LOW TEMPERATURE PLASMA FUNCTIONALIZATION OF ZnO WITH AMINE GROUPS FOR BIOAPPLICATIONS

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Low temperature plasma processing is of great interest due to its numerous advantages compared with the chemical processing usually used nowadays for improving the properties of the materials. Specific surface functionalization of nanomaterials shows great potential for all kind of bioapplications. In this study, we propose the use of low temperature microwave excited surface wave plasma to functionalize ZnO particles with amine groups. Owing to its properties, ZnO would be more suitable for applications such as quantum dots, considering that currently used materials exhibit high toxicity. The outcome of plasma functionalization was analyzed with chemical and physico-chemical methods, all the results showing the successful amine group functionalization of the ZnO materials by dry plasma processing. The results are encouraging for the further usage of such materials for bioapplications.

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1. Introduction

Bioimaging techniques offer the possibility of characterization and visualization of biological processes at nanometer scale giving precise and real time information. Due to the fact that most biological processes take place at nanometer scale, the use of nanomaterials with specific function is of great interest for ideal imaging resolution. By adding functional groups to the surface of nanoparticles (NPs), the sensitivity and selectivity for various biospecies can be improved and new technologies can be developed [1, 2]. Different functional groups can be added to their surface depending on the desired outcome [3-5]. Our interest focuses on amine groups, due to the previous successful results in our group on NPs for bioapplications 0. The amine groups on the surface of the NPs can be later used as linkers for biomolecules, as it is described in Fig. 1, facilitating the implementation of the functionalized NPs in bioapplications.

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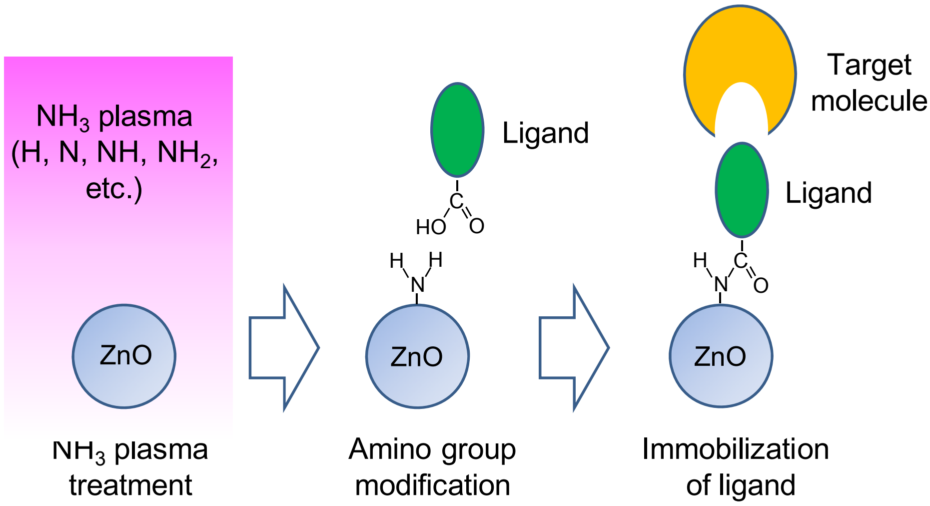

Fig. 1 Illustration of biofunctionalization process of ZnO NPs and utilization in bioapplications.

Functionalization of such NPs has been already done by wet chemical techniques 0. The huge issues with these techniques are imposed by the complex processing procedures and other limitations among which particle agglomeration is a very serious one. To overcome some of these problems, we propose the use of low temperature plasma processing as an alternative. This technique has been proven its numerous advantages in the modification and functionalization of different nanomaterials and biomolecules in a dry chemical reactive environment 0.

On the other hand, zinc oxide (ZnO) has been one of the most studied materials in the last decades. Being a versatile semiconductor and possessing interesting properties like the large band gap (3.37eV) at room temperature, high exciton binding energy (60meV) that ensures an efficient luminescence in the UV-blue region 0, ZnO is a suitable candidate for a large range of applications such as the fabrication of sensors, solar cells, drug delivery and biomedical applications. Compared with other semiconductor-based quantum dots (e.g. CdSe, CdTe, ZnS) currently used in bioapplications 0, ZnO is less toxic and compatible with the living organisms, which promote it to be used as an alternative for the existing materials 0. Surface functionalization of the ZnO nanoparticles is an important issue for improving their sensitivity and selectivity for various biomolecules and creating reactive sites for covalent binding 0. Low temperature surface wave plasma (SWP) proved to be suitable so far for other treatments on nanomaterials. Regarding ZnO, however, there is a lack of studies on plasma functionalization.

