High and low-frequency discharges for biomedical applications and nanostructuring
FINAL REPORT

The aim of the project was the understanding of operation of different high and low-frequency discharges, and the role of the chemical processes that can occur in the discharges and afterglows applicable for nanostructuring and in various areas of biomedicine. We aimed to build discharge systems, study their applicability and test their efficiency. The fields planned to be covered by our work were: *1. Biological decontamination, 2. Medicine, 3. Biocompatible model surfaces and 4. Nanostructuring*. Additionally, we have got involved also into *Plasma agriculture*, a new developing field. We report our results following the plans presented in our research proposal.

1. Biological decontamination

1. We built a post-discharge system based on a flowing surface-wave microwave discharge.

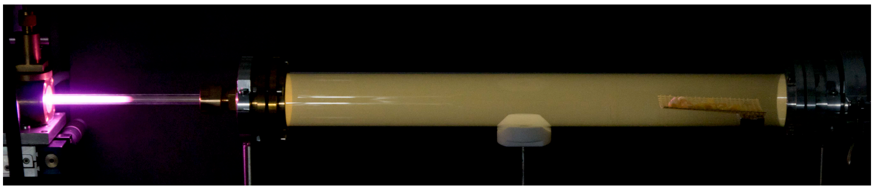

Figure 1: The post-discharge system with the surface-wave microwave discharge operating in N_2-20%O_2 mixture during seed treatments.

2. The etching of the proteins fibrinogen and albumin were studied by exposing protein films to neutral reactive species in early and late afterglow of oxygen surface-wave microwave discharge. The etching rates were determined at different pressures between 50 and 400 Pa. The etching rates for both proteins were found to increase with the increase of pressure, however at higher pressures the saturation of the etching occurs.The etching rates are found to be higher in the early afterglow than in the late afterglow. The concentration of all available species in the afterglow, i.e. neutral oxygen atoms in the ground state, neutral molecules in the first excited state, and ozone were determined either by catalytic probe measurements and through post-discharge system modelling. The etching rates are explained by the synergistic effects of atoms and metastable molecules.

   In order to get an insight into the interaction of active species with bacteria treated in the afterglow plasma, E. coli bacteria were treated in the late afterglow of binary (Ar-$^{15}N_2$, Ar-$^{18}O_2$) and ternary (Ar-15N_2-18O_2) mixture microwave discharges. It was found that only low pressure dehydration and reactive species present in the late afterglow affected the bacterial integrity. A possible additional effect of a 35- 40^{0}C temperature was observed only for O-containing mixtures, which may induce higher ROS binding kinetics or activation of other degradation processes. By using an optimized sample preparation protocol for NanoSIMS50 characterization, the binding of nitrogen coming directly from the late afterglow, was evidenced both on the outer structure and inside the bacteria. The binding of ^{18}O was attributed to the oxidizing and etching of the bacterial membranes and cell wall. In ternary gas mixtures the UV irradiation coming from NO(A,B) contributed to enhance the etching rate of the bacterial material, and improve the removal of oxygen atoms, while NO(X) could also play a role in the higher degradation rate of bacteria. The ^{18}O incorporation rate was found to be higher than the ^{15}N for binary gas mixtures. The atomic oxygen was shown to play the main role in the degradation of bacteria by chemical etching. The quantity of ^{18}O or ^{15}N incorporated/fixed on the bacteria in relation to the plasma treatment time was evaluated by NanoSIMS. The RNS and ROS binding areas on bacteria were localized by coupling the NanoSIMS HSI and AFM images.

   A. Vesel, M. Kolar, N. Recek, K. Kutasi, K. Stana-Kleinschek, M. Mozetic: Etching of Blood Proteins in the Early and Late Flowing Afterglow of Oxygen Plasma *Plasma Process. Polym.* **11** 12 (2014).

   D. Duday, F. Clément, E. Lecoq, C. Penny, J-N. Audinot, T. Belmonte, K. Kutasi, H-M. Cauchie, P. Choquet: Study of Reactive Oxygen or/and Nitrogen Species Binding Processes on E. coli Bacteria with Mass Spectrometry Isotopic Nanoimaging *Plasma Process. Polym.* **10**, 864 (2013).

