PLASMA TREATMENT IN SURFACE MODIFICATION OF SILICA DIOXIDE NANOFIBERS

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Abstract

In this work, silica dioxide nanofibers (SiNFs) were prepared by sol-gel method followed by electrospinning to be further functionalized by silanization. To improve the chemical modification, plasma treatment of various parameters was applied and the effect on the surface properties before and after plasma activation was studied. The increase of the amine groups accessible on the surface after the combined physico-chemical modification, together with the improved hydrophilicity of the so-modified inorganic nanomats, were demonstrated. Finally, the optimal parameters of the SiNFs plasma activation have been applied and their biocompatibility, as well as the time stability of the physical modification, have been proven.

Keywords: Silica dioxide nanofibers, silanization, plasma treatment

1. INTRODUCTION

Due to their unique physical and chemical properties, especially biocompatibility with low imunogenicity and eventual biodegradability, silica dioxide nanostructures are promising candidates for biomedical applications such as treatment of infection associated surgery [1], tissue engineering [2], or pharmaceutical drug-delivery vehicles [3], [4]. Over the past 10 years, research studies have focused on silica nanofibers (SiNFs) which may be an excellent material for regenerative medicine, especially for wound healing [5], [6]. The potential in biomedicine is often conditioned by a certain surface modification of a carrier material. Different methods to modify surface of the nanofibers for biological application are known. They can be of physical (e.g. plasma or UV modification), chemical (via chemical agents), mechanical or biological character and they can significantly influence wettability, functionality, stability and compatibility towards living cells. Concerning drug carrier applications, reactive surface groups are especially needed for attachment of bioactive molecules. When reinforcing them into nanofibrous matrices, not only dispersion, but also strong covalent attachment to the matrix is required [7]. Primary amine groups are widely used to enhance binding potential of the material, silanization being one of the well-known method for their chemical incorporation [8], [9]. At the same time, SiNFs are known for their hydrophobicity diminishing the efficiency of their secondary chemical treatment. In paralel, plasma treatment is a technique applied not only in the industrial fields [10] but also in the biomedical research to increase the hydrophilicity of materials [11]. The advantage of this process is also that it may enhance cell adhesion and biocompatibility of the material [12] while not affecting its properties due to low depth of ion penetration [13].

In order to improve the efficiency of previously applied simple silanization of SiNFs, we realized the combination of both types of modifications, plasma as the physical and silanization as the chemical one. Different parameters of such treatment were tested (time for plasma modification using different atmospheres) as well as its impact on the morphology of the nanomaterial studied. We also proved no changes in biocompatibility of silylated SiNFs that had been plasma treated before. Finally, time stability of plasma pre-treatment was studied.

2. MATERIALS AND METHODS

2.1. Chemicals

Fluorescein isothiocyanate (FITC, fluorescent label), tetraethyl orthosilicate (TEOS, silica fibers precursor), (3-aminopropyl)triethoxysilane (APTES, the coupling agent) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The remaining chemicals, all of analytical reagent grade, were supplied by Penta (Prague, Czech Republic). Nitrogen gas was provided by Linde Gas, a.s., Czech Republic.

2.2. Preparation of nanofibers

The silica nano-fibrous sheets of surface density of 470 g/m^2 were electrospun under stable conditions, at 22°C (air-conditioned space) using the NanoSpider device (NS 1WS500U, Elmarco Ltd.). The distance between an electrode and a collector was 175 mm. Spinning solution was prepared by sol-gel method using TEOS as a precursor, voltage of 70kV was applied. Finally, electro-spun sheets were thermally treated (180°C) to ensure their prolonged stability.

2.3. Physical modification - Plasma treatment

In order to physically modify the surface, plasma treatment with various parameters was used in this study. Three types of discharge were tested: the Gliding Arc (GA) with 25sccm of air in nozzle and 50mm/s speed of treatment (SurfaceTreat a.s.), the corona discharge (KO) with 800W power generator and 16.7mm/s speed of treatment (CXi, TUL), and the low pressure microwave plasma (MW) with radiofrequency of 2.45 GHz and working pressure of 100 Pa (CXi, TUL). The treatment time was 1, 2 or 5 minutes. Besides the air or nitrogen gas was applied as a working atmosphere.

2.4. Chemical modification - Silanisation

Silica nanofi-brous mats (pristine or plasma modified) were cut into 5 x 5 cm sheets and immersed into 3 % (v/v) APTES solution containing 4 % of water in ethanol (v/v). The pH was adjusted to 5.3 using acetic acid. After 2-hour silanization of nanofibers at a laboratory temperature under continual shaking, the samples were washed by the solution containing 4 % water in ethanol (v/v). Finally, the nanofibers were dried at 110°C/30min. Control samples were prepared evenly but immersed in the mixture water/ethanol (4/96) with pH 5.3 only, without APTES.

