Report on Visit to Ruhr-University Bochum by International Training Program

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As a long-term placement program of ITP (International Training Program), I had studied in the Prof. Awakowicz's laboratory (AEPT) in RuB (Rurh-University Bochum) of Germany for two months, from November 23, 2012 up to January 21, 2013. This is my IPT life report.

Research

(a) Research theme

In my recent study, plasma etching of gallium compound semiconductor is analyzed by using the plasma beam-apparatus. My research aim is to realize damage-less etching technology for nano-meter scaled electronic devices. In perspective views in this field, I recently knew that the plasma can kill bacteria but no comprehensive understanding on the killing mechanism by the plasma. Therefore I focus on interaction between ion and bio-polymer employed by the means of our conventional approach on semiconductor research.

The application of technical plasma for biological and medical purposes has grown over the last few decades. [1] Especially atmospheric pressure plasmas gained research interest, offering a wide range of possible applications, e.g. in wound treatment or skin treatment in general. Besides, low-pressure plasmas for sterilization and decontamination of various surfaces are already in use, or show promise for the future.

Low pressure plasma treatment offers certain advantages compared to other established sterilization methods, like avoiding toxic chemical agents, or the possibility to treat sensitive and thermolabile materials. Different low-pressure plasma setups have been investigated since many years for sterilization purposes [2-3], offering the opportunity to inactivate e.g. bacteria, bacterial spores, fungi, as well as to remove biological material from surfaces, e.g. proteins and prions. Recently, the first low-pressure plasma reactor for sterilization in pharmaceutical industry was presented. [4]

Atmospheric pressure plasmas are still far from actually being applied in medical practice and are currently subject of research. There is a wide variety of different discharge types like dielectric barrier discharges (DBD), plasma jets, hollow cathodes, etc., aiming to disinfect skin and wounds, support wound healing, accelerate blood coagulation, reduce cancer cells or have positive cosmetic effects. Those plasma sources can be used outside or even inside the human body.

In order to understand the different plasma processes and the interaction of technical plasmas with biological systems, detailed knowledge of the plasma itself is indispensable. Plasma characterization is the key to understand and to optimize plasma for sterilization, disinfection, decontamination and for the application in therapy for humans. Absolutely calibrated optical emission spectroscopy (OES) and plasma simulations offer deep insight into plasma processes and the interaction of plasmas and cells. These diagnostic methods complement each other and provide information concerning photon and radical fluxes on surfaces of treated objects and can be used for optimization of sterilization efficiency.

In this contribution three different plasma sources and their characterization are presented: two low-pressure plasma reactors, one for basic research, the other one a prototype plasma reactor developed for use in doctor's surgeries and one atmospheric dielectric barrier discharge for human skin treatment. A plasma simulation and a OES characterization of the laboratory setup are performed. Radiation intensity, which is important for fast plasma sterilization, is measured at selected positions and calculated in whole volume around payload object.

Sterilization efficiency of considered plasma devices under variable plasma conditions is studied.

(b) Experiment

Two different low-pressure regime setups and one atmospheric pressure setup for sterilization and disinfection are presented in this contribution namely a "Double Inductively Coupled Plasma" (DICP), a "Very High Frequency Capacitively Coupled Plasma" (VHF-CCP) and a "Dielectric Barrier Discharge" (DBD) operational under atmospheric pressure conditions. The DICP serves as a laboratory setup for basic research, whereas the VHF-CCP has its focus on commercial application. The DBD aims to work in skin contact for wound healing and wound disinfection.

DICP : A detailed description of the reactor and a plasma characterization in different argon mixtures has already been presented. The DICP consists of a stainless steel cylinder enclosed with quartz windows. Coils behind these windows are driven with a RF current at 13.56 MHz under ICP excitation. It is operated in a low pressure plasma regime with a volume of 0.025 m^3. Different gases (Ar, N_2, O_2) and their mixtures enter the reactor through a gas shower. The working pressure is adjustable between 5 and 20 Pa.

This discharge is characterized by OES and Langmuir probe. Two different OES measuring positions are used: One near the active plasma zone and another one is located at middle height of the reactor far from active plasma zones. The Langmuir probe is attached at middle position. To mimic a sterilization process, a cylindrical glass object is placed in the middle of the DICP as an actual sterilization payload.

VHF-CCP : The VHF-CCP plasma rector is a low-pressure capacitively coupled plasma source. The plasma chamber has an inner size of 320 mm x 220 mm. The distance between grounded and driven electrode is 80 mm. Both electrodes have the same size as the chamber.