3. Related to the previous task, we studied the possibility of tuning the species densities and density distributions in the afterglow of Ar-O_2/O_2 and Ar/N_2/O_2 mixture discharges. In the first system the surface-wave microwave discharge was perpendicularly positioned to the reactor. The density distribution of O-atoms along the reactor has been measured with a catalytic probe. The measured distributions served for validation of the model, which made possible the calculation of 3-D distribution of all the species. The calculations revealed that due to the perpendicular injection of the plasma into reactor the gas temperature is close to the room temperature in most part of it, except a 5 cm region around the inlet. The surface recombination probability of atoms varies along the afterglow tube due to the surface temperature gradient, as well as due to the conditioning of the surface resulting from the continuous operation of the system. It has been shown that by tuning the gas flow rate equidensity surfaces can be obtained in the case of the O-atoms and O_2(a) molecules. In the case of O-atoms the densities obtained at the two pressures investigated, i.e. 100 Pa and 50 Pa, and their evolution along the reactor are very similar, while the density of O_2(a) molecules decreases considerably with pressure.

   In the second system, where a 0.5 cm diameter quartz tube of 50 cm in length - where the discharge is generated and the early-afterglow develops - is connected to a 40 mm diameter afterglow reactor, we studied the possibility of tuning the plasma composition in the afterglow of a flowing surface-wave microwave discharge by the different discharge and system parameters in the case of 90%Ar-10%(N_2-O_2) and N_2-O_2 mixtures. The validity of the model used for calculations was proven by the agreement of the calculated atomic densities with those measured by mass spectrometry. Due to the pressure drop along the tube, the position of the discharge (which defines also the lengths of the early-afterglow, t_{aft}) and the discharge pressure (p_{dis}) can be set with the position of the wave coupler - surfatron - along the tube at constant gas flow rate (which defines the pressure in the reactor, p_{reac}). It has been shown that the relative densities of species at the end of plasma column, which constitute the initial condition for the afterglow, depended on the discharge pressure. Therefore, at constant gas flow rate with the position of the surfatron the plasma composition in the reactor is changing due to the variation of both the p_{dis} and t_{aft}. The evolution of the plasma composition has been also studied when both the surfatron's position and the gas flow rate are changed, realizing conditions (*i*) with the same p_{dis}, and different t_{aft} and p_{reac}, and (*ii*) with the same t_{aft}, and different p_{dis} and p_{reac}. Comparing the N_2-O_2 binary and the ternary mixture discharges, it has been shown that the atomic densities obtained in binary mixtures can be reproduced in ternary mixtures with different N_2:O_2 ratios. Furthermore, according to the spectra measured in the afterglow reactor comparable UV radiation can be achieved in both ternary and binary mixtures with the same O_2 content.

   K. Kutasi, R. Zaplotnik, G. Primc, M. Mozetic: Controlling the oxygen species density distributions in the flowing afterglow of O_2/Ar-O_2 surface-wave microwave discharges *J. Phys. D: Appl. Phys.* **47**, 025203 (2014).

   K. Kutasi, C. Noel, T. Belmonte and V. Guerra: Tuning the afterglow plasma composition in Ar/N_2/O_2 mixtures: characteristics of a flowing surface-wave microwave discharge system *Plasma Sources Sci. Technol.* **25**, 055014 (2016).

2. Medicine

1. We built two atmospheric pressure plasma systems: *a surface-wave microwave discharge plasma jet and a radio frequency plasma needle*.We investigated the possibility of using the atmospheric pressure surface-wave microwave discharge plasma jet for treating biological samples, however we found it not suitable for that application. We found this system applicable to treat water creating plasma activated water, which has perspectives for activation of seeds germination and disinfection of seeds. For treating human cells we have chosen the rf plasma needle.

2. The plasma needle consists of a 0.3 mm diameter central electrode made of wolfram, which is covered by a ceramic tube placed in a glass tube. The central electrode is powered by a 13.56 MHz power generator. The discharge is ignited between the needle and a grounded electrode in Helium gas, which flows between

the ceramic and the glass tube. For cell treatments we determined an optimal configuration, which assured a stable glow discharge and a good access of the liquid covered cells in small diameter wells.