In this paper we test the possibility of introducing chemical functionalities, amine groups, on the surface of ZnO particles by dry plasma processing. To use these materials for bioimaging applications, other biomolecules of interest should be covalently bound in a second processing step on the surface of the particles having as intermediary the amine functionalities. To resume, the novelty proposed by our work consists in proposing the use of ZnO for bioapplications and the functionalization of ZnO by dry plasma processing, instead of existing complex chemical wet techniques. In this paper we focus on the first step that is ZnO surface modification by amine group functionalization. To test this possibility, we firstly used commercially available micro-sized ZnO powders. After confirming the amine groups addition on their surface, ZnO NPs were fabricated for the same purpose using a pulsed laser ablation (PLA) technique. Subsequently, the possibility of ZnO NPs functionalization was again investigated in different conditions.

2. Experimental

All the treatments of the ZnO commercial micro-sized powder and of the ZnO NPs prepared by PLA method were performed in the microwave excited SWP device that is schematically presented in Fig. 2. The experimental setup consists of a cylindrical stainless steel chamber with a diameter and hight, each of 400 mm. 2.45 GHz microwaves are guided through a rectangular wave guide and introduced into the chamber through slot antennas on a quartz plate.

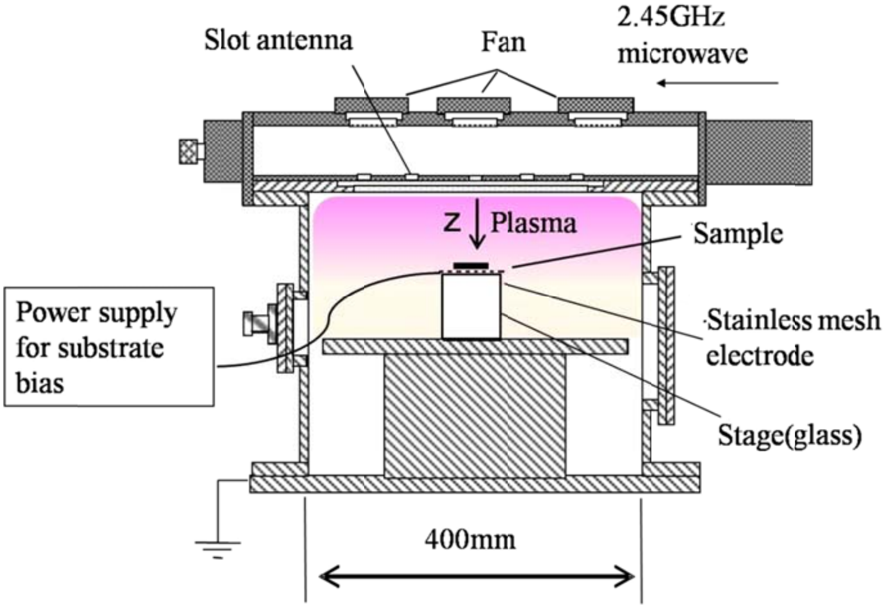

Fig. 2 Schematic drawing of the surface wave plasma device.

The processing conditions used to produce SWP are as follows: discharge pressure 13.3 Pa, constant ammonia gas flow rate of 100 sccm, and a distance between sample stage and the quartz plate of 100 mm. Three parameters were changed in order to compare different conditions. First, the value of the microwave power used to produce SWP was changed between 700 and 1000 W. Secondly, pulsed bias of -100 V and -150 V at 5 kHz was applied on a mesh electrode placed under the samples to stimulate the defects production on the surface of ZnO powders by intensified ion bombardment. In all cases the effect of different processing intervals on surface functionalities was studied. Information on the excited species in the plasma discharge was obtained by emission spectrometry measurements of the SWP using Acton SpectraPro 2300 (Princeton Instruments) equipped with 1200 grooves/mm grating and a spectral response range from 200 nm to 900 nm.

