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Transient Receptor Potential (TRP) Ion Channel - Dependent Toxicity of Silica Nanoparticles and Poly(amido amine) (PAMAM) Dendrimers

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Abbreviation: Silica nanoparticle (SNP); Mesoporous silica nanoparticle (MSNP); poly(amido amine) (PAMAM); Transient receptor potential (TRP); TRPV (vanilloid), M (melastatin), A (ankyrin); Human Embryonic Kidney (HEK); Cetyltrimethylammonium bromide (CTAB); Tetraethyl orthosilicate (TEOS).

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Abstract:

Fundamental to the design and development of nanoparticles for applications in nanomedicine is a detailed understanding of their biological fate and potential toxic effects. Transient receptor potential (TRP) ion channels are a large superfamily of cation channels with varied physiological functions. This superfamily is classified into six related subfamilies; TRPC (canonical), V (vanilloid), M (melastatin), A (ankyrin), P (polycystin) and ML (mucolipin). TRPA1, M2 and M8 are nonselective Ca\(^{2+}\)-permeable cation channels which regulate calcium pathways under oxidative stress, while TRPV4 can be activated by oxidative, osmotic, and thermal stress as well as different fatty acid metabolites. Using a series of well characterized silica nanoparticles with variations in size (approximately 50-350 nm in diameter) and porosity, as well as cationic and anionic poly(amido amine) (PAMAM) dendrimers of similar size, we examined the toxicity of these nanoparticles to HEK-293 cells overexpressing different TRP channels. The data show that the toxicity of mesoporous silica nanoparticles was influenced by expression of the TRPA1 and TRPM2 channels, while the toxicity of smaller non-porous silica nanoparticles was only affected by TRPM8. Additionally, TRPA1 and TRPM2 played a role in the cytotoxicity of cationic dendrimers, but not for anionic dendrimers. TRPV4 does not seem to play a significant role in silica nanoparticle or PAMAM toxicity.

Keywords: TRP ion channels, Silica nanoparticles, Dendrimer, Nanomedicine, Toxicity.
1. Introduction:

Silica nanoparticles (SNPs) and poly(amido amine) (PAMAM) dendrimers have shown potential for use in nanomedicine as drug delivery systems, imaging agents, biosensors, and theranostics among other applications. (Sadekar and Ghandehari, 2012; Lu et al., 2017; Moghaddam et al., 2018; Saikia et al., 2018; Shao et al., 2018) To fully exploit this potential, a detailed understanding of their potential to cause toxicity as a function of nanoparticle physicochemical properties is needed. A better understanding of the structural components that promote toxicity of these particles will help in the design of safe nanoparticles for use in nanomedicine.

Transient receptor potential (TRP) ion channels are a superfamily of polymodal cellular sensors located on the plasma membranes of many mammalian cell types that nanoparticles can potentially interact with. Different TRPs are involved in different physiological processes such as the regulation of local and cellular calcium levels and associated signaling, control of Na$^{+}$ and Mg$^{2+}$ levels, transduction of sensory signals, cell proliferation, cell death, migration, and general homeostasis.(Fleig and Penner, 2004; Nilius, 2007; Venkatachalam and Montell, 2007; Gees et al., 2010; Andres et al., 2016; Markó et al., 2017) The TRP superfamily can be divided into seven subfamilies: TRP Vanilloid (TRPV), TRP Ankyrin (TRPA), TRP Canonical (TRPC), TRP Melastatin (TRPM), TRP Mucolipin (TRPML or MCOLN) and TRP Polycystin (TRPP or PKD and PKDL).(Fleig and Penner, 2004; Gees et al., 2010; Khalil et al., 2018) Most TRP channels are preferentially calcium permeable, and calcium signals originating from the opening of these channels have been reported to be important in modulating cell metabolism and general homeostasis.(Mulier et al., 2017)
In prior studies, it has been demonstrated that silica nanoparticle (SNP) uptake disrupts cellular calcium homoeostasis and also alters the expression of calcium channel related genes. (Yang et al., 2009; Ariano et al., 2011; Duan et al., 2016) There have also been a few reports on alterations to TRPC and TRPV4 channel activities and C60 fullerene- and silica nanoparticle-induced cytotoxicity. (Dryn et al., 2018) A role of the TRPM subfamily channel in SNP-induced oxidative stress and cell death has also been reported. It was shown that the TRPM2 channel was required for reactive oxygen species (ROS) production in HEK-293 cells and the subsequent increase in intracellular Ca\(^{2+}\) induced by SNPs via changes in NADPH oxidase activity. (Yu et al., 2015) However, limited information exists regarding the influence of variation in particle size and surface charge on NP toxicity via TRP channels. In this manuscript, we report on possible roles for TRPA1, TRPM2, TRPM8 and TRPV4, in regulating the cytotoxicity of SNPs with systematic variations in size and porosity, and of PAMAM dendrimers with variations in surface charge, using Human Embryonic Kidney (HEK-293) cell lines stably over-expressing these human TRPs. We chose the HEK-293 line as a model since these cells have few endogenous TRP channels which allowed for a more clear determination of the precise functions of these channels with respect to SNP and PAMAM toxicity.