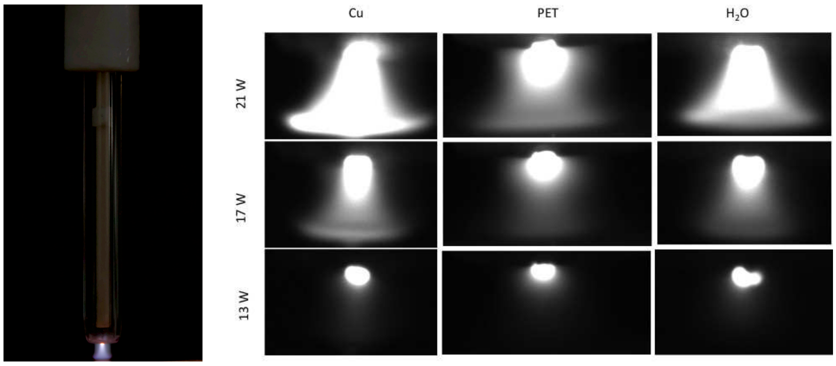

Figure 2: The plasma needle and discharge images with the plasma needle in interaction with different surfaces.

With our study we aimed to verify the influence of a non-thermal atmospheric pressure plasma on the wound healing process. In this process the major contributors are the keratinocytes, which migrate to fill in the gap created by the wound. Therefore, we performed the direct treatment of HPV-immortalized human keratinocytes, protected by a layer of phosphate buffered saline (PBS) solution, with the glow discharge generated in flowing helium by a plasma needle. To mimick a wound, a 4 mm scratch was performed on the cell culture (scratch assay). We conducted two types of experiments: (*i*) cell proliferation and (*ii*) wound-healing model experiments. The plasma needle configuration, the plasma treatment conditions and the thickness of the protecting PBS layer were set based on viability experiments. The proliferation studies showed that short, 5-10 s, and low power treatments, such as 18 W and 20 W input power, could positively influence the cell proliferation when keratinocytes were protected by PBS. On the other hand, the plasma treatment of cell medium covered keratinocytes resulted in the decrease of proliferation. The wound-healing model (scratch assay) studies showed, that there was a maximum in the wound reduction as a function of the input power and treatment time, namely, at 18 W and 5 s. Furthermore, the wound reduction strongly depended on the treated cell - PBS interaction time. To mimic an infected wound, the scratch assay was covered with a 1×10^9 cfu/ml *Propionibacterium acnes* suspension. The plasma treatment of this infected assay resulted in closing of the scratch, while in the non-treated assay the wound did not close at all.

I. Korolov, B. Fazekas, M. Széll, L. Kemény, K. Kutasi: The effect of the plasma needle on the human keratinocytes related to the wound healing process, *J. Phys. D: Appl. Phys.* **49**, 035401 (2016).

3. We have investigated the effect of three different surfaces: Cu, PET and distilled water, on the plasma needle operation and plasma composition for different needle-surface distances. We followed the evolution of the discharge through the discharge images recorded with a CCD camera and the optical emission spectra. The images show the transition of the plasma from the corona to glow mode with increase of the power for all surfaces. The measured absorbed powers show that the power coupling into discharge is most efficient in the case of the conductive surface, while in case of dielectrics it depends on the distance between the needle and the grounded electrode. The time dependent OES show the dependence of the plasma stability on the gas flow rate used. The recorded time dependent intensities of different species revealed the change of plasma composition with time in case of different surfaces.

   K. Kutasi, N. Puač, N. Škoro, Z. Lj. Petrović: Influence of different types of sample surfaces on a plasma needle, Proc. of "21st Symposium on Applications of Plasma Processes (SAPP XXI), 13-18th January, Strbske Pleso, Slovakia", 5pp (2017).

   K. Kutasi, N. Puač, N. Škoro, Z. Lj. Petrović: Interaction of atmospheric pressure plasma needle and DBD jet with different surfaces, to be submitted to Spectrochimica Acta B.

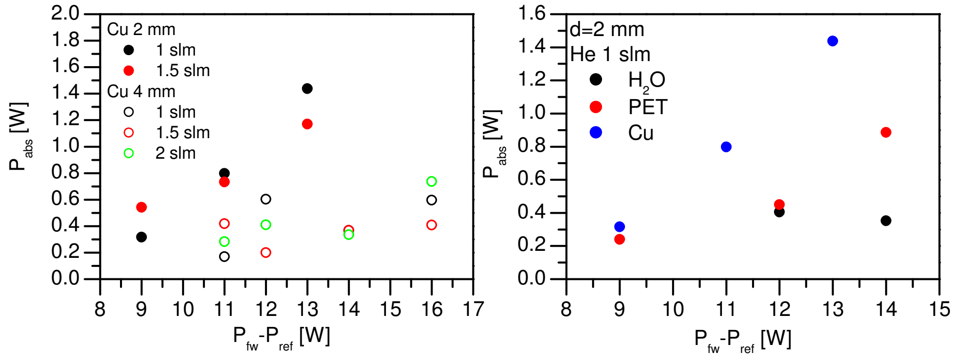

Figure 3: The absorbed plasma power as a function of input rf power in case of different interacting surfaces.

