Abstract

The technological potential of non-thermal plasmas for the antimicrobial treatment of heat sensitive materials is well known and has been documented in a great number of research activities, but the realisation of industrial plasma-based decontamination processes remains a great challenge. One of the reasons for this situation is the fact that an antimicrobial treatment process needs to consider all properties of the product to be treated as well as the requirements of the complete procedure, e.g. a reprocessing of a medical instrument. The aim of the BMBF-funded network project PLASMOSE is to demonstrate the applicability of plasma-based processes for the antimicrobial treatment on selected, heat sensitive products. Modular and selective plasma sources, driven at atmospheric pressure are used. This basic approach shall combine the technological advantages of atmospheric pressure plasmas (avoidance of vacuum devices and batch processing) with the flexibility and handling properties of modular devices.

Two different objectives were selected: the outer surface treatment of medical products and the treatment of hollow packaging for pharmaceutical products. The outer surface treatment of medical products, in particular catheters for intracardial electrophysiological studies, is investigated by means of RF-driven plasma jets in argon. Due to its compact design they are predestined for modularisation and can be adapted to nearly any complex 3-dimensional structure as given by the medical products. The realisation of an antimicrobial treatment process of hollow packaging for pharmaceutical products has quite different demands. Such a process is needed to be implemented in in-line filling procedures and to work without additional process gases. The idea is to use an atmospheric air, microwave-driven self propagating discharge. The plasma process is optimized for the decontamination of 200 ml bottles by field simulation studies combined with optical emissions spectroscopy and micro-biological tests.

Keywords: plasma jet, microwave plasma, sterilization, decontamination, non-thermal plasmas
ten Prozesses zur antimikrobiellen Behandlung von thermolabilen Produkten. Dazu werden modulare selektive Plasmaquellen bei Atmosphärendruck eingesetzt. Der Ansatz beruht auf der Kombination der technischen Vorteile eines Atmosphärendruckplasmas mit den Vorteilen bzgl. der Flexibilität und der Handhabungseigenschaften von modularen Vorrichtungen.

Zwei unterschiedliche Aufgaben wurden als Zielstellung ausgewählt: die Außenbehandlung von Medizinprodukten und die Innenbehandlung von Hohlkörpern als Primärverpackung für pharmazeutische Produkte. Die Behandlung der äußeren Oberflächen von Medizinprodukten, in diesem Fall Kathetern für intrakardiale elektrophysiologische Untersuchungen, wurde mittels eines RF angeregten Plasmajets in Argon durchgeführt. Aufgrund seiner kompakten Ausführung ist er prädestiniert für eine Modularisierung und kann dadurch nahezu an jede dreidimensionale Struktur angepasst werden.

Die Realisierung der antimikrobiellen Behandlung der als Primärverpackung dienenden Hohlkörper hat deutlich andere Prozessanforderungen. Dieser Prozess muss in einer mit hohen Taktraten arbeitenden Abfüllmaschine integrierbar sein und darf aus Kostengründen keine teuren Arbeitsgase benötigen. Daher wurde zur Realisierung eine mikrowellenangeregte Laufentladung eingesetzt, die in Umgebungsluft arbeitet. Der Prozess wurde für die Behandlung von 200 ml PET Flaschen optimiert. Hierzu wurden sowohl Simulationen des elektrischen Feldes als auch optische Emissionsspektroskopie durchgeführt. Begleitet wurden diese Untersuchungen durch mikrobiologische Tests zur Bestimmung der Abtötungskinetik.

Schlüsselwörter: Plasma Jet, Mikrowellenplasma, Sterilisation, Dekontamination, nicht-thermisches Plasma

Introduction

The technological potential of non-thermal plasmas for the antimicrobial treatment of heat sensitive materials is well known (see e.g. [1], [2], [3], [4], [5], [6]. However the realisation of industrial plasma-based decontamination processes is still a challenge for research and development. This intended the BMBF-funded network project “PLASMOSE” (German: Plasmagestützte Oberflächenmodifizierung mittels modularer selektiver Plasmaquelle) consisting of ten partners with the aim to demonstrate the applicability of plasma-based processes for the antimicrobial treatment on selected, heat sensitive products. Modular and selective plasma sources, driven at atmospheric pressure are used, since they combine the technological advantages of atmospheric pressure plasmas (avoidance of vacuum devices and batch processing) with the flexibility and handling properties of modular devices. Since several years an increasing demand for new antimicrobial processes is registered. Among others the following two objects are of great interest:

1. The complexity of medical devices is continuously increasing and contains the use of thermo labile materials (Figure 1), which can not be treated by means of classical sterilisation methods.