**Materials and methods:**


1.1. Chemicals and reagents:

DMEM/F12 media, LHC-8, retinoic acid and epinephrine were purchased from Invitrogen (Grand Island, NY). Fetal bovine serum (FBS) was purchased from ATCC (Rockville, MD). TrypLE Express was purchased from Gibco (ThermoFisher, Waltham, MA). Phosphate Buffered Saline (PBS) Biotechnology grade tablets were received from VWR (Radnor, PA). Human TRPA1 cDNA was obtained from Open Biosystems (Huntsville, AL). Human TRPM8 cDNA was obtained from Origene (Rockville, MD). Human TRPV1 cDNA was amplified from fetal brain mRNA (Stratagene; La Jolla, CA). Hydrochloric acid was obtained from BDH Aristar. Absolute ethanol (200 proof) and ammonium hydroxide were from Decon Labs, Inc. (King of Prussia, PA, USA) and EMD Millipore Corp (Billerica, MA, USA), respectively. Cetyltrimethylammonium bromide (CTAB, ≥99.0%) and Tetraethyl orthosilicate (TEOS, 98%) were purchased from Sigma-Aldrich (St. Louis, MO). Commercially available ethylene diamine-core PAMAM dendrimers were purchased from Dendritech (Midland, USA). Diameter of PAMAM is theoretical values based on perfect dendrimer synthesis which is 36 Å and 45 Å for PAMAM G3.5 (COOH terminal) and G4 (NH2 terminal), respectively. All chemicals were used as received.

1.2. Synthesis and characterization of nanoparticles:

SNPs with variations in size and porosity were synthesized and characterized by a modified Stöber method as reported previously. (Stöber et al., 1968; Huh et al., 2003; Yu et al., 2011; Saikia et al., 2016; Yazdimamaghani et al., 2018a) Briefly, amorphous spherical nonporous SNPs of approximately 50, 100, 200, 350 and 500 nm in diameter were prepared by changing TEOS, water and ammonium hydroxide contents as shown
in Table 1. Approximately 500 nm mesoporous SNPs (MSNPs) were synthesized by the CTAB emulsifier templating combined with modified Stöber method. (Saikia et al., 2016) Mesoporous SNPs were further subject to CTAB removal using acid extraction at 60 °C for 6 h. The SNPs were fully characterized using Transmission Electron Microscopy (TEM) on a JEOL JEM 1400 microscope (JEOL Ltd., Tokyo, Japan) operating at 120 kV. One drop of colloidal solution of SNP sample was placed on a copper formvar-carbon-coated TEM grid. The excess liquid was removed using a piece of filter paper, the grid was allowed to dry for 5 min, and then the SNPs on the dried grid were imaged using a TEM instrument. The surface morphology of nanoparticles were studied using Scanning Electron Microscopy (SEM). One drop of colloidal solution of SNP sample was placed on a bilateral tape and gold sputter-coated utilizing an SPI-Module Sputter Coater. Each sample was observed under a FEI Quanta 650 FE-SEM scanning electron microscope operating at 20 kV (Hillsboro, OR). Dynamic Light Scattering (DLS) and Zeta potential measurements were carried out by Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, U.K.). Nitrogen adsorption–desorption isotherm analysis was conducted on a Micromeritics ASAP 2020 (Norcross, GA) at −196 °C to measure internal and external surface area and pore size.

