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PhD Thesis

**Synthesis of complex polymeric
structures by plasma techniques for
medical applications**

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Summary

The PhD thesis is structured on five chapters and an appendix that presents the protocol of magnetic beads functionalization with myoglobin and their labeling with smaller magnetic beads.

The aim of this thesis was to improve the properties of certain polymeric materials and to synthesize new materials with specific properties by plasma techniques in order to use them for various medical applications.

In *Chapter I* are presented main material types from complex polymeric structures category and their medical applications. There are also described the main techniques used to analyze surface or bulk properties of modified or synthesized materials by plasma techniques.

Complex polymeric structures includes both polymers modified by different techniques (UV radiation, thermal, plasma, etc.), or synthesized by nonconventional methods that can induce them specific properties, and mixtures between polymers and other materials such as metals, semiconductors, magnetic nanoparticles, ceramics or polymers. Moreover, due to variety of requests from medical and pharmaceutical domains this category of materials is a very actual subject.

To be able to control the interface processes it is very important to know very well the surface properties of biomaterials. The physico – chemical surface and bulk properties of polymeric materials obtained during PhD were studied by different techniques such as: contact angle measurements, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), scanning electron microscopy (SEM), electro kinetic technique for ζ potential measurement, Fourier transform infrared

spectroscopy (FTIR), X-ray diffraction (XRD), impedance spectroscopy.

Modification of surface properties by different techniques is a method for improving the quality of these materials and for raising their probability of success in adequate medical applications. Accordingly to scientific literature, the surface properties modification techniques are classified into two categories: conventional techniques that include chemical oxidations, functionalization with chemical reactive, or polymeric chains addition by covalent bonds, and dry techniques such as UV, X or gamma treatments, plasma treatments, laser, electron or ions beams, etc.¹⁻³ By these techniques it can be modified surface roughness, hydrophilicity, electrical charge, surface energy, cross-linking, reactivity, biocompatibility, the control of these properties being a very actual research domain for materials' science and engineering. In other words, polymers' surface can be changed so that they could enlarge their applicability by increasing their surface adhesion, improving their wettability, increasing their chemical stability.

In *Chapter II* are presented the main characteristics of atmospheric pressure plasma, especially dielectric barrier discharge plasma and its medical applications that include both plasma effects on biomolecules and living organisms, as well as modifying or obtaining new materials with specific properties with it. There are also presented plasma's effects on polymeric surface and methods of diagnosis used throughout the thesis to characterize and control plasma parameters.

Thus, the main effects of plasma on polymeric surface are cleaning, activation and functionalization, crosslinking, or corrosion. Also, plasma, both at atmospheric and low pressure can be used as an energetic medium to produce physico-chemical reactions to synthesize polymeric films by introducing monomer's vapor in plasma's bulk. In these studies we used dielectric barrier

discharge plasma working at atmospheric pressure in various geometries to obtain polymeric structures with specific properties for various biomedical applications.

In *Chapters III - V* are presented the main experimental results obtained during the PhD period.

Chapter III presents the experimental results of the surface properties modifications study for certain polymeric materials by their activation in plasma. Polymeric surfaces activation by plasma is the result of the following processes: the removal of weakly bound layers at the surface, the crosslinking of molecules at the surface and generation of polar groups by surface oxidation.

For plasma treatments we used a dielectric barrier discharge at atmospheric pressure in helium, with the tip to plan geometry. Applied signal was sinusoidal, 4kV voltage, about 15mA current and 2 kHz frequency. The electrodes gap was 5 mm.

We studied the energetic characteristics modifications of plasma treated polymers and time stability of these properties by contact angle measurements. Plasma modified polymers were poly (methyl methacrylate) (PMMA), polysulfone (PSU), poly (tetrafluoroethylene) (PTFE). These were selected due to their different chemical structure, various sensitivities to treatment and their importance for biomedical applications.

The response of polymers on plasma treatment depends on both the chemical structure and the duration of each treatment. Modification of surface hydrophilicity by plasma treatment was very fast so that after treatment 3s was recorded an increase of adhesion work of water of about 75% and of surface polarity of 90% for PMMA, with 40% of the adhesion work of water and with 250% of surface polarity for PSU, and 160% of the water's

adhesion work and 490% of surface polarity for PTFE. These modifications in surface properties by plasma treatment is higher for a longer treatment, but the difference between the responses to different treatment durations of polymers is low, for example, from 3s to 10s of treatment the adhesion work increase was of approximately 0.6% for PMMA, 1.2% for PSU and 9% for PTFE.

The lowest increase of surface hydrophilicity was recorded for PSU, due to the hydrophilic character of the untreated polymer and so after 10s of plasma treatment the water droplet spreads completely, the polymer surface becoming so super-hydrophilic. In the case of PMMA, although the contact angle of water on its surface initially was $80^\circ \pm 2^\circ$, after plasma treatment it decreased to $12^\circ \pm 2^\circ$ value, probably due to strong oxidation that occurred at the interface plasma - polymer. Although the surface properties of PTFE are also highly modified in plasma, however, the contact angle of water on its surface after 10s treatment was $60^\circ \pm 2^\circ$ due to strong hydrophobic characteristics of the polymer. The time stability study of these characteristics induced by plasma established that the degree of recovery to the initial characteristics of the surface is low. Moreover, the lowest recovery was recorded in case of 10s of plasma treatment.

