MECHANICAL DEGRADATION OF SILICA NANOFIBERS IN AQUEOUS MEDIA

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Abstract

Pure silica nanofibers prepared by electrospinning from the sol-gel were reported to be successfully used in tissue engineering due to their biocompatibility, non-toxicity and biodegradability. This study deals with the possible mechanical degradation of silica nanofibers in vitro that have been pulverized down to the size of nanoparticles. The resulting fragments prepared in distilled water were compared to those that were removed up after two weeks of dissolution in saline adjusted to pH 7.4 and at 37 °C (values simulating the environment of some body fluids). The nanoparticles were measured using the Zetasizer device and compared to the data obtained by scanning electron microscope (SEM) and optical microscope.

Keywords: Silica nanofibers, silica nanoparticles, degradation, aggregation

1. INTRODUCTION

Silica nanofibers are relatively new inorganic material prepared by various methods. One of them is the sol-gel method that uses electrospinnig [1] [2]. Depending on the method of production, their diameter ranges from 70 to 1500 nm. Fibers of micrometer dimensions (tens to hundreds of micrometers) could be prepared by the melt-spinning approach. The silica fibers are utilized in diverse fields, such as electronics, optics, and most recently, also in medicine and tissue engineering. Glass and ceramic materials find application in the form of bones and joint implants [3], even though, the responses are not consistent and some works report high toxicity of the released silica [4]. There is an ongoing research of the possible use of silica nanofibers in wound healing [5] [6].

Silicon is a trace element that is important to nail, hair, bone and skin health. It assists synthesis of elastin and collagen, which are present in blood vessel walls and hence considered beneficial for the cardiovascular system. It is mainly a natural part of vegetable nutrition. The excess of dissolved silica is usually eliminated from the body by kidneys. Silicon, respective the silica, has been studied for many decades in connection with the disease silicosis that develops from the chronic exposure to the silica dust (e.g. miners). Inhalable silica particles (max. size about 10 micrometers) [7] can pass through into the pulmonary alveoli, where a prolonged irritation causes a chronic inflammation, and in the end, extensive nodular fibrosis. A way of removing silicon from the body can be illustrated by experiments performed in laboratory dogs [8]. After the application of silica into the lungs of dogs, the renal excretion increased. Application of the soluble form (silicic acid) led to the rapid increase of silicon in the urine, and 66% of its elimination by the kidneys within 24 hours. When silica was applied to the lungs in the insoluble crystalline form (particle size about 3 microns), it resulted in only 2% increase of the silica content in the urine, which is attributed to the possible content of the low proportion of very small particles in the suspension. Nevertheless, increased silica content in the urine of miners after one week without any contact with dusty environments (compared to volunteers) suggest that larger silica particles captured in the lungs gradually dissolve and are removed from the body. [8]

The health hazard of particles that penetrated to the alveoli may depend not only on their chemical composition, but also on the dose, exposure time, size, shape and overall surface area. For example, the structures contributing to the lung tumor risk appear to be long (>5 μm) and thin (0.4 μm) fibers whose potency appears to rise with increasing length [9]. The study [10] demonstrated that the cytotoxicity of monodisperse amorphous silica nanoparticles with the same morphology was strongly related to their particle size. The smaller particles

showed significantly higher toxicity than the bigger ones in the contact with human endothelial cells [11]. Several studies have shown translocation of ultrafine particles from the lungs to extrapulmonary organs via the systemic circulation [12].

Prevailing theories suggest that acicular, or fiber-like, particles induce enhanced toxicity over isotropic materials through barrier of phagocyte-mediated clearance mechanisms and through the mechanical interactions with cells. It is generally agreed that thinner and longer fibers are typically more toxic to pulmonary cells as well as more persistent in the lungs [13]. Currently, the degree to which either of these mechanisms operates is not well understood. It appears that the important factor in the particles' toxicity is the area of their surface because the cytotoxicity in experiments was dependent on the cell contact with the surface of particles, while the effect of the shape was not so significant [14]. The results indicate that particles induced an increased lactic acid dehydrogenase release and interleukin (IL) - 8 expression from mesothelial cells as markers of the cytotoxicity and inflammation, regardless of the shape. The decreased particle size seems to attenuate the effect of particle shape regarding phagocytosis resistance [15]. The studies showed a strong relationship between toxicity and biopersistence of fibers in lungs [16]. When inhaled, high biopersistence of inorganic particles and fibers in lung can cause a range of chronic fiber-related lung diseases like silicosis, chronic bronchitis, asthma or tumors [17]. Besides other factors, the shape can affect the particle aggregation and play a significant role in their removal and degradation (phagocytic clearance) [14].