For confirming the possibility of ZnO amination, we first used commercial ZnO powder (Nilaco Co. P-15 #137) with micrometer size particles pasted on silicon wafers. After successfully binding amine groups on the surface of these particles, and considering the good results previously reported on the production of ZnO NPs by PLA 0, we started producing our own controllable size NPs. The experimental setup used is schematically presented in Fig. 3. A ZnO target produced from commercial powder is ablated in a pure reactive oxygen atmosphere with a Nd:YAG laser having a wavelength of 1064 nm and an energy of about 7 J/pulse.

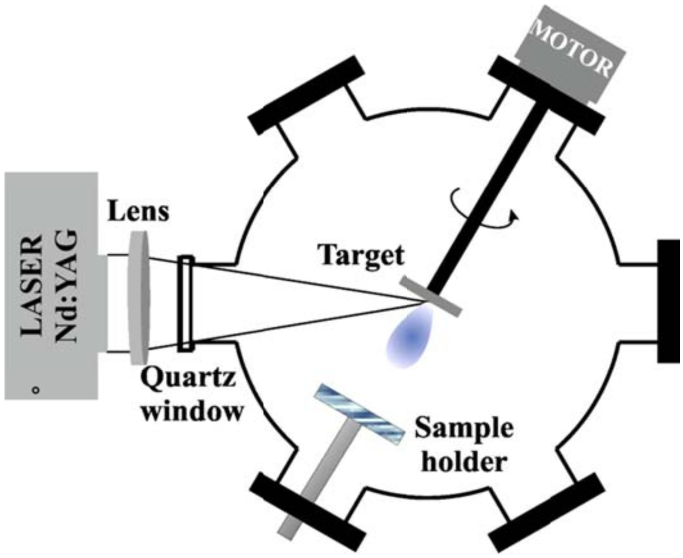

Fig. 3 Schematic representation of the Nd:YAG PLA experimental setup.

The outcome of plasma functionalization was analyzed with chemical and physico-chemical methods. To investigate the surface elemental and chemical composition of the samples X-ray photoelectron spectroscopy (XPS) measurements were carried out using ESCA-3400 spectrometer (Shimadzu Corp.). For a further confirmation and quantification of the functionalities we have chosen a chemical method: chemical derivatization by ninhydrin assay 0. Ninhydrin is able to specifically connect amine groups, the reaction releasing a purple (when reacting with primary amines) or yellow (when reacting with secondary amines) compound. The quantification of the method is done using a UV-VIS spectrophotometer. The absorbance measured at 570 nm is proportional with the number of primary amine functionalities, while the absorbance at 440 nm indicates the number of secondary amine functionalities.

Transmission electron microscopy (TEM) measurements were carried out both for the commercial powder and the prepared NPs to examine the fine details of the samples. The possibility of further biomolecule connection on the functionalized NPs was also checked using a fluorescent dye (Alexa Fluor 488, Life Technologies) that specifically binds amine functionalities and emits green fluorescent light (520 nm) when excited with 494 nm radiation. The result can be visualized using a fluorescent microscope (DMI 4000, Leica).

3. Results and discussions

Plasma emission spectrum was recorded to obtain information on the radiative species. Figure 4 shows the typical emission spectrum of the ammonia SWP produced in the conditions described in the previous section, in the UV-visible range.

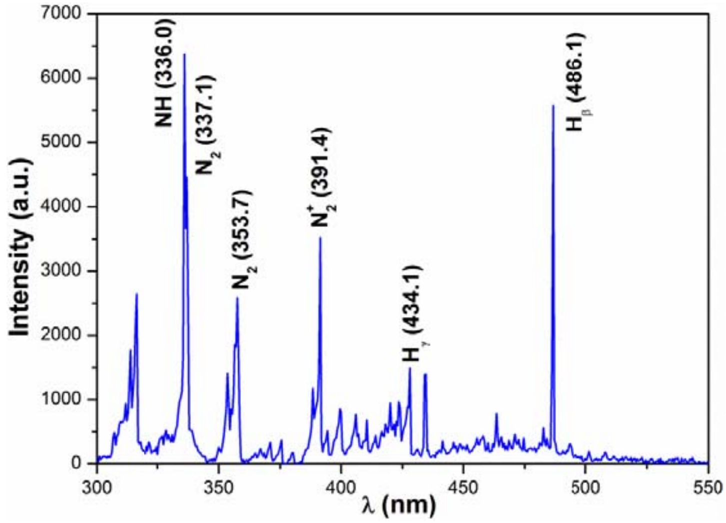

Fig. 4 Optical emission spectrum of ammonia surface wave plasma.

