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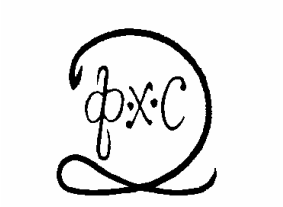

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MESOPOROUS SILICA DECREASE CELL VIABILITY *IN VITRO* IN DOSE DEPENDENT MANNER

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ABSTRACT
Mesoporous silica renders chemical and mechanical stability under biological conditions, but due to its physicochemical properties, could be potentially harmful to exposed cells. The aim of the current study was to estimate SBA-15 (concentration of 50, 100 and 250 μg/mL) impact on human peripheral blood mononuclear cells to induce cyto- and genotoxicity *in vitro* after 72 h treatment, as well as lipid peroxidation in serum samples *ex vivo*. SBA-15 mesoporous silica treatment impact on cell viability was performed by XTT assay, lipid peroxidation was estimated by determining malondialdehyde and 4-hydroxyalkenals levels and genotoxicity by micronucleus assay. SBA-15 treatments displayed genotoxic potential at the lowest concentration, while highest tested concentration led to decrease of cell viability and increase of lipid peroxidation. Chemical modification of SBA-15 material that could influence its physicochemical properties could be useful way to lower its toxicity.

INTRODUCTION
Santa Barbara Amorphous (SBA-15) mesoporous silica was synthetized and characterized by a well-ordered hexagonal structure with a uniform pore size up to 6 nm [1]. Their high surface areas enable large pharmaceutical adsorption capacities referring to high application potential. Biocompatibility, low toxicity and the presence of micropores, promote their application as carriers in drug formulations of prolonged release [2]. Despite all named quality properties, it was shown that 24 h exposure to 10 - 80 μg/mL of SBA-15 caused oxidative stress, i.e., increased reactive oxygen species (ROS) in the cell culture medium, in a concentration dependent manner. Increased ROS production could provoke cyto- and genotoxicity by altering important cellular macromolecules, which is why initial toxicity screening is very important. Moreover, impurities that could be introduced into mesoporous silica during the production process, such as catalysts, could display intrinsic toxicities [3]. Thus, the aim of the present study was to estimate potential cyto- and genotoxicity of SBA-15 and their relation to oxidative damage.

METHODS
SBA-15 is synthesized according to the standard procedure [4], by using Pluronic P_{123} (non-ionic triblock copolymer, $EO_{20}PO_{70}O_{20}$, BASF) as a surfactant and tetraethoxysilane (TEOS, 98 %) as a source of silica [4]. Peripheral blood samples were obtained from three healthy donors [5]. Aliquots of heparinized whole blood were set up for cytokinesis-block micronucleus assay (CBMN) [6]. Samples were centrifuged at 400 x g, plasmas were collected, and sediments diluted in PBS and used for further peripheral blood mononuclear cells (PBMC) separation by Ficoll™ density gradient media. PBMC were resuspended in RPMI 1640 medium supplemented with 1 % penicillin–streptomycin, 10 % fetal bovine serum (FBS) and 5 μg/mL phytohemagglutinin (PHA-M) mitogen for the stimulation of cell proliferation. Plasma samples were used as a medium for oxidative capacity estimation. Whole blood, PBMC and plasma samples were treated with increasing concentrations of SBA-15 (50, 100, 250 μg/mL and untreated control) for 72 h at standard cell culture conditions, i.e.

37 °C 5 % CO_2. Viability XTT assay was performed according to Roehm et al. [7]. From plasma samples, lipid peroxidation products (LPP) assay was done according to Tsikas [8]. All experiments were set up in duplicates and repeated three times. Statistical analysis was done using ANOVA test and Pearson correlation coefficient. Data are presented as the mean percentage ± SD, relative to control.

RESULTS AND DISCUSSION

Increase in cell proliferation was induced only by the lowest tested SBA-15 concentration. Upon 100 µg/mL SBA-15 treatment no impact on cell viability was detected, while the highest tested concentration of SBA-15 (250 µg/mL) significantly reduced cell viability (Figure 1.). The lowest tested SBA-15 concentration had no impact on LPP values, while higher SBA-15 concentrations elevated LPP in dose dependent manner (Figure 1.). However, the lowest concentration led to a significant increase in micronuclei formation by almost 50 % (Figure 2.), while other two concentrations didn't significantly affect MN frequency. Viability rates correlate positively with micronucleus frequency ($p < 0.01$; $r = 0.702$), indicating that genotoxicity assessment of SBA-15 treatments could rely on a number of cells that past cell division, and that higher concentration didn't display genotoxic effect due to more pronounced cytotoxicity.