1.3. Cell culture TRP channel cloning:

Cells were maintained in a humidified cell culture incubator at 37°C with a 95% air:5% CO₂ atmosphere. HEK-293 cells (ATCC; Rockville, MD) were cultured in DMEM: F12 media containing 5% fetal bovine serum and 1x penicillin/streptomycin. Human TRP channel overexpressing HEK-293 cells (TRPA1, TRPM2, TRPM8, and TRPV4) were generated as previously described, (Deering-Rice et al., 2011; Deering-Rice et al., 2012)
and cultured in DMEM: F12 media containing 5% fetal bovine serum, 1x penicillin/streptomycin, and geneticin (300 μg/mL).

1.4. Cytotoxicity assays:

The in vitro cytotoxicity of nanoparticles was assessed by Cell Counting Kit-8 assay (Dojindo Molecular Technology, USA). Briefly, cells (4 × 10^3 per well) were seeded in 96-well microliter plates for 24 h before treatment. Cells were exposed to freshly prepared different concentrations of nanoparticles and incubated for 24 h. The media was removed and 100 mL of medium solution containing 10% (v/v) CCK-8 reagent was added. The cells were kept at 37 °C for a further 1–2 h incubation. The absorbance of the supernatant was obtained by scanning with a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm. Each experiment was performed at least three times in triplicate. Control experiments were performed using cells treated with complete medium without particles as the negative control and 0.01% (v/v) Triton X-100 as the positive control. The difference between values was considered significant at the level of p ≤ 0.05.

2. Results and Discussion:
Figures 1, 2 and Table 2 outline the characteristics of the synthesized SNPs. Stöber SNPs (~50, 100, 200, 350 and 500 nm) with zeta potential values of approximately -40 mv to -50 mv were prepared to investigate the influence of size. In addition, the effect of porosity was examined by comparing similar size Stöber SNPs (500 nm) with MSNPs. Porosity increased the surface area of the particles by approximately 40-fold in comparison to nonporous particles. MSNPs had approximately 3.05 nm pore size which was confirmed by TEM in at least 50 different particles.

The human TRPM subfamily was named from the founding member melastatin (TRPM1) and consists of eight members that can be grouped into four pairs (TRPM1 and TRPM3; TRPM2 and TRPM8; TRPM4 and TRPM5; and TRPM6 and TRPM7). (Fleig and Penner, 2004) It is well-known that TRPM2 channels are expressed by many cell types in the immune system and play a crucial role in producing pro-inflammatory cytokines in response to oxidative stress and inflammatory stimuli. (Jiang et al., 2010; Mortadza et al., 2015; So et al., 2015) TRPM8 is activated by moderately cool temperatures and also by compounds that elicit a sensation of coolness, such as menthol. (McKemy, 2007; Sabnis et al., 2008; Liu et al., 2013) The cytotoxicity data in Figures 3 and 4 show that TRPM2 and TRPM8 affect the cytotoxic properties of 500 nm MSNPs, 50 nm SNPs and PAMAM G4-NH2 dendrimers, albeit in different ways and with some degree of selectivity. Specifically, overexpression of TRPM2 attenuated the cytotoxicity of 500 nm MSNPs and PAMAM G4-NH2 dendrimers in comparison to normal HEK-293 cells. However, the viability of TRPM8-overexpressing HEK-293 cells treated with 500 nm MSNPs and 50 nm SNPs significantly decreased relative to normal TRPM8-deficient HEK-293 cells. Published data suggests that TRPM8 channels form calcium permeable channels in the
ER membrane and plasma membrane of prostate cancer cells and are involved in proliferation, differentiation and cell death. (Fliniaux et al., 2017) Here, our data showed cell viability was significantly reduced by exposure to SNPs in a concentration-dependent manner in the cells expressing a high level of TRPM8 channels. On the other hand, TRPM2 channels might mediate a defense mechanism in the cells that have taken up the nanoparticles and increase their survival. The increase in cell viability of TRPM2-overexpressing cells in our current studies is consistent with previous reports examining smaller SNPs. (Yu et al., 2015) This study showed that the ROS production in HEK-293 cells expressing a low level of TRPM2, resulted in cell death, but high TRPM2 expression led to inhibition of ROS production and attenuated cell death. (Yu et al., 2015) We have observed the same protective effect of TRPM2 with MSNPs and positively charged PAMAM G4-NH2 dendrimers. Thus, it can be concluded that the protective mechanism of TRPM2 against cell death induced by SNPs and PAMAM dendrimers is highly dependent on physiochemical properties.