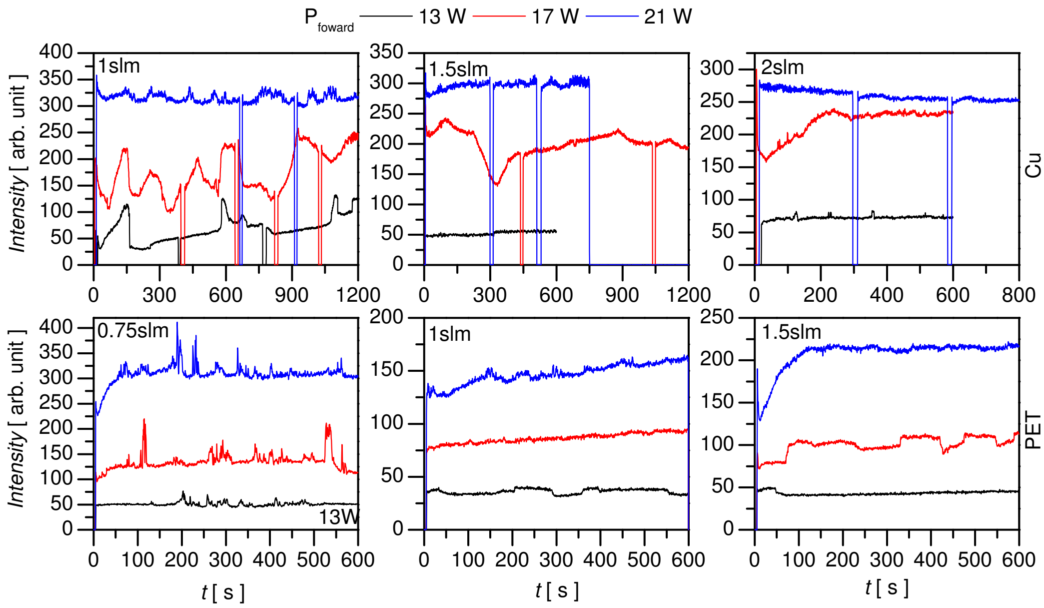

Figure 4: The intensity of the N_2 band as a function of plasma operation time showing the stability of the discharge. The discharge was turned off and on to verify the memory effect.

3. Biocompatible model surfaces

1. Applying the flowing afterglow of an N_2-O_2 surface-wave microwave discharge we have shown, that using 500-1000 sccm gas flow rates the afterglow can efficiently travel through a 4.5 mm polymer tube placed into the afterglow reactor, as well as simultaneously inside and around the tube. This proves the system to be appropriate to treat polymer tubes. In order to get an insight into the evolution of the system when loaded with small diameter tube, we used a 5 mm I.D. quartz tube and followed the afterglow through the UV emission of NO(B) molecules created in the three-body re-association process of N and O-atoms, and by modelling calculations. We have shown that along the tube the absolute density of atoms decrease due to the pressure drop there, which can be controlled through the system's pumping efficiency. We have demonstrated that several tubes can be treated at the same time.

   K. Kutasi, I Korolov : Characteristics of the flowing afterglow of a surface-wave microwave discharge in a reactor loaded with a small diameter tube *Plasma Process Polym.* e1700028, DOI: 10.1002/ppap.201700028 (2017).

2. An air diffuse coplanar surface barrier discharge was used to activate the surface of polytetrafluoroethylene (PTFE) samples, which were subsequently coated with polyvinylpyrrolidone (PVP) and tannic acid (TAN)

single, bi- and multilayers, respectively, using the dip-coating method. The XPS measurements showed that with plasma treatment the F/C atomic ratio in the PTFE surface decreases, due to the diminution of the concentration of CF2 moieties, and oxygen incorporation through formation of new C-O, C=O and O=C-O bonds. In the case of coated samples, the new bonds indicated by XPS show the bonding between the organic layer and the surface, and thus the stability of layers, while the gradual decrease of the concentration F atoms with the number of deposited layers proves the creation of PVP/TAN bi- and multi-layers. According to the ATR-FTIR spectra, in the case of PVP/TAN multilayer hydrogen bonding develops between the PVP and TAN, which assures the stability of the multilayer. The AFM lateral friction measurements showed that the macromolecular layers homogeneously coat the plasma treated PTFE surface.