These modifications of surface properties by plasma treatment are due to highly reactive character of plasma in contact with a polymeric surface. Thus, on polymer's surface physical or chemical bonds break and new polar groups are generated. These modifications of surface composition, especially the presence of oxygen - based functional groups (-OH, -C=O, etc.), induced the increase of the polar component of surface energy, as confirmed by the presented results. Responsible for these changes in the surface are ionized or excited particles, photons, and radicals from plasma, each of them having enough energy to induce chemical reactions on the polymeric surface.

The study L-asparaginase immobilization on plasma modified surfaces showed that enzyme is immobilized in clusters on the untreated surface, while on plasma treated surface L-asparaginase is distributed uniformly over the entire analyzed surface. We also studied the cellular viability for untreated and plasma treated ELISA plates' wells coated with L'asparaginase molecules. It was recorded a decrease of cell viability for treated wells than for untreated ones, but the difference is quite low, of about 4% or 7% in wells with a L-asparaginase solution concentration of 5 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$, respectively. At higher concentrations, 500 $\mu\text{g/ml}$, it was reached a saturation of immobilized enzyme amount and the cell viability was about the same for both types of wells.

Plasma activation of materials used in dentistry showed an improve of their adhesion properties, increasing so their ability to form a stable salivary pellicle on the surface and to form stronger bonds between these materials and dentin surface preventing their edge linkage when they are used as restorative materials in the oral cavity on the other hand.

In *Chapter IV* are presented the experimental results for obtaining and characterization of thermosensitive polymer films based on N-isopropyl acrylamide by plasma polymerization (ppNIPAM). Plasma was generated in a dielectric barrier discharge with a plane - plane symmetric geometry. Working gas used was helium with a spectral purity of 99.999%. The helium flow rate was 3l/min and for monomer was 5 $\mu\text{g/min}$. Polymer deposition time was 10 min, and the distance between electrodes was 5mm. The discharge was monitored by both electrical and optical diagnostic methods. The physico-chemical properties of the obtained films were analyzed by different techniques.

Thus, XPS analysis showed a higher content of oxygen then the classical pNIPAM. Also, 17.4% of the carbon atoms were

involved in covalent bonds with nitrogen, C-N, and 25.8% in C = O double bonds, the remaining 56.8% being involved in C-C and C-H bonds⁴.

If we compare the distribution of chemical bonds in the ppNIPAM matrix to that of the same polymer obtained by classical methods^{5,6}, (66.7% C-C and C-H, C- N 16.7%, and 16.7% C = O), the proportion corresponding to C = O and C-N is higher into ppNIPAM structure due to the incorporation of oxygen and nitrogen from the atmosphere into the polymer structure during polymerization reaction.

Both FTIR and XPS spectra led us to the formation reaction mechanism. Thus, in monomer IR absorption spectrum a peak corresponding to C = C bond at 1620 cm^{-1} was present while in the IR spectrum of the polymer this peak disappeared. Also, strong monomer peaks at 1411 cm^{-1} , 991 cm^{-1} (and harmonics 964 cm^{-1}) and at 918 cm^{-1} corresponding to the same double bonds were no longer present in the polymer spectrum. In other words, the ppNIPAM forms by C = C double bond breakage and linkage between the resulting radicals, result confirmed also by the XPS spectrum of polymer, where the double bond was not identified⁴.

The frequency dependence of the dielectric properties of PET substrate and PET coated with p-NIPAM films was determinate at room temperature in a large frequency domain. At low frequency, the ppNIPAM sample showed an increased of permittivity up to 6, but at higher frequency permittivity decreases to 4.6. This phenomenon is due to dipolar relaxation of oxygen absorption ppNIPAM matrix during the polymerization, the result can be correlated with XPS analysis results showing an increased amount of oxygen in the polymer composition.

By comparison with other results from literature^{7,8}, plasma obtained polymeric films were more hydrophilic due to permanent contact with the plasma.

In *Chapter V* was made a comparative study between polystyrene (PS) materials obtained by two methods: spin-coating (scPS) and plasma polymerization (ppPS) to use it as a substrate for myoglobin biosensor. Thus, we analyzed the physico-chemical properties of PS and its interaction with myoglobin coated magnetic beads. Nonspecific interactions between myoglobin coated magnetic beads and polystyrene surface were studied using a rotating magnetic field.

Higher surface polarity recorded for ppPS films was due to the mechanism of plasma polymerization at atmospheric pressure by incorporating atmospheric oxygen in the polymer matrix. This result was also confirmed by XPS analysis of obtained polymer films. Moreover, this surface property of ppPS was time stable, one month after obtaining, its polarity still having a high value compared with the scPS.