The TRPA1 cation channel is a molecular sensor for electrophiles and oxidants in lung cells. (Bessac and Jordt, 2010) TRPA1 is the only TRPA protein present in humans and other mammals. (Venkatachalam and Montell, 2007) TRPA1-overexpressing HEK-293 cells were treated with SNPs, MSNPs, and PAMAM dendrimers, and their viability in the presence and absence of nanoparticles, was measured. All SNPs showed concentration-dependent toxicity in HEK-293 cells and in HEK-293 cells overexpressing the TRPA1 channel (Figure 5). Among all the SNPs studied, HEK-293 cells overexpressing TRPA1 were less susceptible to toxicity caused by mesoporous 500nm SNP and PAMAM G4-NH2 dendrimers. At a dose range of 0.09-0.37 mg/mL, the degree of toxicity caused by
mesoporous 500nm SNPs in TRPA1-overexpressing HEK-293 cells was significantly (P≤0.05) less than for normal HEK-293 cells MSNP (Figures 5). The same trend was observed for the positively charged PAMAM dendrimer in the concentration range of 0.78-3.13 mg/mL. These results suggest that overexpression of TRPA1 on the cell membrane, and perhaps intracellularly, can induce resistance to the toxic effect of MSNPs and PAMAM G4-NH2 dendrimers. To the best of our knowledge, there are no reports on TRPA1 regulating the cytotoxicity of SNPs in any model system. Finally, previous observations showed that TRPA1 may mediate some of the adverse effects of wood smoke nanoparticles in humans. (Shapiro et al., 2013) One explanation for our results is that overexpression of TRPA1 in HEK-293 cells can facilitate rescue from cell death signaling which is triggered by SNPs uptake. More mechanistic studies are needed to investigate the molecular pathways related to TRPA1, novel agonists such as SNPs, and modulation of cell viability.

TRPV4 was originally identified to be involved in the regulation of osmotic homeostasis. Involvement of TRPV4 channels was reported for gonadotropin hormone releasing hormone (GnRH) release by neurons when treated with 50nm SNPs. (Gilardino et al., 2015) Further studies on human bronchial epithelial cell lines demonstrated the inhibition of TRPV4 in the presence of 10.2 nm silica nanoparticles, suggesting that these nanoparticles may impair the positive regulatory action of the stimulation of TRPV4 channels on ciliary function of 16HBE human bronchial epithelial cells. (Sanchez et al., 2017) Consistent with previous reports (Gilardino et al., 2015) we did not observe a correlation between the size of SNPs with TRP-related toxicity in TRPV4-overexpressing HEK-293 cells (Figure 6A and B). Our results show that there is no significant difference
for cytotoxicity between normal HEK-293 cells and TRPV4-overexpressing HEK-293 when exposed to SNPs, MSNPs, or PAMAM dendrimers of either surface charge (Figure 6A and B). These results suggest that the effect of TRPV4 on nanoparticles effects may be cell-type specific and/or dependent upon differences in the zeta potential values.