The discharge chamber itself is composed of PEEK, a high-performance plastic, and shaped like a drawer. The concept is designed to meet industrial needs, using the drawer as easy and safe to handle sterile container. It is connected to a rotary vane pump (Trivac D65B, Oerlikon Leybold Vacuum, Cologne, Germany) and a gas inlet system through a gas shower inside the grounded electrode in order to achieve a homogeneous discharge. The gas inlet system is composed of mass flow controllers (MKS MFC Type 1179, MKS Instruments, Munich, Germany) for O_2, and H_2 with a constant total gas flow of 20 sccm and a pressure of 5 Pa, or 10 Pa respectively. The VHF-CCP is driven by an RF source, operating at a frequency of 81.36 MHz with maximum power output of 500 W. It is matched to the discharge through a variable matching network in T-type configuration (both Aurion, Seligenstadt, Germany). With this setup, we have the advantages of a CCP, i.e. high electric field for a better penetration of small lumen, combined with a higher electron density and lower ion energy, due to the high frequency employed. The capability to enter small lumen, provided by this setup, is proofed by a so called PCD.

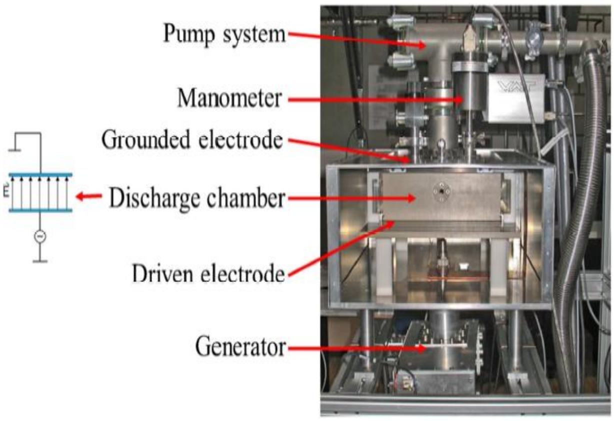

Fig. 1 VHF-CCP

DBD : The DBD used at our institute comprises a single aluminium oxide coated copper electrode with 10 mm total diameter. Every object with high capacitance like human tissue serves as a counter electrode. Owing to the fact that plasma is ignited directly on the human body surface it is a so called direct treatment. The discharge is ignited in the gap between electrode and tissue in ambient air as process gas. High voltage supply with peak amplitude of -13 kV and oscillation frequency of 100 kHz is pulsed with a frequency of 300 Hz. This setup leads to current peaks with short durations which are essential to keep the mean power low and the treatment pain-free for the patient.

The DBD is characterized using optical

emissionspectroscopy, current-voltage measurements, numerical simulation, and microphotography. All these methods complement each other to allow determination of averaged plasma parameters such as gas temperature, electron density, and electron velocity distribution function in the active plasma volume.

(c) Result

DICP : In order to have a closer look at relative sterilization efficiency of UV/VUV radiation different filter experiments are performed. Spores are sprayed in a square of 1 cm^2 on glass slides and covered with different cut-off filters: MgF_2 (115 nm) and two glasses (235 and 300 nm respectively). The filters are separated from the glass slides by glass rings. These samples are treated in the DICP in $Ar/N_2/O_2$ 100/4/1 sccm at 10 Pa and 750 W for 60 s. The plasma condition is chosen so that a similar amount of photons in each spectral interval (115-235 nm, 235-300 nm and 300-450 nm) is reaching the sample. The spores are recovered and counted as mentioned above. Further description of the procedure and the spectrum of the used plasma can be found elsewhere. Figure 2 shows the relative sterilization efficiency of A. *brasiliensis* and B. *atrophaeus.* For this, the log-reduction R is divided by the number of photons reaching the samples, the highest value is normalized to 1. The result shows that B. *atrophaeus* endospores are sensitive to radiation in spectral range 235-300 nm, in contrast A. *brasiliensis* spores are much less sensitive to radiation above 235 nm. These spores are more sensitive to a radiation in a spectral range of 115-235 nm. This result encourages to build a fast sterilization reactor with high UV/VUV emission.

VHP-CCP : Hydrogen, oxygen, and a mixture of both, are investigated as process gases for plasma sterilization. Since VUV/UV radiation is known to be one of the most efficient sterilization agents in the initial stage of plasma treatment , the discharge can be tuned to achieve efficient sterilization. However, oxygen offers a high amount of radicals and reactive species, so it should be considered for removal of biological material. A hydrogen discharge is known to emit a high quantity of photons in UV-C and VUV/UV range, among others due to the hydrogen continuum H_2(a-b) $\lambda = 158 - 350$ nm. The emission spectrum obtained by the measurements start at a wavelength of 200 nm. As shown in the radiation in the spectral range 200-280 nm contributes only 13 % of the total photons emitted in the VUV/UV range. Since the characteristics of this continuum are well known, the band from 158 to 200 nm can be fitted to the measured spectrum. With a hydrogen discharge (20 sccm H_2, 5 Pa, 400 W), a total UV radiation of 13.59 $Jm^{-2}\ s^{-1}$ is reached, with 6.76 $Jm^{-2}\ s^{-1}$ in the most efficient UV-C range, and 10.56 $Jm^{-2}\ s^{-1}$ considering H_2(a-b) continuum in VUV range. In comparison, pure oxygen discharge has a total UV dose of 7.67 $Jm^{-2}\ s^{-1}$, with 3.44 $Jm^{-2}\ s^{-1}$ in the UV-C range.