The identified emission lines indicate that there are various excited species required for amine group functionalization of NPs that would be processed in the ammonia plasma. These are mainly NH, but N_2 and N_2^+ might also make a contribution to induce amine and amide group grafting on the surface of NPs as found in other studies 0. This possibility is strengthened by the presence of hydrogen indicated by the strong H_β and H_γ lines. One should also consider the non-radiative species that could be responsible for functionalization, such as NH_2, NH_2^+, NH^+ and so on. In this chemical reactor, ZnO particles set at a distance of 10 cm under the quartz plate were processed in different discharge conditions. To accompany the plasma treatment by ion bombardment, a negative pulsed voltage was applied to a Si substrate under the samples. All the results were compared to those of direct plasma processing without biasing.

Figures 5(a) and (b) show the N 1s XPS spectra of the untreated ZnO micro-particles and the ZnO samples treated by ammonia SWP at 700 W, respectively. N 1s components are clearly enhanced in the case of ammonia SWP treated sample compared with the case of untreated samples. N 1s peak can be deconvoluted in two components centered at 397.6 eV and 399.7 eV 0 and corresponding to the secondary amines (=NH), and primary amines (-NH_2), respectively, as shown in Fig. 5(b). All the spectra of the processed samples have a similar deconvolution structure. Thus plasma processing is able to add not only primary amines but secondary amine groups as well on the surface of commercial micro-sized ZnO particles. Considering the reactive species evidenced inside the plasma reactor, these results are reasonable and expectable.

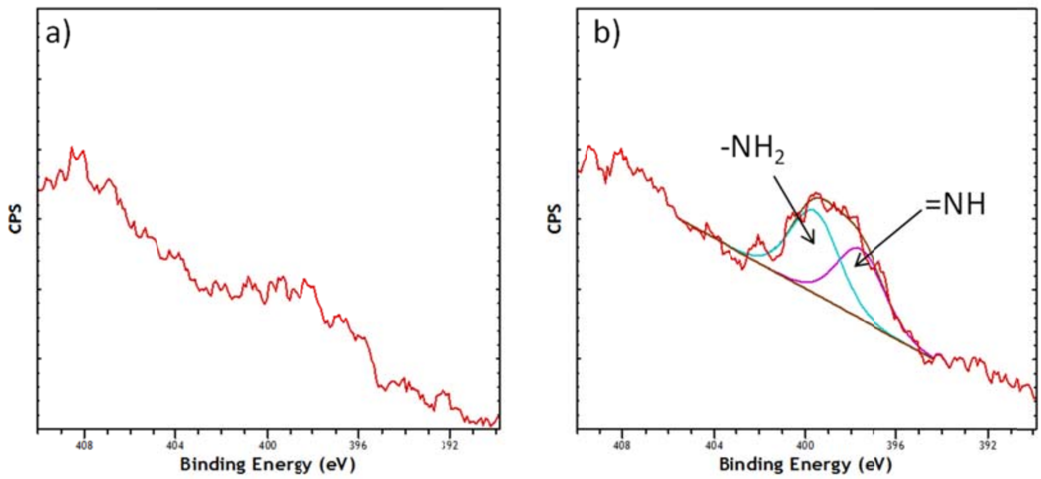

Fig. 5 XPS spectra of N 1s for a) untreated ZnO particles and b) ZnO particles treated by ammonia SWP at 700 W.

To examine the effect of ion bombardment on the surface modification, we compared the nitrogen content for three cases: no bias, biased at -100V and -150 V. It is found from Fig. 6 that the highest nitrogen content for the same processing time, 5 min, was obtained in the case of the highest bias voltage, -150V. These results show that increasing ion energy favors the connections between the reactive functionalities and ZnO materials. So it is considered that reactive sites creation over the surface of ZnO particles due to the strong ion bombardment promote the subsequent reaction of functional moieties with the reactive sites.