Overall, our data shows that among a series of well-characterized SNPs with systematic variations in size and porosity, the toxicity of smaller and MSNPs is significantly affected by TRPM2, TRPA1 and TRPM8 channels in HEK-293 cells. On the other hand, TRPA1 and TRPM2 play an important role in the cytotoxicity of amine-terminated PAMAM dendrimers G4-NH2, but not on carboxyl-terminated negatively charged PAMAM G3.5-COOH dendrimers of approximately similar size. Finally, TRPV4 does not seem to play a significant role in SNP, MSNP, or PAMAM dendrimer toxicity.

It is known that the cellular uptake of nanoparticles also can induce different stresses to cells. Depending upon the severity, duration, and mode of stress, the cumulative cellular response can range from activation of pro-survival pathways or unprogrammed cell death.(Fulda et al., 2010) It has been demonstrated that SNP uptake can disrupt cellular calcium homoeostasis.(Yang et al., 2009; Ariano et al., 2011; Duan et al., 2016) Also, calcium can trigger a signaling cascade in which CAM-K kinase activates Protein Kinase B pathways and protects cells from apoptosis.(Yano et al., 1998) Here, our data show that overexpression of different TRP/Ca++ channels (TRPA1 and M2) on the membrane of epithelial cells may help cells to overcome or avoid the cytotoxic effects of certain forms of particles, as a function of the nanoparticles' physicochemical properties. The next challenge is to understand the specific calcium-sensitive pathways that are modulated by the various nanoparticleless as a function of the presence or absence of
specific TRP channels, to understand the related cell survival and death molecular pathways.

We have previously shown that transepithelial transport and toxicity of PAMAM dendrimers depend on their size, surface charge, concentration and incubation time of these particles (Greish et al., 2012; Sadekar and Ghandehari, 2012; Thiagarajan et al., 2013) and that cellular uptake, in vivo biodistribution and toxicity of silica nanoparticles depend on their size, porosity, surface functionality, and geometry among other factors (Yu et al., 2012; Herd et al., 2013; Yazdimamaghani et al., 2018b). However, little is known about the detailed mechanisms of interaction of these nanoparticles with ion channels, such as the TRPs, which have varied physiological functions such as being cellular redox potential sensors or in modulating various phases of immune responses. While our data do not address these topics, it shows that size, porosity and surface functionality influence the toxicity of SNPs and PAMAM dendrimers via selective interactions that are, in part, related to the expression and function of different TRP channels. As such, these early, but enlightening studies have implications with respect to the design of silica and dendritic nanoconstructs for transepithelial transport and their immunotoxicity.

3. Conclusion:
Together, these findings provide the first evidence of differential involvement of TRPA1, M2 and M8 in SNPs and PAMAM dendrimer toxicity in a model system of human HEK-293 cell lines overexpressing TRP channels. Specifically, size and surface properties of the nanoparticles seem to have important roles on the effects of different TRPs on the acute cytotoxicity of these materials in HEK-293 cells. A better understanding of the underlying molecular mechanisms of cation TRP channel-related nanoparticle toxicity will be necessary to further elucidate the critical aspects of how TRPs influence nanoparticle toxicity. However, we anticipate that these novel findings will provide fresh insight into the design of safe and effective nanoparticles for use in nanomedicine applications.

Authorship Contributions:
Participated in research design: Raziye Mohammadpour, Mostafa Yazdimamaghani, Christopher A. Reilly, Hamidreza Ghandehari

Conducted experiments: Raziye Mohammadpour, Mostafa Yazdimamaghani, Christopher A. Reilly

Performed data analysis: Raziye Mohammadpour, Mostafa Yazdimamaghani, Christopher A. Reilly, Hamidreza Ghandehari

Wrote or contributed to the writing of the manuscript: Raziye Mohammadpour, Mostafa Yazdimamaghani, Christopher A. Reilly, Hamidreza Ghandehari
References


**Footnotes:**

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Conflict of Interest: The authors declare no conflicts of interest.

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Figure Legends:
**Figure 1:** Transmission Electron Microscopy images of Stöber (A-E) and mesoporous silica nanoparticles (F-G) with variations in size and porosity. Increasing the size of the Stöber particles from 50 to 500 nm results in an increase in the smoothness on the surface of the particle. Uniformly aligned mesopores along and perpendicular to the axes of mesoporous SNPs were observed (F and G). High-resolution TEM images showed 2D-hexagonal mesopores in the close-packing structure for mesoporous SNPs(G).