2. In packaging industry for food and pharmaceutical products biological decontamination is requested in order to reduce or even to omit preservatives.

For both above mentioned objectives the antimicrobial process must be able to treat thermo labile materials such as polyethylene (PE) or polyethylenterephthalat (PET). The high complexity of the most medical products, e. g. instruments for micro invasive surgery, requires an antimicrobial process which is capable of the treatment of small gaps and lumina which causes the challenge for most new technologies. The packaging materials are in most cases simple structures as foils or hollow compartments, e. g. bottles, and require a cost efficient and very
fast process. In beverage industry, for example, the treatment time for a new technology has to be below 3 s for a single bottle to be competitive to existing processes mainly based on hydrogen peroxide.

In order to fulfill the divergent requirements of biological decontamination processes for the two above mentioned selected examples individual solutions have to be investigated. Although low pressure plasma devices are widely examined and the processes causing the antimicrobial effect are fairly well understood the main drawback is the missing ability of treating gaps and lumina with high aspect ratios. The reason for this is, that the main effect is based on UV interaction in combination with the surrounding air is observed.

However, even in this case the plasma is significantly shortened, but the impedance matching of the nozzle outlet. In this configuration the plasma jet has a length of about 25 mm measured from the nozzle outlet and a diameter of about 8 mm for the conditions being considered. It has been shown by spatially resolved optical emission spectroscopy and short exposure time photos that the plasma is filamentary and “active”, i.e. high energetic electrons are present at the entire length of the jet (Figure 2, right) [7]. Along the plasma jet a gradual mixing of the argon gas with the surrounding air is investigated.

Instead of using the substrate as virtual grounded electrode a grounded ring electrode can be placed directly at the nozzle outlet. In this configuration the plasma jet is significantly shortened, but the impedance matching of the system is more stable and less sensitive to stray capacitances. However, even in this case the plasma is filamentary, but additionally, a diffuse shine is observed at the nozzle outlet. Due to the reduced length of the plasma outside the nozzle less mixing of the argon gas with the surrounding air is observed.

Several single plasma jet modules can be arranged in arrays to treat surfaces of objects having complex geometries such as the catheters. Therefore the jets can be mounted in a ring-like structure for example (Figure 3). For the outer treatment of catheters or cables the jet nozzle can be equipped with a “T-type” adapter made of glass alternatively (Figure 4). The plasma is generated inside the glass adapter, while the catheter is guided via the apertures at both sides of its vertical part. The generated plasma surrounds the outer surface of the catheters more or less completely.

These different jet configurations can be implemented as modules in a treatment device, shown in Figure 5 and Figure 6, to demonstrate the applicability to real medical devices as e.g. catheters (Figure 1). In this device the catheter is fixed in horizontal direction and a plasma module is moved along the catheter.

**Results and discussion**

Optical emission spectroscopy (details in [7], [8]) has shown that several possible microbicidal components are generated by the plasma jet, namely

1. VUV-radiation by means of emissions mainly from Ar* eximers
2. UV-radiation by means of molecular bands emissions mainly from of excited NO, OH and N₂ molecule
3. reactive oxygen species such as O-atoms and OH-radicals and
4. low molecular chemical products, e.g. nitric oxide NO.

In fact ozone O₃ is produced as well, but not determined by its optical emissions.

To test the antimicrobial effect of the plasma jet, PE-strips (8x32 mm) are inoculated punctually (diameter of contaminated area about 5 mm) with a suspension of vegetative bacteria (Escherichia coli) or endospores (Bacillus atrophaeus), respectively, dried under aseptic conditions and treated by the plasma jet modules, described in Figure 2. The results are shown in Figure 7 and Figure 8. Plasma jet treatment of spores and vegetative bacteria on PE-strips results in an increasing time-dependent reduction of the microorganisms. Depending on the initial contamination and the detection limit of microorganisms, recovery given by the methods (colony counting method: 100 cfu/strip (cfu – colony forming units), see [7]; membrane filtration: 1 cfu/strip) a maximum reduction between 4 and 6 orders of magnitude is investigated. In further studies discussed elsewhere the best decontamination was achieved by a direct plasma treatment of the PE-samples. It was pointed out that UV-radiation cannot solely be responsible for the inactivation of microorganism [7]. Thus, a co-action of excited reactive species, partially assisted by UV and VUV radiation, heat (a moderate but significant heating of the jet can give a certain effect on heat-sensitive bacteria) and low-molecular chemical products can be assumed. However, comparing Figure 7 and Figure 8, the plasma jet with the ring electrode (jet 2) seems to be less effective than the jet without ring electrode (jet 1). For example for E. coli after 4 min of treatment with the jet 1 the detection limit of...
100 cfu/object is reached. Using jet 2 this value is reached after 7 min. This difference can be understood by taking into account the reduced production of reactive oxygen species and chemical compounds as well as the lower energy input on the contaminated area by using jet 2. Furthermore the initial contamination can hardly be controlled in our procedure and thus, it is slightly different for both experiments. This may have an influence on the reduction of microorganisms, too.
Figure 5: Principle of the treatment device