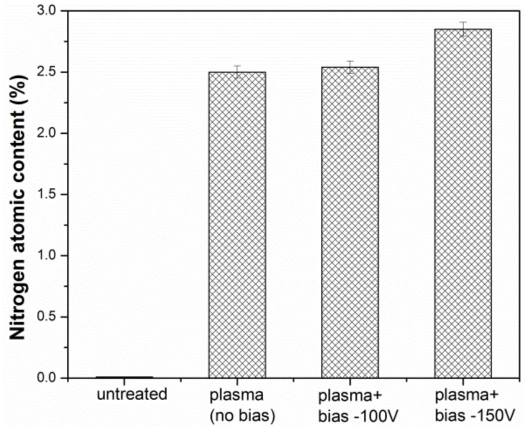

Fig. 6 Nitrogen percentage analyzed from XPS N 1s spectra for untreated and ammonia SWP treated ZnO samples biased at -100 V and -150 V for the same treatment time (5 min).

The same primary amine and secondary amine functionalities bonded on the surface of ZnO were detected by chemical derivatization. Fig. clearly shows the situation for the direct plasma processing without bias as a function of processing time. As described in the experimental section, according to ninhydrin assay, the absorbance determined with the spectrophotometer at 570 nm is proportional with the number of primary amine groups, while the absorbance at 440 nm is proportional with the number of secondary amine groups in the sample. According to the results in Fig. 7, the number of both nitrogen-containing functional groups in the samples increased with the processing time. Also there seems to be a saturation of functional group addition after the first few minutes since the values determined for 5 and 10 minutes are not considerably different. All values detected for the SWP-processed ZnO samples show absorbance for both wavelengths in contrast with the untreated ZnO that doesn't exhibit absorbance for neither values, indicating that in all studied cases the successful functionalization of the ZnO materials by SWP processing was achieved and it is not related with the unprocessed samples.

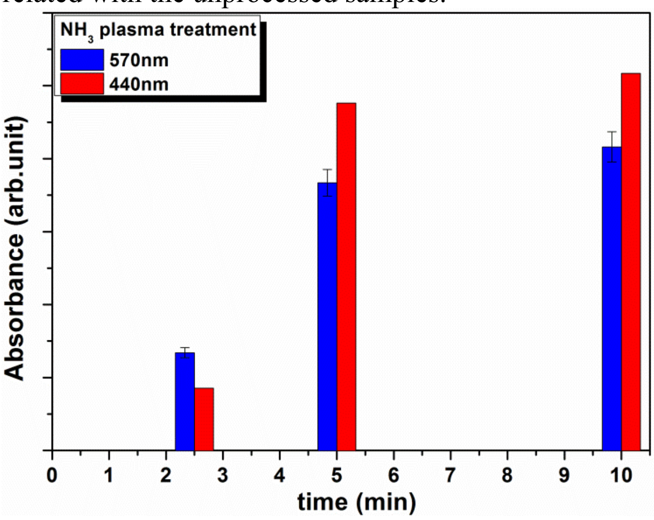

Fig. 7 Ninhydrin assay results: sample absorbance as function of processing time.

As stated before, our interest focuses on improving the properties of ZnO nanomaterials by plasma functionalization. Considering the above results obtained for the processing of commercial powder, we got to the next step of this research that is testing the possibility of functionalization of our ZnO NPs fabricated by PLA. The NPs were first analyzed by TEM, the results in

Fig (b) showing the image of ZnO NPs produced by PLA as compared to those of ZnO commercial powder in

Fig (a). The size difference is obvious, the latter being micrometer while the NPs produced in our lab have nanometer size.

