electrostatic force caused by charge accumulation on the outer surface of the membrane could overcome the tensile strength of the membrane and cause its rupture. Another explanation might be that electrons attach to the membrane structure and change the membrane via electron reduction. This increases permeability of the membrane and eventually leads to the destruction of the cell. Research is needed to validate these hypotheses.

Plasma sterilization research provides some insight in the mechanisms that play a role in the effect of plasma on cells. Although it is very important to realize that the aim of sterilization research is very different than that of medical research. Plasmas used in sterilization research are designed to maximize bacterial cell death and thus possesses lethal intensities. Furthermore sterilization can be done under low pressure conditions while treatment of tissue can only be done at atmospheric pressure.

One step beyond sterilization of non living material is the idea of actually disinfecting or more in general (since the effects of plasma on living tissue are unclear at this moment in time) treating living tissue. Plasmas used for these purposes are designed to keep cells alive. Effects induced by such plasmas are therefore termed non lethal effects or sub lethal effects. Intensities of these medical plasmas are much lower (~100 mW) than those used in sterilization research (~ 1 W). And because of the extreme non linearity of plasmas and the complex cellular machinery one cannot easily extrapolate effects from sterilization research. Furthermore bacterial and mammalian cells are completely different, which justifies this statement even more. It is therefore impossible to predict the effects of low intensity plasma on tissue.

## 2. Materials and method

Making the agar plates

1. Remove sterile Petri dishes (VWR 25384-208) from plastic bag.

2. Pour a thin layer (5mm) of LB Agar (~10mL) into each plate.

3. Swirl plate in a circular motion to distribute agar on bottom completely.

4. Each plate cool until its solid (~20 minutes) then flip so as to avoid condensation on the agar.

5. Store plates in plastic bags in fridge with: name, date and contents (note any additive).

Pseudomonas Aeruginosa (gram negative) and Staphylococcus Aureus (gram positive) were cultured and counted before inoculation. More dilutions were made in the range 10-1000 bacteria / agar plate. Plasma treatment of the plate was applied.

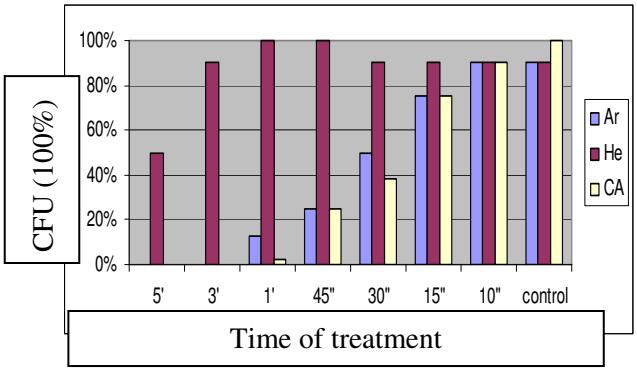
We used for our measurements 3 types of gases: Helium, Argon and compressed air. The treatment time varied between 1 minute and 5 minutes of treatment.

Plates were incubated overnight after plasma treatment. Next day, the Colony Forming Units (CFU) were counted in order to check the efficiency of bacterial inactivation using cold plasma treatment.

## 3. Results and discussion

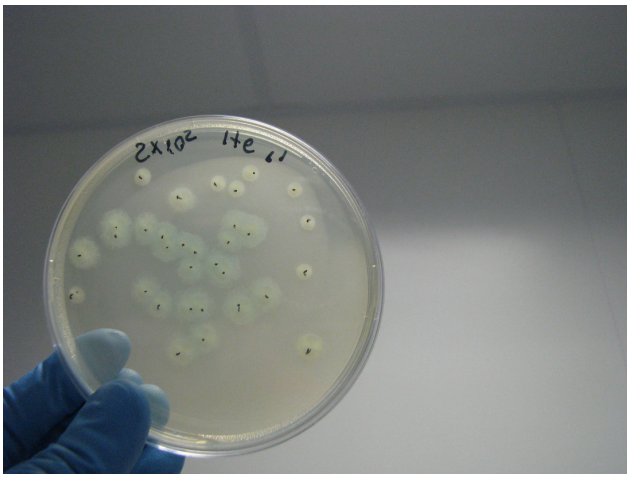
In Fig. 1 one can see the effects of cold plasma treatment on S. Aureus.

**Figure 1.** Effects of c*old plasma treatment effects on S. Aureus.*




In the graph the result of the counting of the CFU after plasma treatment is presented. One can see that, compared with the control, plasma treatment always results in a reduction of the number of CFU's. Helium seems to be the least efficient. Another observation is the fact that the shape of the colonies also changes after the plasma treatment. Argon is a more efficient bacterial inactivator. After 1 minute of argon plasma treatment, one can see a reduction in the number of colonies. For our experiments, compressed air plasma gave the best results in bacterial inactivation. Already after one minute of compressed air plasma treatment there is almost no bacteria left on the plate. Increasing the treatment time to 3 minutes or 5 minutes leads to complete inactivation.