Figure 6: Realisation of treatment device: left: 3D drawing of the construction, right: photograph of the treatment device

Figure 7: Median number of surviving microorganisms after treatment with plasma jet 1 (jet without ring electrode, 20 W, 20 sIm Ar, 25 mm distance nozzle-substrate) of B. atrophaeus spores and E. coli on test strips (dashed line: detection limit of microorganism recovery, 100 cfu/strip)

Figure 8: Median number of surviving microorganisms after treatment with plasma jet 2 (jet with grounded ring electrode, 20 W, 20 sIm Ar, 6 mm distance nozzle-substrate) of B. atrophaeus spores, E. coli and S. aureus on test strips (dashed line: detection limit of microorganism recovery, 1 cfu/strip)
To investigate the efficiency of antimicrobial plasma treatment by means of plasma jets on catheters they are divided in 6 sections (each 6 cm long). Each section is contaminated with a suspension of vegetative bacteria (Staphylococcus aureus). The first five sections of the catheter are treated with the plasma module (1 or 2 cycles). The 6th section is not treated and used to determine the initial contamination (control). The treatment was performed by the “T-type” jet module (10 W, 20 slm argon with and without air admixture of 0.25 vol.%) are shown in Figure 9. A reliable reduction of microorganisms between 4 and 6 orders of magnitude on the catheter sections is shown in Figure 9. On some catheter sections no viable counts are found after plasma treatment, i.e. the minimum values are at the detection limit for all conditions. But on some objects there are still survivors, thus the maximum and the median values (data points) are somewhat higher. The spread between minimum and maximum values decreases if air is admixed to the argon gas. In this case 3 or more objects of 5 are free of viable microorganisms, thus the median is at the detection limit. This tendency may be explained by a higher number of reactive oxygen species due to the air admixture. These results demonstrate that the findings of the treatment of plastic strips can principally be transferred to the treatment on a real medical instrument, but however the stability and reproducibility is not satisfying at the moment. The investigations lead to the assumption that a proper conditioning of the process is necessary. To investigate the effect of a hydrated surface or environment of the microorganisms a first preliminary investigation has been performed. About 1 ml of distilled water was filled in a petri dish and treated by the plasma jet (jet 2) for up to 20 min. After several times of treatment the pH of the water was measured by test strips. The results are shown in Figure 10 (left). It is clearly seen, that an acidification is caused by the argon plasma jet treatment. In the experiment, the initial pH of 5.5 decreases to 2. It has to be mentioned that the amount of water in the Petri dish was reduced during the treatment due to evaporation and nebulation. Therefore the slope of the curve in Figure 10(left) is not linear. The correlation of acidification and antimicrobial effects is shown in the right diagram of Figure 10. In this experiment an initial number of B. atropheus spores was given into solution with a defined pH for several minutes and the recovery of the microorganisms was checked. For pH <5 a significant reduction effect is investigated. For pH=2 about 6 orders of magnitude reduction is observed. It is known that nitride oxides in contact with wet films or droplets lead to an acidification through different mechanisms. In the gas phase nitrogen dioxide NO₂ can react with OH-radicals forming nitric acid HNO₃ which dissolves into the aqueous phase. NO₂ can be dissolved into aqueous phase forming nitrous acid HNO₂ and HNO₃ by the reaction with H₂O [9]. In a similar way the formation of HNO₃ via reactions with nitrogen peroxides N₂O₅ or NO might be possible. Another possibility are reactions or processes involving (hydrated) ions. However, the interaction between a plasma and a liquid is quite complex and further investigation are necessary on this issue.