A. Tóth, K. Szentmihályi, Zs. Keresztes, I. Szigyártó, D. Kovacik, M. Černák, K. Kutasi: Layer-by-layer assembly of thin organic films on PTFE activated by cold atmospheric plasma *Open Chem.* **13** 1-7 (2015).

3. Polyvinylpyrrolidone (PVP) and tannic acid (TAN) macromolecules were attached to polymethyl methacrylate (PMMA) surface in a two-step approach in order to establish a tailored tissue-friendly surface layer. First PMMA surface was activated by an air diffuse coplanar surface barrier discharge plasma and subsequently coated with these macromolecules. PVP and TAN which has antibacterial effects are widely used in food and drug industry, however, have never applied for polymer surface modification. Throughout characterization measurements confirm that during plasma treatment oxygen containing functional groups (-OH, -C=O, -COOH) incorporate in the surface. ^{1}HNMR spectroscopy verified the strong H-bonds between PVP and TAN, which rendered the layer-by-layer assembly of these molecules. The roughness increased considerably during plasma treatment, which can promote the further reactions with organic molecules.

   Sz. Klébert, Z. Károly, A. Késmárki, A. Domján, M. Mohai, Zs. Keresztes, K. Kutasi: Solvent- and catalysts-free immobilization of tannic acid and polyvinylpyrrolidone onto PMMA surface by DBD plasma *Plasma Process Polym.* **14** e1600202 (2017).

4. The DBD atmospheric plasma treatment of diamond like carbon (DLC) (deposited in capacitively coupled rf (2.4 MHz) plasma deposition system by decomposition of pure methane) and pyrolytic carbon (PyC) were performed with the aim of grafting macromolecules on the surfaces. The surfaces before and after the treatments were characterized by XPS, ATR-FTIR, SEM, AFM, contact angle measurements. The IR

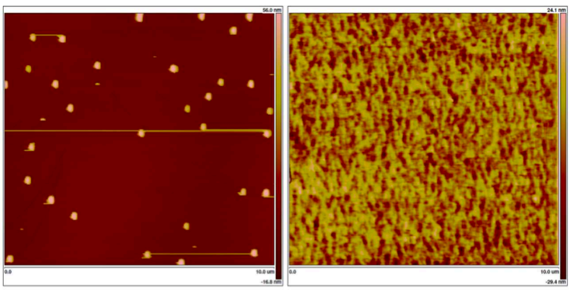

Figure 5: AFM scan of Diamond like carbon before (left) and after(right) the plasma treatment.

spectroscopy was found not to be suitable for following the changes in these kinds of carbon materials. SEM could show the signs of etching but the height differences and increase in roughness is under the detection limit. AFM proved to be more sensitive and we found that the plasma etched the surface and increased its roughness. XPS analysis showed increase in the surface oxygen content in compliance with the decrease in the carbon content. The oxygen's bonding state is found to be mostly composed of carbonyl groups offering the possibility to functionalize the surface. Due to the appearance of functional groups the contact angles show a decrease after the treatment. Experiments were also carried out to design reliable methods to evaluate the concentration of newly formed chemical groups on the surface using UV-Vis spectrometry with special dyes, that have specific tendency to form complexes or bonds with different chemical groups. This new method combined with XPS scans can help the interpretation of the findings.