2.5. Quantification of amine groups

Quantification of primary amine groups was realised according to the work [14] with some modifications. At first, the 3.2 mM stock solution of FITC in ethanol was prepared. Subsequently, silica nanofibers were immersed into 25 fold diluted (130 μM) solution of FITC and incubated in dark overnight under gentle shaking. After removing all unreacted FITC from the nanofibers by thorough washing with ethanol, 0.2M NaOH was added and the samples were incubated in dark under vigorous shaking until the nanofibers fully dissolved. Fluorescence of prepared solution was measured at F_{ex}: 485/20nm, F_{em}: 528/20nm using Multi-Mode Microplate Reader (BioTek Instruments, USA).

2.6. Morphology characterization

Scanning electron microscopy, SEM (Vega3 SB, TESCAN Ltd.), was used to analyse silica nanofibers prior and after the modification process. Samples were coated with 5nm Au/Pd using a sputter coater equipment (SC7620 Mini Sputter Coater, Quorum Technologies Ltd.). Average fiber diameters and standard deviations (SD) were determined from at least 50 randomly chosen measurements of SEM images using VegaTC software.

2.7. Water contact angle measurement

To study the hydrophilicity/hydrophobicity rate, the contact angle was measured at different positions on the samples at room temperature using a Kruss Drop Shape Analyzer DS4. A total volume of 2 μL of distilled water

was dropped on the surface of a dry membrane surface, and the average values of the contact angle were calculated.

2.8. Cytotoxicity testing

Biocompatibility of the functionalized nanofibers was evaluated *in vitro* by the „direct contact" method. Human keratinocytes HaCaT were seeded into 24-well plate (40.000 cells) and cultivated for 48 hours in DMEM medium supplemented with stable glutamine dipeptide alanyl-glutamine and 10 % FBS. Nanofibrous discs of 6 mm in a diameter were placed on the cell layer and covered with a fresh medium. After 24 hours of exposition, the cell viability was evaluated by MTT method. Resultant viability was expressed as a portion of viable cells compared to the cell control (CC). Tested samples were also compared to negative (NC) and positive (PC) controls and untreated silica nanofibers prior and after silanization. Plasma treated samples were tested 15 days after the treatment was performed. Each sample was tested in triplicate.

3. RESULTS AND DISCUSSION

Among the different discharges tested, microwave plasma promoted the highest level of surface activation for further chemical functionalization. Under the conditions tested, this type of plasma discharge allowed an increase of grafted primary amine groups by 22±3 % in comparison to silanization of pristine SiNFs, while the corona and gliding arc offered an increase only by 12±2 % and 4±1 %, respectively (**Figure 1**). For further surface pre-treatment, microwave plasma was tested with or without a specific atmosphere of nitrogen gas. The specific atmosphere did not provide any benefit in activating the surface for further silanization. Not even an increasing treatment time reaching 5 min offered any special improvement in incorporation of amines via the coupling agent reaction. The results of the effect of various time and atmospheres are summarized in **Figure 2a**, where an increase of primary amines is represented by percentage vis-a-vis the functional groups grafted on pristine SiNFs (column labeled „APTES"). The effect of microwave plasma pre-treatment on the silanization was similar using both atmospheres, which is why a hypothesis regarding possible functional group grafting provided via nitrogen gas atmosphere [15] under used conditions was rejected. The functional impact ranged between 46 % and 51 % increasement in both cases. Nonetheless, the smallest values were associated to 1-minute plasma exposure which, regarding economic aspects, were the reason to choose a 2-minute activation procedure for further research. Herewith, based on gathered knowledge, the activation time did not exceed 5 minutes [16].

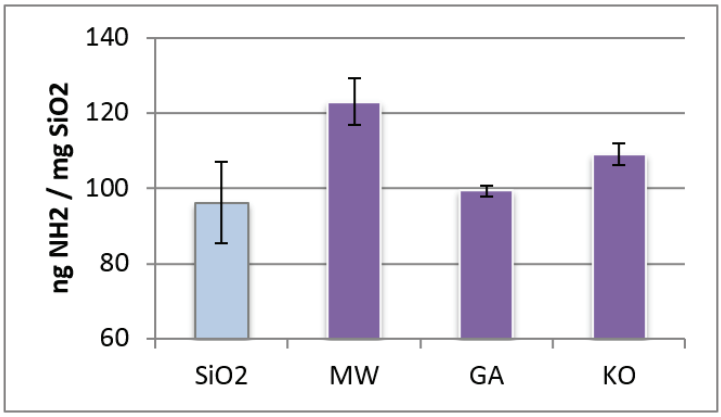

Figure 1 Effect of plasma treatment (with various discharge) on silanization of SiNFs: SiO2-pristine SiNFs, MW-microwave plasma treated, GA-Gliding Arc treated and KO-corona discharge treated SiNFs