It seems that the biopersistence of silica nanofibers can be affected already during their production, when their thermal stabilization (up to 500 degrees) leads to the faster degradation. According to the in vitro tests, the half-life of silica nanofibers in saline solution at 36 °C was for about 20 days [6]. Also study [18] concluded that pure silica nanofibers with the mean value of fibers diameter about 170 nm (stabilized for 2 hrs. at 180 °C and dissolved in the fluid that imitated pulmonary fluid with its pH 7.6), could be relatively safe because of the dissolution rate, which was determined by the order in the tens $ng \cdot cm^{-2} \cdot h^{-1}$. Even in the dynamic test of solubility fibers silica dissolved completely within 7 days. The safety criterion is based on recommendations of the study [16] that the fiber dissolution rate in the tens and hundreds $ng \cdot cm^{-2} \cdot h^{-1}$ can be considered safe.

Tests of the solubility of pure silica nanofibers prepared by electrospinning (mentioned above [6] [18]), that demonstrate their high degradability, short half-life and thus a relative safety and non-toxicity, do not take into account other physico-chemical factors in which fibers are subjected to addition of enzymes in vivo, mainly pH and interaction with a variety of other chemical substances in the body fluid. The saline solution of body temperature [6] simulates only the osmotic environment and the presence of main extracellular cation (sodium). The „simulated lung fluid" used in [18], in infact, does not simulate any other attributes besides the elevated pH (7.6). he lungs have unique features such as the lung surfactant and a negligible amount of the aqueous fluid that are difficult to replicate in vitro. The lung surfactant consists of lipid-rich lipoproteins with the lipid composition dominated by phosphatidylcholine. Therefore considering the high dipalmitoyl content, designing a standardized dissolution method applicable to the lungs is not easy. A majority of lipids in the surfactant form phosphoglycerols with cholesterol. Also, proteins (albumin, apoproteins), ions and a number of other substances are present as well [19]. However, considered these solutions were sufficient for the basic and very simplified idea of the potential behaviour of silica fibers in a living system and therefore, our mechanical in vitro tests were carried out under the similar conditions.

This study is based on the assumptions that silica nanofibers, which come into direct contact with tissues and blood (either in an inhalation form of individual fibers into the lungs, or as a covering material for wound treatment in the form of fibrous layers), are exposed to the combination of physicochemical and immune factors, in addition to the mechanical strain and stress in the presence of a slightly alkaline pH of pulmonary fluid or blood plasma. The larger fibers are fragmented and eroded into smaller fragments and particles, which are finally completely dissolved or phagocyted. In this study, a layer of pure silica nanofibers prepared by the sol-gel electrospinning was used. These fibers were pulverized in an aqueous environment for different periods of time using high speed impeller and sonication. The size and stability of the resulting fragments were

measured using Zetasizer device and their shape was evaluated by use of an electron microscope. This procedure was also performed on the silica nanofibrous layer which was previously dissolved for 2 weeks at 37 °C in the saline solution with the pH adjusted to 7.4. This should, in accordance with [6] [18], simulate osmotic and pH conditions in the lungs or in the contact with body fluid, assuming their partial dissolution or erosion to the safe form for contact with the organism. The dependence of fragments size on the breaking time was studied and both types of fragments were compared. The results were summarized in the discussion.

2. EXPERIMENTAL PART

2.1. Material

The layer of silica nanofibers which was prepared by electrospinning of a sol-gel formed by hydrolysis and polycondensation of tetraethoxysilane and then stabilized at 180 °C for 2 hours; NaCl, Na_2CO_3 (Lach-Ner).