4. Nanostructuring

1. We built a low pressure inductively coupled radio frequency discharge system, operating in flowing gas. We have studied the formation of oxide structures on copper plates in the discharge sheath and in the afterglow region at different gas mixtures, input power and treatment time. Similarly we have studied the oxidation of copper in the afterglow of the surface-wave microwave discharge and compared the two systems. In the sheath of the rf discharge regular shapes have been formed, with initiative growth of nanowires as shown in Figure 6. Higher power, which results in higher temperature contributed to the thicker layer formation, while lower powers to the structuring of the oxide layer. The oxidation in the afterglow was found to be much faster, in few minutes a thick layer was formed, which after a threshold thickness detached. Depending on the oxygen content and gas temperature different structures could be created. At lower O_2 content mixture: 50 sccmAr-10 sccm O_2 larger individual structures have been formed, with the attempt of wires growing on them. At the same low flow rate with further decrease of input power similar wall structures were found as in the afterglow of a MW discharge at 500 sccm N_2 - 120 sccm O_2. Figure 8 shows the restructuring of the copper-oxide layer created in RF afterglow with the N_2-O_2 MW afterglow, showing the wall shape structuring of the initial structures. In case of Ar-O_2 MW discharge the oxidation rate is very low due to the lower temperature comapring to the N_2-O_2. We have found that the wall structure, that is the basic element the of the structures can be created at lower oxidation rate, which is related to lower temperature and lower O-atom density. In case of a surface-wave microwave discharge system this can be easily tuned with the gas flow rate and the position of the wave launcher along the discharge tube.

   K. Kutasi and T. Belmonte: Effect of the RF and MW discharge parameters on the formation of copper-oxide structures, under preparation

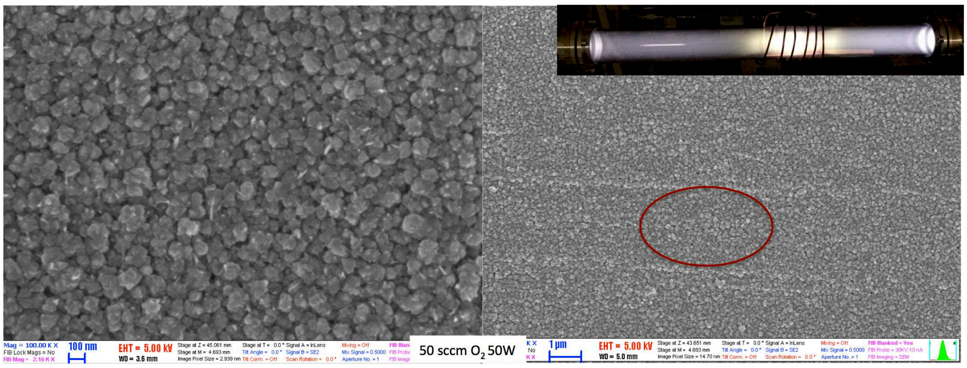

Figure 6: Copper-oxide surfaces created in RF discharge region.

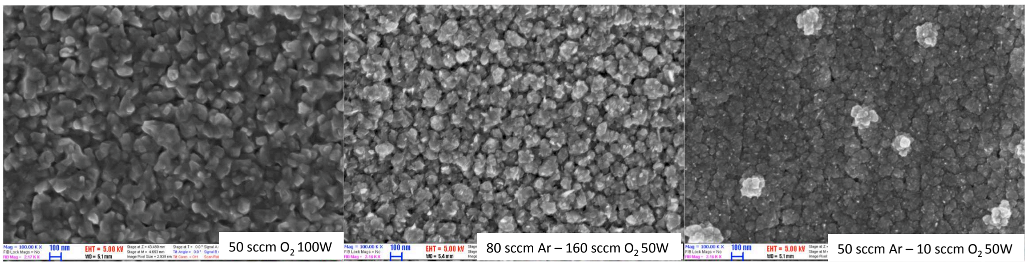

Figure 7: Copper-oxide surfaces created in RF afterglow region.

2. We have characterised the afterglow of an Ar-O2 surface wave discharge in order to understand the plasma chemical processes, which lead to nanoparticle formation during the interaction of the afterglow plasma with (3-Aminopropyl)triethoxysilane(APTES). We suggested that the fragmentation of APTES starts with its interaction with O-atoms, while further oxidation occurs with O_2 molecules in any state.

   M. Gueye, T. Gries, C. Noel, S. Migot-Choux, S. Bulou, E. Lecoq, P. Choquet, K. Kutasi, T. Belmonte: Interaction of (3-Aminopropyl)triethoxysilane with Pulsed Ar-O2 Afterglow: Application to Nanoparticles Synthesis *Plasma Chem Plasma Process* **36** 1031-1050 (2016).