Stability of plasma activation on SiNFs was followed during one week. Each activated SiNFs sample was chemically modified after a specific time period ranging from 5 minutes to 7 days. Surface NH_2 quantification

showed that 10 % decrease in activation occurred only within the first hours, while the plasma effect on the surface remained stable within the next days (**Figure 2b**). These results are in a good correlation with previous studies dealing with long-term stability of plasma-grafted functional groups [16]. The consequent morphology study of the samples showed that none of the surface modifications (neither via plasma, nor via silanization nor via combination of both) did not generate major mechanical damages on the tested substrate, only unchanged surface morphology was observed, no differences could be seen in means of fibre morphology (scanning electron micrographs are summarized on **Figure 3**). It can be suggested that the material crystallinity was largely unaffected by the applied conditions of plasma activation. The effect of plasma modification on hydrophilisation of the substrate was explored by measuring the water contact angle. Without treatment, the contact angle reached approximately 119.3°, and decreased to 27° after 1-minute plasma exposure (**Figure 4a**), and complete wetting was observed after all longer exposure times. Improved hydrophilicity is supposed to have a positive effect on further silanization.

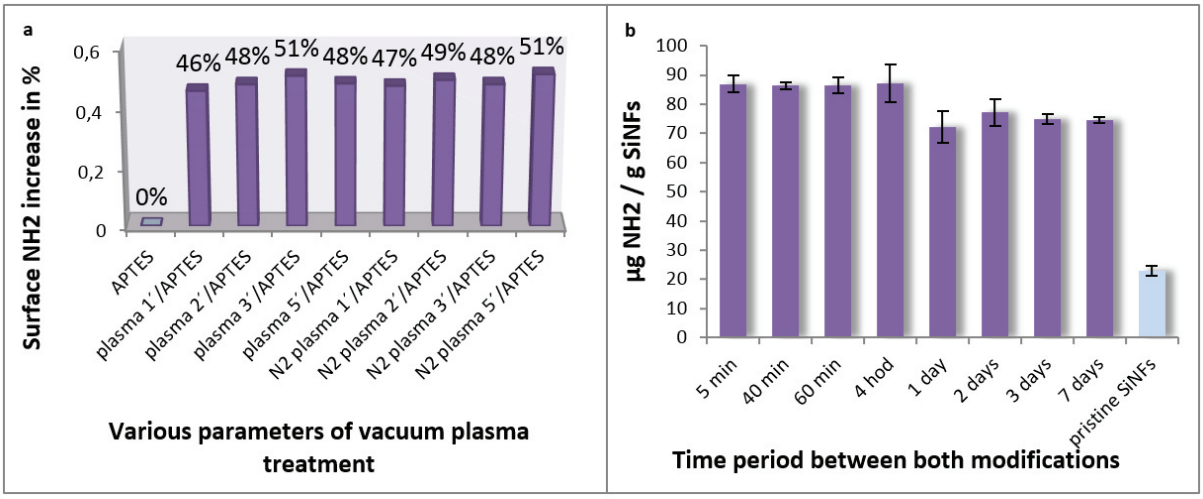

Figure 2 a) Effect of MW plasma treatment (with various time and/or atmosphere) on silanization of SiNFs; **b)** Temporal stability of plasma activated SiNFs via their posttreatment by silanization