The nonspecific interactions between magnetic beads functionalized with myoglobin and polystyrene surface were studied by analyzing the percentage of beads that are bound or not to the polymeric surface. Thus, we studied the variation of the bound beads fraction by buffer solution's ionic strength. A small percentage, about 2% of magnetic beads coated with myoglobin started binding to the scPS when they are suspended in solutions with ionic strength below 10 mM, for an ionic strength higher than 20mM all the myoglobin coated magnetic beads are non-specifically bound to the surface. In case of ppPS the magnetic beads started binding to surface in solution with 35mM ionic strength and they were all bound to polymeric surface in solution with 100mM ionic strength. The dependence of bound beads fraction to ionic strength of the solution is explained by the fact that in a solution with low ionic strength, i.e. low ions' concentration, electrostatic repulsion between functionalized beads and polystyrene surface kept them at a finite distance from each other, so beads can rotate in rotating magnetic field. By

increasing the concentration of ions in solution surface potential is shielded so that the distance between the two interacting bodies decreases. When the protein layer on magnetic beads reaches the surface they can nonspecifically bind to substrate by van der Waals interactions.

The total interaction energy of magnetic beads with the PS surface was calculated by DLVO theory, and taking into account the gravity, buoyancy and magnetic interactions^{9,10}. The calculation relation is:

$$E_{DLVO}(x) = -A_{1m2} \frac{R}{6x} + RZe^{-kx} - 4\pi R^3 g(\rho_{part} - \rho_{fluid})(R+x) - \frac{4\pi R^3 \chi B \nabla B}{3\mu_0 \mu_r} x$$

where:

- A_{1m2} - Hamaker constant for the interaction between magnetic beads (polystyrene) - water – polystyrene surface
- R - radius of the magnetic particle
- x - distance between the spherical particle and a flat surface
- ρ_{part} - magnetic beads' density
- ρ_{fluid} - solution's density
- g - gravitational acceleration
- χ - magnetic susceptibility
- B - magnetic field
- μ_0 - magnetic vacuum permeability
- μ_r - relative magnetic permeability of the environment

Due to oxygen content in ppPS matrix its surface potential is lower than the scPS. Energy barrier increases with decreasing surface potential, so the probability that myoglobin coated magnetic beads to nonspecific bind to the substrate decreases for ppPS.

The main **conclusions** of the experimental results during PhD are presented below.

1. The mechanisms responsible for modifications induced by plasma at interface with polymeric materials are complex, and they are due to ionized or excited particles, photons, radicals, electrons. All these species have enough energy to induce chemical reactions at the polymeric surfaces, but their roles in modifications of surface properties are different. Experimental results for plasma treatment of various polymers study showed a different response depending on treatment time, structure and chemical composition of these compounds.
2. Protein immobilization on polymeric surfaces study is of great importance for medical applications. To obtain the best results in this respect is necessary to improve the surface properties without changing bulk properties of these materials. Plasma treatment of polymer surfaces can be a solution for changing these properties and, depending on the nature of the induced functional groups at polymeric surface, the adsorption of proteins could be controlled.
3. Dental material properties can be improved by plasma treatment in order to increase their period of usage in oral environment. This is due to a protective saliva biofilm on dental materials' surface by increasing the saliva adherence and the linkage between plasma treated dental materials and organic material surface, decreasing the probability of edge detachment.
4. We obtained for the first time pNIPAM termosensitive films by using dielectric barrier discharge at atmospheric pressure. Among the most important advantages of this technique is short time for obtaining thin uniform and stable films, with superhydrophilic character on various substrates.
5. The chemical composition, analyzed by XPS and FTIR techniques, was in good agreement with those reported in the literature for the pNIPAM obtained by other methods. Dielectric barrier discharge plasma at atmospheric pressure

- can be a good solution for obtaining thermosensitive films for biomedical applications.
6. This polymer was obtained to use it for certain biomedical applications consisting on controlling the attachment / detachment of cells.
 7. To minimize nonspecific interactions between myoglobin coated magnetic beads and the surface of a biosensor's substrate we investigated their dependence on the ionic strength of the solution for scPS and ppPS. It has been shown that the probability that magnetic beads bind to the surface increases with increasing ionic strength, a result that is in good agreement with DLVO theory. Moreover, the probability that magnetic beads coated with myoglobin to bind ppPS is lower than for scPS. This result is correlated with the oxygen content of ppPS and can be explained by using DLVO theory.
 8. A solution for minimize the nonspecific interactions between myoglobin coated magnetic beads and ppPS is to increase the PS oxygen content by increasing the oxygen content of plasma working gas. This idea is strengthened by the results presented by M. Kempler, D. Spridon et al.: for two minutes UV/O₃ treatment (oxygen content into PS matrix was approximately 20%) all magnetic beads rotated in the rotational magnetic field, so the number of nonspecific interactions are reduced to 0¹¹.

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