**Figure 2:** Scanning electron microscopy images of (A,B) Stöber SNPs with average diameter of 46 nm (Stober50), (C,D) Stober SNPs with average diameter of 91 nm (Stober100), (E,F) Stöber SNPs with average diameter of 205 nm (Stöber 200), (G,H) Stober SNPs with average diameter of 432 nm (Stöber 500), (I,J) Mesoporous SNPs with average diameter of 466 nm. Data for SNPs 50nm, 500nm and MSNP s were reported previously (Saikia et al., 2016; Yazdimamaghani et al., 2018b).

**Figure 3:** Comparison of cytotoxicity between HEK-293 cells and HEK-293 cells overexpressing the human TRPM2 ion channel after treatment with different concentrations of SNPs and PAMAM dendrimers for 24 h. Data show a significant (P≤0.05) TRPM2-dependent reduction in mesoporous 500 nm (MSNPs) and PAMAM G4-NH2 cytotoxicity relative to normal HEK-293 cells. Values are mean ± SD (n = 3).

**Figure 4:** Comparison of cytotoxicity between HEK-293 cells and HEK-293 cells overexpressing the human TRPM8 ion channel after treatment with different concentrations of silica NPs (nonporous 50 and 500nm; mesoporous 500nm) and
PAMAM G4 dendrimers for 24 h. Data show a significant increase in the cytotoxicity of the 500 nm mesoporous (MSNP 500) and 50 nm non-porous SNPs (P≤0.05), but not PAMAM G4-NH2 dendrimers in TRPM8-overexpressing cells. Values are mean ± SD (n = 3).

**Figure 5:** Comparison of cytotoxicity between HEK-293 cells and HEK-293 cells overexpressing the human TRPA1 ion channel after treatment with different concentrations of silica NPs and PAMAM dendrimers for 24 h. Data show a significant (P≤0.05) TRPA1-dependent reduction in mesoporous 500nm (MSNPs) and PAMAM G4-NH2 cytotoxicity relative to normal HEK-293 cells. Values are mean ± SD (n = 3).

**Figure 6:** Comparison of cytotoxicity between HEK-293 cells with HEK-293 cells overexpressing the human TRPV4 ion channel after treatment with different concentrations of SNPs (A) and PAMAM dendrimers (B) for 24 h. Data show no significant difference in cytotoxicity between the two cell lines for any of the nanoparticles tested. Values are mean ± SD (n = 3).
Tables:

Table 1. Synthetic conditions of nonporous and mesoporous SNPs based on molar ratio of solution.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Ethanol</th>
<th>H₂O</th>
<th>TEOS</th>
<th>NH₄OH</th>
<th>Stirring rate (rpm)</th>
<th>Temp (°C)</th>
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<tbody>
<tr>
<td>Stober 50*</td>
<td>1000</td>
<td>91.5</td>
<td>9.7</td>
<td>37.7</td>
<td>400</td>
<td>22</td>
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<tr>
<td>Stober 100</td>
<td>1000</td>
<td>91.5</td>
<td>9.9</td>
<td>60</td>
<td>400</td>
<td>22</td>
</tr>
<tr>
<td>Stober 200</td>
<td>1000</td>
<td>102</td>
<td>10.5</td>
<td>83</td>
<td>400</td>
<td>22</td>
</tr>
<tr>
<td>Stober 350*</td>
<td>1000</td>
<td>245</td>
<td>11.8</td>
<td>75</td>
<td>400</td>
<td>22</td>
</tr>
<tr>
<td>Stober 500*</td>
<td>1000</td>
<td>176</td>
<td>15</td>
<td>271</td>
<td>400</td>
<td>22</td>
</tr>
<tr>
<td>MSNPs 500*</td>
<td>0.14</td>
<td>1000</td>
<td>0.98</td>
<td>9.8</td>
<td>300</td>
<td>22</td>
</tr>
</tbody>
</table>

*Data for synthesis and characterization of these particles was reported previously. (Saikia et al., 2016)
Table 2: Physicochemical characterization of Stöber and mesoporous silica nanoparticles in different solutions: water, cell culture media (RPMI) with serum (FBS) and without serum. Values are mean ± SD (n = 3).