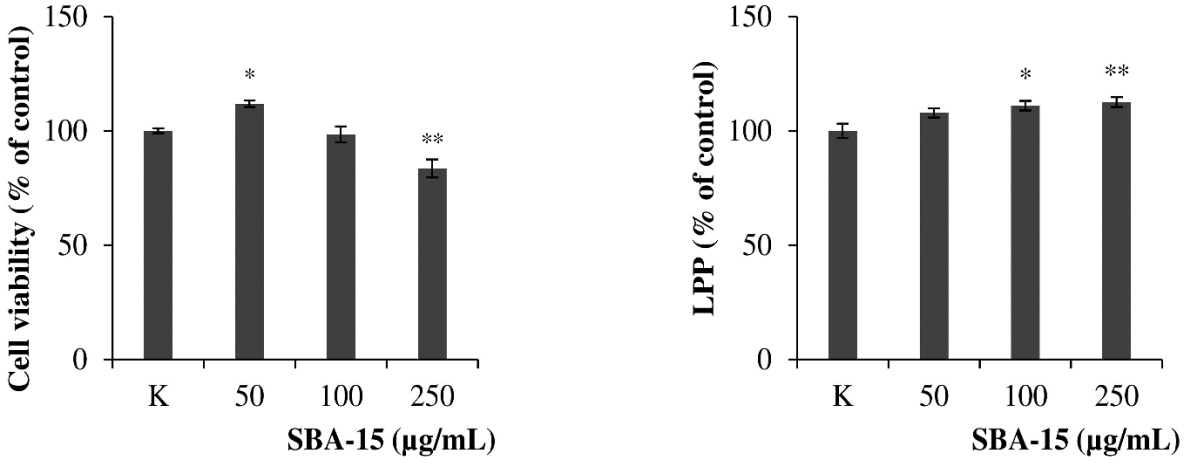

Figure 1. Cell viability (left) and level of lipid peroxidation products (right), expressed as percentage of control. Level of significance is marked as $^{*}p < 0.05$, $^{**}p < 0.01$.

Physicochemical properties of test materials that can contribute to their toxic effects include particle size and shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, and porosity [9]. Since size of SBA-15 aggregate prevents cellular uptake (Figure 2.), mechanical damage induced by its rod-like shaped structure could be the factor contributing to its cytotoxicity. Rise of LPP could be indicative of oxidative damage that also affects the cell viability, and presumably contributes to decreased cellular survival in higher concentration treatments. Detected changes in all of the tested parameters could arise from potentially present impurities that tail SBA-15 synthesis and concentration increment. Sodium, zinc, potassium, aluminum, calcium, magnesium, and iron were reported as abundant in SBA-15 [3].

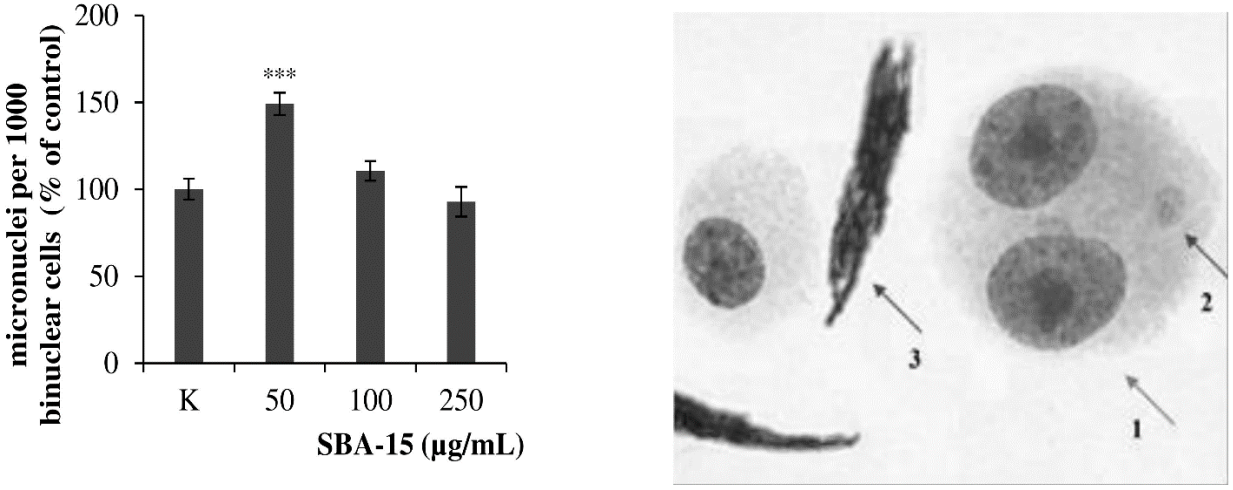

Figure 2. Frequency of micronuclei expressed as percentage of control. Level of significance is marked as $^{***}p < 0.001$ (left); Representative photomicrograph of binuclear cell (1), with micronuclei (2) and rod-like SBA-15 structure (3), (right).

CONCLUSION

SBA-15 treatments displayed genotoxic potential and decreased cell viability. Additional test should be performed to exclude potential influence of trace impurities that could contribute to SBA-15 toxic influence. Modification of SBA-15 material that could influence its shape without changing surface area potentially could be useful way to lower toxicity.

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