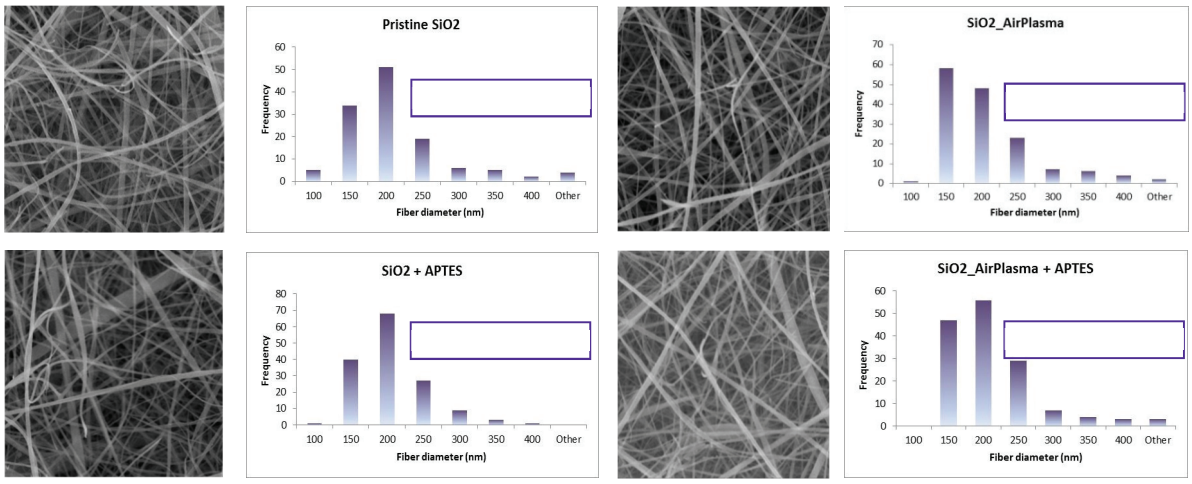

Figure 3 SEM analysis (magnification 10000x) - comparison of pristine SiNFs (SiO_2) with the physically and/or chemically modified nanosheets

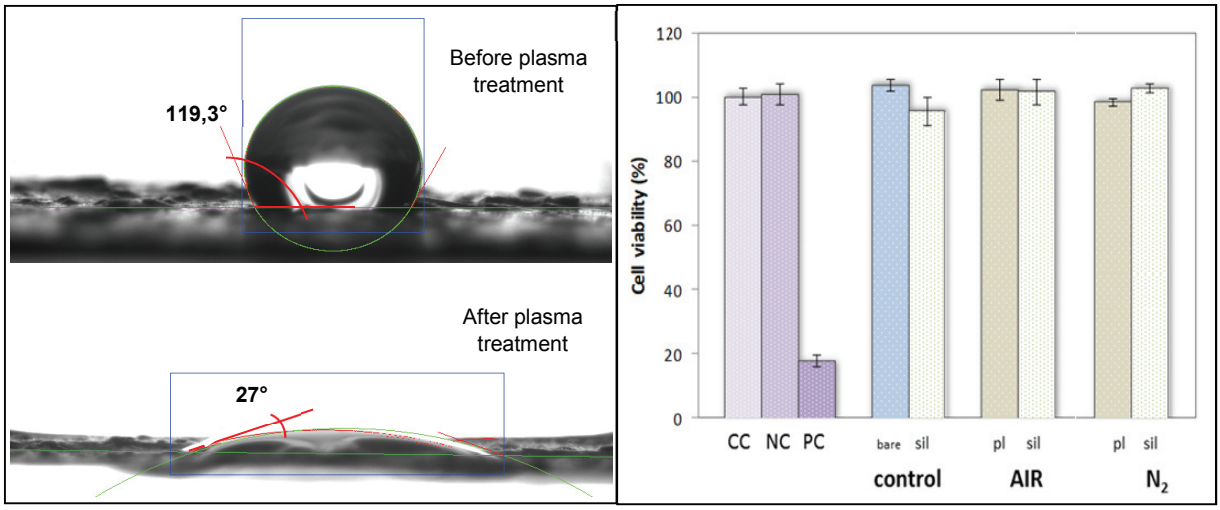

Figure 4 a) Contact angle of pristine and plasma treated SiNFs; **b)** Viability of the HaCaT cells obtained after 24hours exposure to the tested samples. (CC, NC, PC- cell, negative and positive controls; bare NF control - untreated silica NFs; pl- plasma treated, sil - samples silanized after plasma treatment).

Finally, all samples tested in a contact with the HaCaT cell line were proven to be biocompatible, as shown on **Figure 4b**. Viability of all the tested samples exceeded 90 % of the CC. According to these results, plasma treated and silanized silica nanofibers are declared cytocompatible to the Hacat cells according to the EN ISO 10993:5. Exposure to untreated silica nanofibers (NF control) and some plasma treated and silanized samples led to increased viability exceeding 100 % of CC. The best results were obtained by treatment in the air atmosphere, where viability after the plasma treatment was 102.1±3.4 % and 101.4±3.8 % after the subsequent silanization.

4. CONCLUSION

In this study, we showed the effects of plasma treatment on a following chemical modification; silanization of silica nanofibers. We demonstrated that among the discharges tested, microwave plasma provided the best results in improvement of further chemical modification of the surface. At the same time, plasma significantly improved hydrophilicity of the nanofibers. Biocompatibility of silanized SiNFs was unchanged after plasma treatment, and time stability of the the plasma-treated nanofibrous sheets was proven as well. Plasma modification followed by silane coupling agent reaction is thus the most promising method for surface functionalization, not only for industrial polymers but also for engineered materials in biomedicine.

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