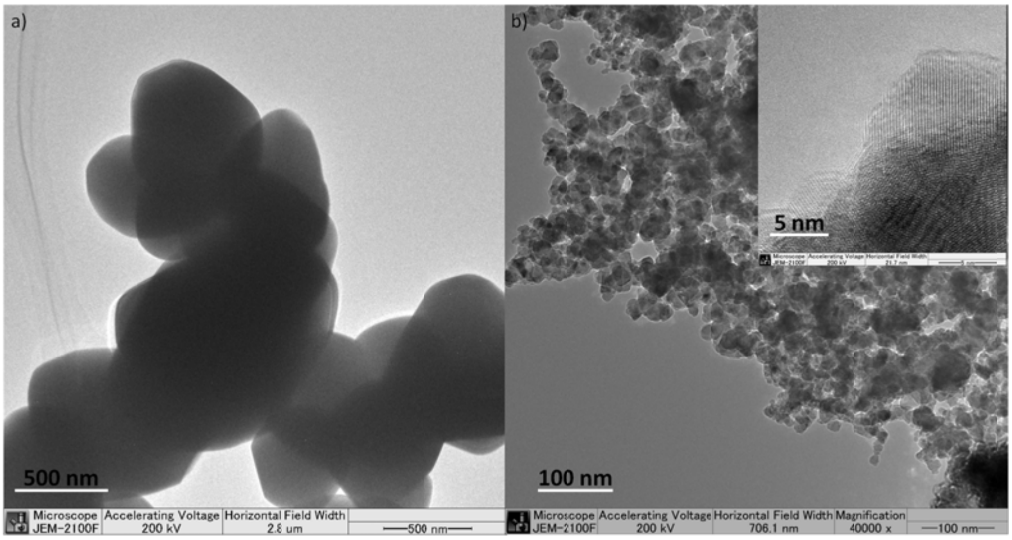

Fig. 8 TEM images of (a) commercial ZnO powder, and (b) ZnO NPs fabricated by PLA.

The same plasma treatment as employed for the commercial ZnO particles was used for the ZnO NPs. As a preliminary test to check the amine group functionalization we used a fluorescent dye that specifically connects the amine groups as described in the experimental section. Fig shows the images of (a) pristine ZnO NPs that were not exposed to ammonia SWP, and (b) ZnO NPs processed in ammonia SWP, both after reaction with the fluorescent dye. It is clearly seen that in the first case there are no amine groups while the green light emission in the second case proves the presence of fluorescent molecules bound on the amine groups on the surface of ZnO sample.

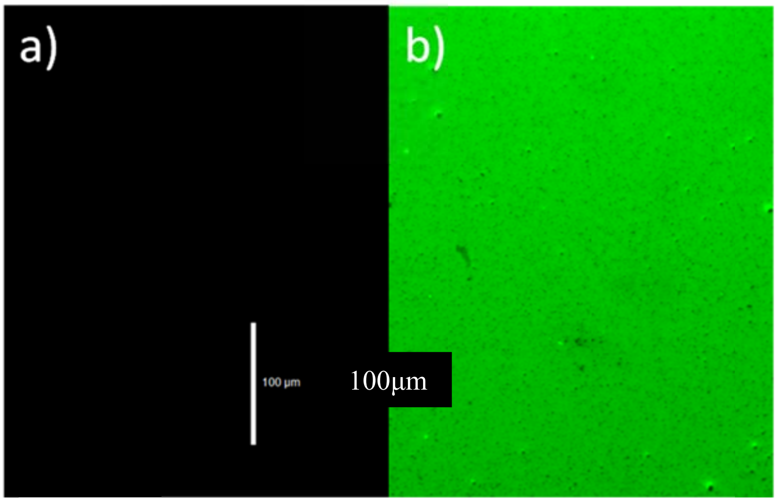

Fig. 9 Fluorescent microscope images for (a) untreated and (b) SWP processed ZnO material produced by PLA.

These results not only prove the successful functionalization of ZnO NPs using in dry plasma processing but also show the possibility of further biofunctionalization. The selectivity of the bonding and the importance of amine groups also reside from these results; the covalent bonding between the dye molecules and the amine groups is proven by the fluorescence measurements. Moreover, the fact that the untreated samples don't exhibit any fluorescence confirms that adsorption or other weak interactions between dye molecules and the ZnO NPs are negligible.

4. Conclusions

We studied the possibility of ZnO functionalization with amine groups in a dry reactive environment represented by ammonia surface wave plasma. All the results indicate that successful functionalization is achieved not only for the micro-size commercial powder but for PLA produced ZnO NPs as well, in all discharge conditions. Increasing the microwave power used to produce SWP results in a better functionalization, probably owing to a higher plasma density. In time, there seems to be a saturation effect, only few minutes of processing being enough for obtaining a strong increase of the functional groups density. Not only primary amine functionalities result from the treatment but also secondary amines, the fact being proven both by physico-chemical and chemical procedures. Applying bias voltage on a substrate situated under the ZnO material results in an increase of functionalities addition. The successful connection of the functionalities with fluorescent dye molecules indicates the possible further usage of such biofunctionalized NPs. The results presented in this paper show that there are very good premises for further use of such ZnO materials in bioapplications. Further experiments are needed to establish the most efficient parameters for the functionalization and also for figuring out the insights of the plasma processing of ZnO.

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