<table>
<thead>
<tr>
<th></th>
<th>Hydrodynamic diameter (nm)</th>
<th>Zeta potential (mV)</th>
<th>Diameter by TEM (nm)</th>
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<tbody>
<tr>
<td></td>
<td>water</td>
<td>RPMI</td>
<td>RPMI and 10% serum</td>
</tr>
<tr>
<td>Stöber 50 nm*</td>
<td>108.1±6.3</td>
<td>95.52±2.5</td>
<td>190.5±4.8</td>
</tr>
<tr>
<td>Stöber 100nm</td>
<td>133.1±2.4</td>
<td>123±0.5</td>
<td>291.9±3.2</td>
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<tr>
<td>Stöber 200nm</td>
<td>248.3±1.7</td>
<td>245.9±5.6</td>
<td>434.3±2.2</td>
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<tr>
<td>Stöber 350nm*</td>
<td>378.3±5.4</td>
<td>402.6±7.3</td>
<td>566.6±17.6</td>
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<tr>
<td>Stöber 500nm*</td>
<td>520.3±4.8</td>
<td>497.7±5.3</td>
<td>539.7±6.2</td>
</tr>
<tr>
<td>Meso 500nm*</td>
<td>799.8±22.9</td>
<td>999±23</td>
<td>915±72</td>
</tr>
</tbody>
</table>

*Data for synthesis and characterization of these particles was reported previously. (Saikia et al., 2016; Yazdimamaghani et al., 2018b)
Table 3: Different TRP ion channel-dependent toxicity of silica nanoparticles and PAMAM dendrimers; Results of the cytotoxicity assay show PAMAM G4-NH2 dendrimers and 500nm MSNPs have different toxicity in TRPA1 and TRPM2 overexpressed HEK-293 cells. Overexpressing of TRPM8 also changed the toxicity of Stöber 50nm SNPs and 500nm MSNPs.

<table>
<thead>
<tr>
<th>Human Kidney Epithelial Cells(HEK-293)</th>
<th>Nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overexpressed TRPV4 channels</td>
<td>No difference in toxicity for any nanoparticles</td>
</tr>
<tr>
<td>Overexpressed TRPA1 channels</td>
<td>Decreased toxicity for PAMAM G4-NH2 Dendrimer and Mesoporous SNP-500 nm</td>
</tr>
<tr>
<td>Overexpressed TRPM2 channels</td>
<td>Decreased toxicity for PAMAM G4-NH2 Dendrimer and Mesoporous SNP-500 nm</td>
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<tr>
<td>Overexpressed TPRM8 channels</td>
<td>Increased toxicity for Stöber SNP-50 nm and Mesoporous SNP-500 nm</td>
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Figures:

Figure 1:
Figure 2:
Figure 3:

![Graph 1: HEK-293, SNPs (50 nm)]

![Graph 2: HEK-293, SNPs (100 nm)]

![Graph 3: HEK-293, SNPs (200 nm)]

![Graph 4: HEK-293, SNPs (350 nm)]

![Graph 5: HEK-293, SNPs (500 nm)]

![Graph 6: HEK-293, MSNPs (500 nm)]

![Graph 7: HEK-293, PAMAM (G4-NH2)]

![Graph 8: HEK-293, PAMAM (G3.5-COOH)]
Figure 4:
Toxicity of SNPs (50 nm) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

Toxicity of SNPs (100 nm) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

Toxicity of SNPs (200 nm) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

Toxicity of SNPs (350 nm) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

Toxicity of MSNs (500 nm) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

Toxicity of PAMAM (G3.5-COOH) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

Toxicity of PAMAM (G4-NH2) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

(A)

Toxicity of PAMAM (G3.5-CODH) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

Toxicity of PAMAM (G4-NH2) in HEK-293 cells compared to HEK-293 overexpressed TRPV4 channels

(B)