2.2. Methods

The distribution of pure silica nanofibers (**Figure 1**) was observed using image analysis of pictures from the scanning electron microscope (Tescan Vega TS 5130). Five mg of silica nanofibers layer were immersed in 25 ml of saline and adjusted with sodium carbonate and acetic acid to pH 7.4. This sample was incubated at 37 °C for 14 days (static dissolving). After 14 days, this fibrous layer was mechanically crushed using the high speed impeller (Ultra Turrax T25 basic, IKA WERKE) at a the rotation speed 17 500 rpm for 1, 3, 5 and 10 minutes, subsequently at 24 000 rpm for 10 minutes and then sonicated for 10 minutes. In these time intervals, the samples were taken for measurements on Zetasizer device and SEM. Simultaneously, this procedure was also applied to the 5 mg of pure silica nanofibers without previous dissolution; these fibers were inserted into 25 ml of distilled water without pH adjustment. The size and stability of the broken silica particles were measured on the Zetasizer device, which evaluates these values on the principle of dynamic light scattering and laser Doppler electrophoresis. A colloidal system with the absolute value of the zeta potential less than 30 mV is considered unstable due to the low repulsion of particles with a low surface charge that occurs in such a system it leads to an easy aggregation and agglomeration of the particles. Pulverized fractions were evaluated with optical microscope Bresser Biolux.

2.3. Results and discussion

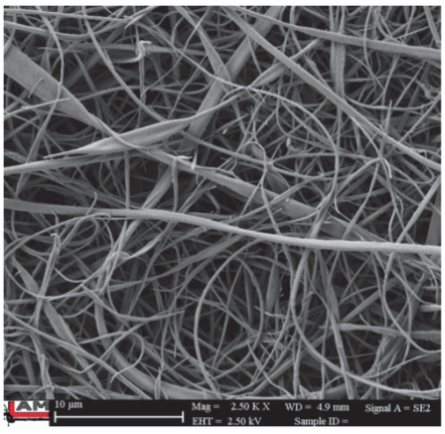

Figure 1 Silica nanofibers

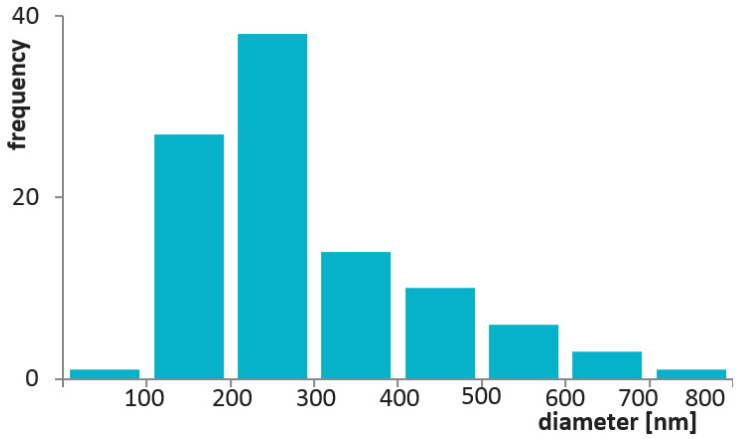

Figure 2 Length distribution of silica nanofibers

As seen in **Figure 2**, most fibers had a diameter of about 250 nm. However, this fibrous layer also contained a low percentage of fibers with the diameter of about 1-2 microns, as well as fibers having the diameter of less

than 100 nm. According to testing of statistical software (QC.Expert), the data correspond to lognormal distribution.

Table 1 Size of pulverized silica particles

Sample	17 500 rpm				24 000 rpm	Sonicator
	1 min.	3 min.	5 min.	10 min.	+ 10 min.	+ 10 min.
SiO_2 (dist.water)	1 305 nm	1 256 nm	1 215 nm	1 135 nm (87 %) 169 nm (13 %)	716 nm (91 %) 82 nm (9 %)	192 nm
SiO_2 (saline)	1 386 nm	1 290 nm	1 278 nm	967 nm	712 nm	241 nm

The measurement results from the Zetasizer showed that the size of silica particles decreased (**Table 1**) with continuing mechanical stress of silica fibers (time exposure increase of impeller, further increase of revolutions and subsequent ultrasonic shattering) to 192 nm or 241 nm. It is evident from **Table 1** that the particle size of individual fractions did not differ much in either of the sample (silica fibers in distilled water vs. silica fibers after 14 days in saline, pH 7.4, at 37 °C) as if the dissolution in the "simulated body fluid" had practically no effect on their strength.