Decontamination of hollow packaging

Methods

The basic scheme of the plasma source here described and the treatment process is given in Figure 11. The device consists of a multimode waveguide structure which serves as process chamber and an ignition device which is mounted on a moveable lance. The microwave radiation is generated by a magnetron (frequency 2.45 GHz; power up to 1.7 kW) and is coupled to the waveguide via a coupling antenna. The alignment of the magnetron frequency to the process chamber geometry is performed via a moveable shorting plunger. The PET bottle is placed in the centre of the process chamber, the lance with the ignition device is driven into the bottle and by applying the microwave field the plasma is ignited in their bottom region (see Figure 11, right). After the ignition of the plasma the lance is moved to its initial position outside of the bottle and the plasma moves freely to the neck of the bottle [10]. The plasma is generated in ambient air (i.e. with certain humidity) at atmospheric pressure. The shape of the plasma is predominantly given by the field configuration in the process chamber, respectively the modes which can propagate in the waveguide. Therefore simulations of the field distribution in the device by means of the CST program MicroWave Studio allow a rapid development and optimization of new designs.

Results and discussion

A very stable plasma ignition at atmospheric pressure is performed by an ignition pin similar to the electrode microwave discharges, described e.g. in [11]. The main difference of the device presented here is the launching of the plasma from the ignition device. This is mainly driven by convection. After the launching the plasma expands and moves self propagating through the waveguide and thus the hollow compartment. In Figure 12 the propagation of the plasma is shown. The plasma luminosity in the centre of the process chamber as a function of position and time is shown in the left plot of Figure 12. At certain times, the snapshot of the full waveguide is shown (plots at the right). As can be seen in the snapshots, the propagating plasma has a diameter of about 40 mm and a length of 50 to 80 mm. The propagation velocity is about 0.5 m/s. The modulation of the plasma propagation and luminosity is clearly seen in the left plot of Figure 12 and corresponds with the maxima and minima of the standing microwave field distribution in the waveguide which is shown in Figure 13. In the vicinity of the ignition pin the plasma is localized.
Figure 9: Median number (n=5) of surviving microorganisms (S. aureus) after treatment with “T-type” plasma jet module (10 W, 20 slm Ar) on catheter parts (error bars: spread between minimum and maximum values; dashed line: detection limit of 1 cfu/object)

MO: Staph. aure.
"T-type Jet":
- P= 10 W
- Q= 20 slm Ar
- f= 27 MHz

Figure 10: Acidification of water by plasma treatment and the effect on B. atrophaeus spores

Figure 11: Propagating microwave plasma device (left) and plasma treatment of bottles (right)
and expansion takes place after launching during the movement to the antenna. The velocity of the plasma propagation can be controlled by the amount of absorbed power in the plasma which heats the plasma and leads therefore to convection. Besides this movement by material transport there is also a propagation of the plasma state. This is caused by the strong absorption of the microwave field in the front of the plasma and therefore a high ionisation rate in this plasma region is generated [12]. This effect can be easily demonstrated by coupling the microwave power from the bottom of the process chamber. Then, the plasma starts after ignition to move towards the microwave source. During this movement the plasma expands and as soon as the convection force becomes dominant, the direction of movement changes. The microwave field distribution has to be adapted to the specific compound and purposes of the treatment; otherwise a local thermal deformation of the bottles occurs.

Optical emission spectroscopy was carried out to characterize the plasma. The radiation emitted by the plasma is coupled to an optical fibre leading to an optical multichannel analyzer (spectrometer with an ICCD-camera as detector). The overview spectrum of the UV-range is shown in Figure 14.
The UV spectrum between 200 nm and 400 nm of the microwave-induced plasma in air is dominated by two molecular bands, namely the γ-system of NO (transition $A'\Sigma^+ \rightarrow X''\Pi$) and the Å-system of OH ($A'\Sigma^+ \rightarrow X''\Pi$). The latter can be used as a “molecular pyrometer” in order to estimate the rotational temperature $T_{rot}$ of the OH-molecules [13], [14], [15], [16]. For the conditions being considered (high pressure and thus high collision rates) $T_{rot}$ is close to the gas temperature. To estimate $T_{rot}$, the OH-spectrum at 309 nm is measured with a resolution of about 0.2 nm (see Figure 15). From the intensity ratio of the peaks $G_{ref}/G_0$ and $G_{ref}/G_1$ respectively, $T_{rot}$ can be taken from the values tabulated in [15], which are based on the assumption of a Boltzmann equilibrium and the calculation of population densities. Although this method has previously been shown to be unqualified to examine small temperature gradients in a plasma, the described approach is a simple and easy method to get information on the gas temperature. It is in agreement with the observed propagation of the plasma forced by convection. Although the temperature of the microwave excited plasma is such high, the treatment of plastic materials is possible with an upper limit for the treatment temperature of about 330 K. This effect may be explained by the diffuse shape of the plasma with a low heat capacity and the fast movement of the plasma.