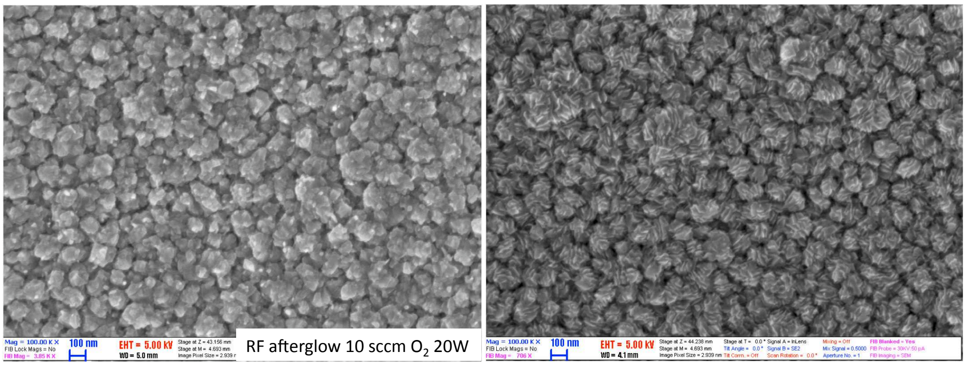

Figure 8: The copper-oxide surface created in RF afterglow and the same surface restructured in the N_2-O_2 MW afterglow.

3. During our discussion with chemist partners it turned out, that they already managed to develop an efficient technology to produce carbon nanotube carpets, and recently the main interest lies in creating long coiled nanotubes, due to their exceptional mechanical, electrical and magnetic properties. Therefore instead of straight carbon nanotubes we investigated the growth of coiled nanotubes on copper-oxide surfaces prepared in Ar-O_2 and O_2 ICP discharges. Different structure surfaces have been used and an effort is made to find the relation between the surface structure and the coiling of nanotubes.

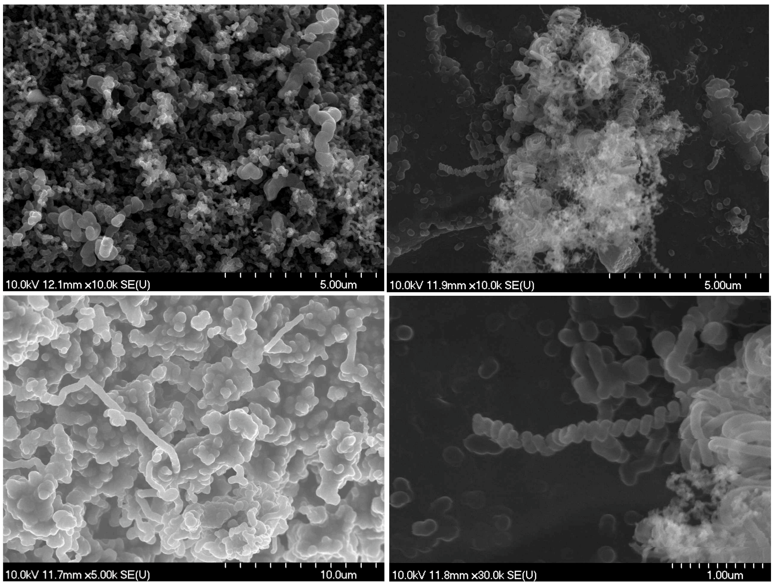

Figure 9: Coiled carbon nanotubes synthesized on structured Cu plates in 100sccm C_2H_2-300sccm N_2 at 800°C.

K. Kutasi, I. Korolov, P. Berki, E. Kecsenovity, D. Fejes, K. Hernádi: Substrate design for growing nanostructured carbon, 7th international conference on Innovations in Thin Film Processing and Characterization, 16-20 November, Nancy, France (2015).

K. Kutasi, P. Berki, E. Kecsenovity, D. Fejes, K. Hernádi: Substrate design for synthesis of coiled carbon nanotubes, under preparation