The comparison with the actual appearance of particles under the microscope (**Figure 3**) and use of the SEM made it evident that the sizes measured with the Zetasizer are greatly distorted and inaccurate in this case. As a matter of fact, the heterogeneous mixtures of fragments with lengths of up to tens of micrometers were formed. Even with the increasing mechanical stress, the fibers were still breaking more and particles thus diminishing. However, even in the most pulverized fraction (after 20 minutes exposure to Impeller and 10 minutes of sonification) when the Zetasizer indicated 100% of particles with size corresponding to a mean diameter of the original nanofibers, we were still finding fragments and needles with a length of 10 to 40 microns (**Figure 4**) and individual fibers with a length more than 100 microns on SEM images (**Figure 5**).

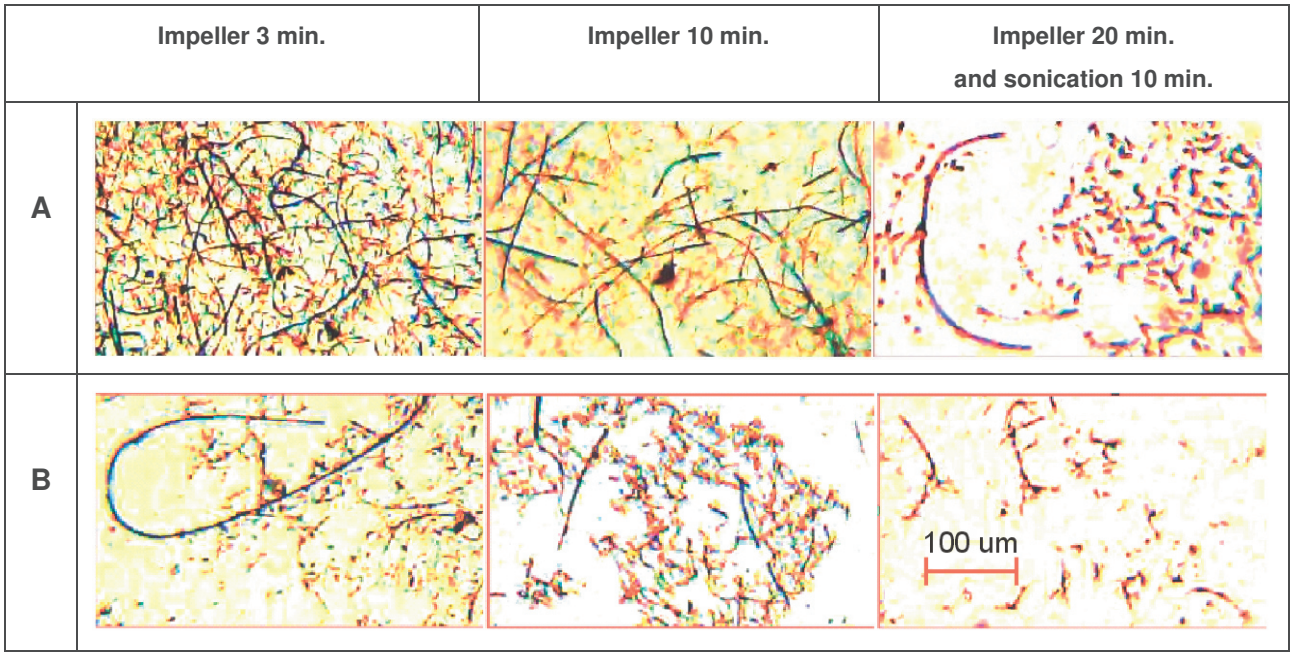

Figure 3 Gradual fragmentation of silica fibers in distilled water (A) and after 2 weeks in saline, pH 7.4 and 37 °C (B),(zoom 200x). Samples B were 10 times diluted because a lot of salt in field of vision