The propagating microwave plasma has many similarities to plasma torches driven at atmospheric pressures [14]. NO is formed due to the plasma chemical reactions at high power input [18]. Thus, the following possible lethal components of the plasma can be identified namely (i) UV-radiation, (ii) low molecular chemical reaction products (mainly nitride oxides NO), (iii) reactive oxygen species (O-atoms and OH-radicals) and heat.
Contaminated PET-bottles are treated by the plasma three times, while each plasma cycle is about 550 ms long and characterized by a dissipated energy of about 300 J/cycle. After plasma treatment the bottles are scrubbed with 10 ml of a rinsing solution (water with NaCl and Tween 80). The resulting elution undergoes the classical microbiological test procedures (count plate method or membrane filtration). The antimicrobial effect of the plasma is demonstrated in Figure 17.

Figure 17: Median number of surviving microorganism after treatment of PET-bottles with microwave plasma in air (error bars: spread between minimum and maximum values), detection limit: 1 cfu/object

Depending on the microorganism, 5 to 7 orders of magnitude of reduction are observed. Note that the total plasma-on time is about 1.5 s only. Therefore, with respect to further needed improvements, the device is applicable in an in-line aseptic filling procedure.

In order to simulate the filling process the time between plasma treatment and the elution (more precisely between the end of the last plasma cycle and filling of the bottle with scrubber solution) was varied. The results of this experiment are shown in Figure 18. The reduction of vegetative bacterium S. aureus is independent of the time between plasma treatment and scrubbing. At least 5 orders of magnitude of reduction is observed, even after one plasma cycle and no matter on the fact, that the bottle is eluted immediately (within about 4 s) or after about 30 min. The more resistant endospores of B. atrophaeus are not significantly reduced by the plasma treatment alone. The reduction is time dependent and even after 15 min there are still survivors in some bottles. This effect may be explained by the action of the NO\(_2\) residual gas on the spores. Furthermore residual nitrous gases can be eluted by the rinsing solution leading to acidification (mainly due to formation of HNO\(_3\)). This could already affect the reduction of bacteria. To exclude such effects buffered rinsing solution was used instead of water. The results are shown in Figure 19. The difference between buffered and non-buffered rinsing solutions is clearly seen for both vegetative bacteria under consideration. With buffer the effect of the residual gas is needed for a significant reduction of the microorganisms. This has also been found for B. atrophaeus endospores. If the bottles are blown off from the residual gas before scrubbing no such a difference is observed. Thus the hypothesis of an acidification of the rinsing solution by residual plasma gas can be confirmed.

The above mentioned effects need to be considered in the further process design. Furthermore they demonstrate that even the microbiological test procedures can have
an influence on the final results or can do a misleading of the process effectiveness.

**Conclusion**

In this work two different types of atmospheric pressure plasma sources are described. In principle both of them can be applied in industrial processes since they are properly adapted on the specific products and process requirements. The products and demanded processes are as different as the plasma sources themselves. On the one side with the PET bottle decontamination a low cost application for in-line treatment with high process rates was presented. On the other side the application of plasma jets in the special reprocessing of expensive medical devices was shown.

In both cases the principle capability and effectiveness of the plasma sources for biological decontamination up to the level of sterilization (i.e. reduction of germs by 6 orders of magnitude) could be demonstrated. It was also shown that the plasma processes can be adapted to real products, e.g. intracardial catheters. But the investigation has also shown, that indirect plasma chemical induced processes (e.g. acidification due to nitride oxides) can have an influence on the microbicidal effects. Even the microbiological analysis procedure can interfere with the plasma process and contort the microorganism reduction results. Such effects need to be known and considered in the design of industrial processes.

In the processes a careful conditioning is necessary in order to provide the process stability and reproducibility.

In order to improve plasma methods to stable and applicable plasma processes, a detailed understanding of the plasmas and its interaction with biological matter is provided.

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