5. Plasma agriculture

Based on our experience gained during the biological decontamination studies on the afterglow plasmas, we have joint another fast developing field, namely *Plasma agriculture*, which is aimed to develop new technology for agriculture. We used the afterglow of a surface-wave microwave discharge to investigate the effect of different afterglow plasmas on cereal crops, more specifically maize and barley. In our study we treated non-infected and infected cereal crops, respectively,in the afterglow of Ar/N_2-O_2 surface-wave microwave discharges at 2-8 mbar pressure, using the following initial gas mixtures: (*i*) N_2-20%O_2, (*ii*) N_2-10%O_2, (*iii*) N_2-2%O_2, (*iv*) Ar-20%O_2, (*v*) Ar-40%O_2 and (*vi*) Ar-20%O_2 + N_2-2%O_2, which made possible to isolate different species and identify their role in the process. We have shown, that the germination and vigour of non-infected seeds are not significantly effected, when barley is treated max 120s at 2 mbar and maize 240 s at 4 mbar. On the other hand, seeds can be disinfected from the germination inhibitors *F. graminearum* and *F. verticillioides*. The most efficient treatment, which also increases the germination of infected seeds above 80%, for barley is the 3 min Ar-20%O_2 afterglow at 4 mbar, while for maize the 4min Ar-20%O_2+2min N_2-2%O_2 at 8 mbar. The high NO content mixtures and the heating of seed surface by the recombination of O and N-atoms inhibit barley germination. We have also performed field experiments by planting plasma treated maize seeds in the experimental nursery field of the Hungarian Academy of Sciences, Agricultural Institute (Martonvásár, Hungary), where the growth and evolution of the plants, the photosynthesis of plants and the production yield is under investigation.

Cs. Szőke, Z. Nagy, K. Gierczik, A. Székely, T. Spitkól, Zs. Tóthné Zsuboril, G. Galiba, Cs. L. Marton, K. Kutasi: Effect of the afterglows of low pressure Ar/N_2-O_2 surface-wave microwave discharges on maize and barley, under acceptance at Plasma Processes and Polymers

Infrastructure development

As presented in the report, according to our plans we have built four discharge systems. Instead of the planned large volume surface-wave microwave discharge we built an inductively coupled radio frequency discharge, which is adapted to the same gas flowing system as the low pressure surface-wave discharge system. For plasma generations we owned the RF and MW power generators. From the project we have purchased the MW wave launchers, two rotary pumps and flow controllers, which allowed to sustain controllable gas flow in the systems at low pressure.

Instead of purchasing vacuum chambers we manufactured a 50 l aluminium reactor, which has been designed for our needs and for future applications.

The modelling calculations have been performed on a Linux PC cluster operated by the Gas Discharge group. From the project we have replaced one module of the cluster, which has been run-down during 5 years of usage.

Research personal

During the project we encountered periodical personal losses due to serious illnesses and maternity leave. One of our senior researchers needed to be replaced during the first year due to his illness and followed retirement. Finding his replacement, as well as the time spent on the relocation of our partners laboratories (Institute of Materials and Environmental Chemistry) our work suffered some delays. With the permission of the office, some of the budget allocated for our partner for the surface treatment tasks, were allocated for purchasing equipment. With the encountered difficulties, we still addressed all the research topics planned in our proposal and managed to involve in our studies researchers from very different fields from: (*i*)Agriculture Institute, Centre for Agricultural Research of HAS, Martonvásár, (*ii*) Institute of Medical Genetics, Faculty of Medicine and MTA-SZTE Dermatological Research Group, University of Szeged, (*iii*) Department of Applied and Environmental Chemistry, University of Szeged and (*iv*) Research Centre for Natural Sciences of HAS, Institute of Materials and Environmental Chemistry. On surface and biological sample analysis we needed to rely on our partners and a new protocol needed to be established for cell treatments, which also delayed our timing.

The work has been done in international cooperation with: (*i*) Institut Jean Lamour, Univ. Lorraine, Nancy, France, (*ii*) Instituto de Plasmas e Fusão Nuclear, Univ. Lisboa, Portugal, (*iii*) Jozef Stefan Institute, Ljubljana, Slovenia, (*iv*) Centre de Recherche Public Gabriel Lippmann, Luxembourg and (*v*) Institute of Physics Belgrade.

Six students joint our project. Their results became part of 1 PhD thesis: Barbara Fazekas: UVB- and plasma radiation-induced cellular responses of human keratinocytes (University of Szeged, 2015) and 2 BSc thesis: Herceg Zoltán: Poli(tetrafluoroetilén) felületmódosítása nem-egyensúlyi hideg plazmával további molekulák hozzákapcsolásának céljából (BME, 2016), Késmárki András: Poli(metil-metakrilát) felületének hidegplazmás funkcionalizálása makromolekulák rögzítése céljából (BME,2016).

Our results have been also presented in 11 invited lectures.