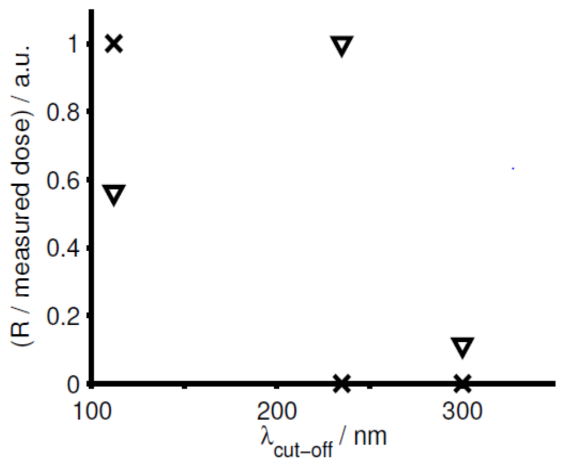

Fig. 2 Relative sterilization effciency of B. atrophaeus endospores (▽) and A. brasiliensis (X) spores

However, oxygen as process gas leads to a high amount of radicals, causing oxidative stress in cells. Additionally, oxygen is known as a good etching agent. For plasma sterilization it is important to design the discharge fitting the application. If solely inactivation of bacteria and bacterial spores is desired, a hydrogen discharge is most efficient. If there is a need to remove bacteria, bacterial endospores, and proteinaceous material, a gas mixture with oxygen might be the most efficient. In order to mimic worst-case-situation for plasma sterilization, a process challenging device is designed, consisting of a metal box with 3 small slits on top. The slits have a width of 0.3 mm. Glass slides are contaminated with B. *atrophaeus* ATCC

51189 as described above. The glass slides are placed inside the metal box. Afterwards, the process challenging device is placed inside the process chamber, and treated for 8 s, 16 s, 32 s, 64 s, 128 s and 256 s with hydrogen plasma. In figure 3 the results of the sterilization tests with the process challenging device are presented. The diagram shows the logarithmic reduction of B. *atrophaeus* spores, treated with hydrogen plasma at a pressure of 5 Pa and a power of 400 W. The black squares show the results for reduction inside the process challenging device, whereas the white squares show the results for samples placed outside the process challenging device, thus treated directly. While sterilization of directly treated B. *atrophaeus* spores happens instantaneously, sterilization inside the process challenging device takes longer. However, after around 4 minutes of plasma treatment, a log 6 - reduction is achieved also inside the challenging device. The presented results demonstrate, that sterilization with the VHF-CCP inside a PCD is possible. In order to optimize the plasma device for commercial use, it is necessary to tune the discharge to achieve fast and sufficient sterilization. With a pure hydrogen discharge, a high amount of VUV/UV radiation is available for fast sterilization. A pure oxygen discharge provides a high amount of reactive species. A combination of both can lead to fast inactivation and removal of endospores and other biological material.

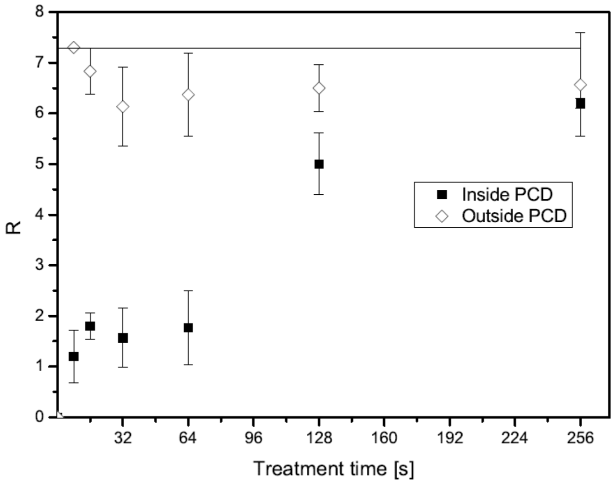

Fig. 3 Logarithmic reduction of B. atrophaeus spores, placed inside a PCD (blacksquares) and in comparison placed outside the PCD (white squares)

College life in RUB

After we arrived on Frankfurt Airport by a plane gone to Düsseldorf using ICE trains and transfer to the RE train move to Bochum. Professor Czarnetzki came to Bochum Central Station for pick-up us. He bring us by hotel and usher nearby facilities such as Uni center. We stayed in same room at the hotel "Schmelkotter" for 2 months. The institution is located on opposite side of university from the station; it takes 15 minutes to the university station by walk.

In the hotel, we could use common kitchen and there are all other things we need. I could stay there without feeling uncomfortable. But there are not washing machine so we had to go to washing room near Bochum city hall on weekend.

Summary

I stayed in Germany two months for study about the application of plasma in the field of bio-materials, discussions with students and professor in English, and life in a foreign country. It was very good experience to broaden my knowledge for two months.

Finally, I deeply appropriate Prof. Awakowicz, Prof. Czanertzki, Prof. Hori, Prof. Toyoda for giving me such a great opportunity, and all of people for assisting my stay.

Reference

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