**Figure 2.** *Cold plasma treatment effects on S. Aureus.*



In Fig. 2 one can see the structure modification of the bacteria due to the helium plasma treatment. We applied 1 minute treatment on bacteria culture. After treatment, the plate was incubated for one day and CFU counted after 24 hours from the treatment.

**Figure 3.** Comparison between the effects of 3 types of plasma treatment on 2 different types of bacteria

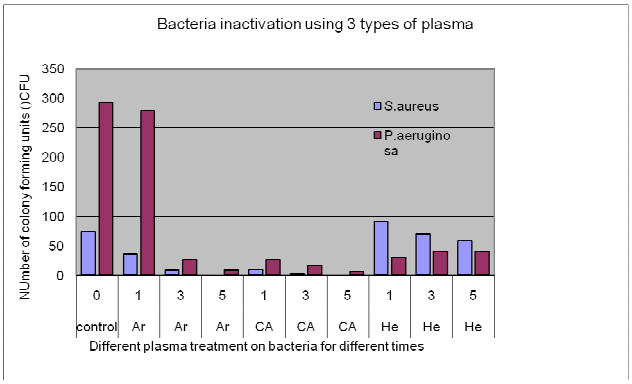
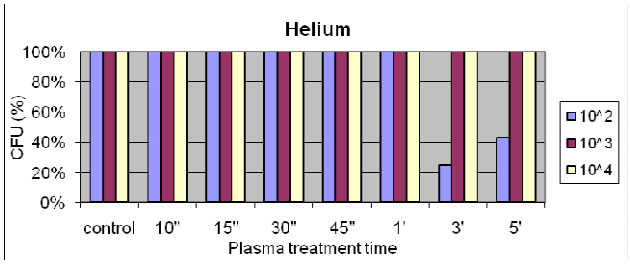
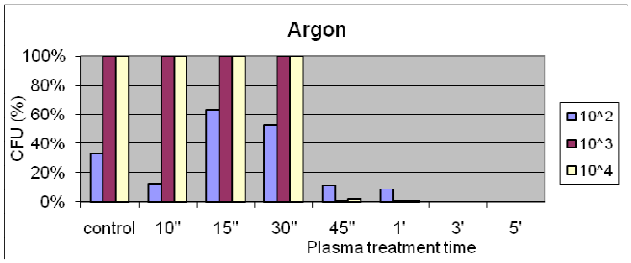



**Figure 4.** *Helium plasma treatment effects on Pseudomonas Aeruginosa.*



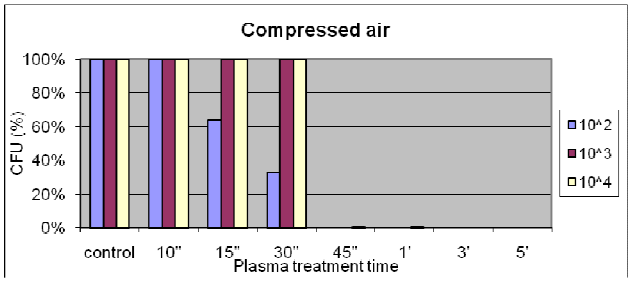

In the graph from In the graph from Fig. 4 one can see that helium plasma treatment was not very efficient. 3 different concentrations of bacteria were used, and even after 5 minutes of treatment many CFU's are present.

**Figure 5.** *Argon plasma treatment effects on Pseudomonas Aeruginosa.*




In the case of argon plasma, even after 45 seconds of plasma treatment one can observe a reduction in the number of CFU. Increasing the plasma treatment time lead to a decrease in the amount of CFU.

**Figure 5.** *Compressed air plasma treatment effects on Pseudomonas Aeruginosa.*




In the case of compressed air plasma, after 45 seconds of plasma treatment one can observe that there are fewer CFU's. 3 minutes of compressed air plasma completely inactivated the bacteria present in the plate.

Gram positive bacteria (S. Aureus) seem to be more sensitive to plasma treatment than gram negative bacteria (P. Aeruginosa).

Different mechanisms have been proposed in the literature [4] to explain different sensitivity of different bacterias against plasma treatment. The cocktail of reactive species produced by the plasma (NOx, H2O2, OH radicals, ozone, UV etc) triggers the breaching of the membrane. Apparently, the thick lipolysacharide outer membrane of gram negative bacteria is a better shield to reactive plasma species than the thinner peptidoglycans membrane of gram positive bacteria. Under high doses, both kind of bacteria suffer damage, which lead to a decrease in the number of colonies. Best results were obtained with Argon and compressed air, while Helium was hardly effective against bacteria.

## 4. Conclusion

Experiments analyzing a comparison between the helium, argon and compressed air plasma effects on bacterial inactivation were investigated. Helium plasmas are not very efficient: even after 5 minutes of treatment a lot of CFU's were still present on the plate. Promising results were obtained for argon or compressed air plasma. The results suggest that the composition and thickness of the outer cell membrane play an important role in the process of plasma based bacteria inactivation.

## References

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