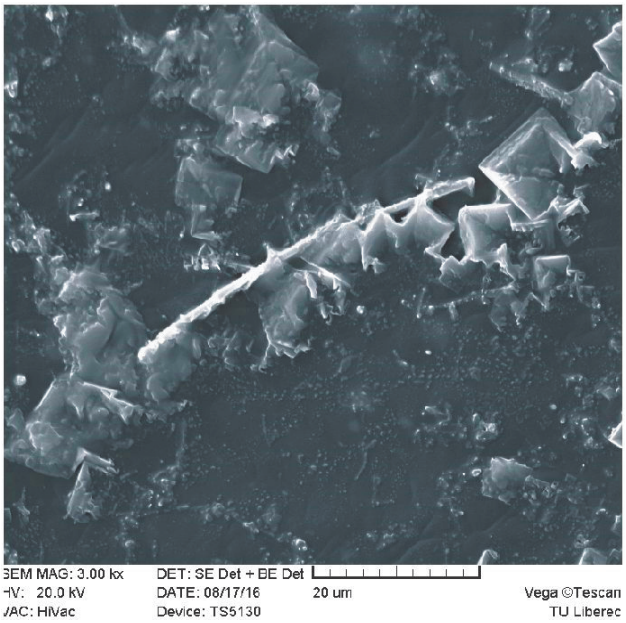

Figure 4 Silica fragment between the crystals of salt (SEM, 3 000x)

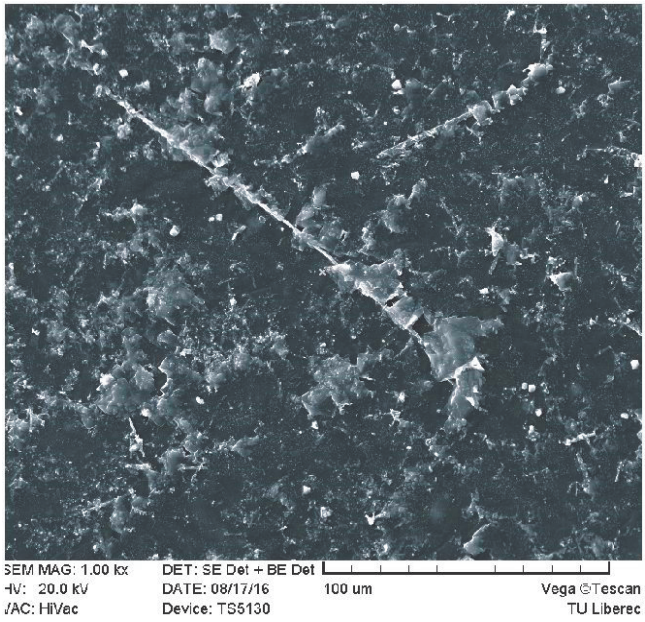

Figure 5 Silica fiber between the salt (SEM 1 000x)

The zeta potential values of silica nanoparticles and fragments in buffered saline were around -28 mV, which does not indicate very high stability of this suspension. The value was measured in suspension of particles after the first 3 minutes of exposure to the impeller (-28.7 mV) and in the finest fraction after 20 minutes of exposure to the impeller and 10 min of sonification (-28.4 mV). The measurements indicated that the change in shape and particle size (by Zetasizer 1290 and 241 nm) had no influence on the surface charge of silica fragments. The visible agglomeration occurred very quickly in all suspensions after the mechanical stress ended.

3. CONCLUSION

It is clear that the interaction of silica nanofibers with tissues and their subsequent elimination from the body is a complex process influenced by a number of physical, chemical and biological factors. In this research, we examined mainly the mechanical aspect of the possible fiber degradation with regard to physico-chemical effect of the prolonged exposure to body fluids and body temperature. Those are the influences to which silica nanofibers are exposed in the body, where they can appear accidentally (for example inhaled into the lungs) or deliberately (in the form of fiber layer used for the treatment of wounds or burns). The removal of foreign particles from the tissues ensures the immune phagocytal system, particularly macrophages. The size of particles absorbed by macrophages was limited by the size of macrophages (about 20 microns). Our results indicate that two weeks of interaction of body temperature, pH and osmotic conditions simulating the blood plasma with silica nanofibers (average diameter of about 250 nm) were not sufficient even for the strongly mechanically stressed silica nanofibers in aqueous media to reach the size and shape that would be safe and theoretically favourable for the absorption by macrophages. Fibrous suspension dissolving for two weeks dissolving in simplified simulated body conditions, and after the maximum mechanical stress, still contained many fragments with a length of more than 100 micrometers. At the same time, the silica (nano)particles and fragments showed a strong tendency to aggregate (aggregation or agglomeration) due to their low zeta potential. This is another possible challenging aspect which makes it difficult to remove them. It is evident that the issue of the interaction of inorganic silica nanofibers, layers, scaffolds, grafts and other carriers with living organisms is a complex process and therefore, it is necessary to study the behaviour of silica nanomaterials from different aspects.

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