Nanoparticles Increase Calcium Internalization via TRPV4: Towards Improved Understanding of Nanoparticle Delivery Technologies

[1]Jaspreet Singh Nagi, Amber Doiron[1]
[1]Department of Electrical and Biomedical Engineering, University of Vermont, Burlington, VT 05405, USA

Statement of Purpose: Nanoparticles (NPs) show significant promise in disease diagnosis and therapy and have many advantages, but our incomplete understanding of the complexity of the biological responses to particles limits their potential. For particles administered intravenously, the endothelium is the primary barrier to NP transport into the tissues. Endothelial cells (ECs) are also subjected to varied shear stress (SS) *in vivo*, ranging from 0.5-7 dyne/cm^2 in veins and peripheral arteries; thus, it is critical to investigate the effects of SS on NP-endothelial interactions. Our study aims to demonstrate the importance of SS in NP uptake and binding on ECs in dynamic flow conditions and it also aims to better understand the impact of 20 nm NPs on calcium (Ca^{2+}) levels to understand their potential for drug delivery applications as well as toxicity mechanisms. Our previous studies indicated an intracellular Ca^{2+} elevation when human umbilical vein endothelial cells (HUVEC) were exposed to 20 nm gold (Au) NPs in flow which was significantly higher as compared to static. Our lab and others have published studies showing a unique effect of 20-30 nm NPs that cause increased permeability also known as the nanomaterial-induced endothelial leakiness (nanoEL) which can be due to degradation of VE-cadherin, elevation in oxidative stress, cytoskeleton rearrangement, and Ca^{2+} signaling. The importance of Ca^{2+} in cell signaling and cell homeostasis is well known, and its concentration in a cell is regulated either from release of intracellular stores or influx through membrane channels. Transient receptor potential vanilloid-type 4 (TRPV4) and Piezo1 are the ion channels that control influx of extracellular Ca^{2+} in ECs Also key in calcium homeostasis are G(q) Protein coupled receptors (GqPCRs), which when activated lead to release of Ca^{2+} from intracellular stores in the endoplasmic reticulum. Based on our previous work, we hypothesize that Ca^{2+} is key in regulating the nanoEL effect, and we sought to understand the mechanism of intracellular Ca^{2+} change.

Methods: The level of intracellular Ca^{2+} was measured with Fluo-3, AM (Invitrogen) in HUVEC cultured with or without 10 mM of Ca^{2+} inhibitor ethylene glycol tetraacetic acid (EGTA) followed by exposure to Au NPs. Further, to test the mechanism of Ca^{2+} ion influx or intracellular release due to exposure to Au, 1 µM GSK 2193874, 3 µM GsMTx4, and 1 µM YM-254890 channel inhibitors were used separately to inhibit TRPV4, Piezo1, and G(q)PCR, respectively. All studies were performed with or without a 24 h exposure to SS of 6 dynes/cm^2 and a 2 h exposure to three different concentrations (10, 20, and 100 µg/mL) of 5, 20, or 50 nm Au NPs.

Results: Across samples treated with different particle sizes and concentrations, a statistically significant trend of intracellular calcium reduction when calcium was removed from media indicated that the source of intracellular calcium change was tied to regulation of an ion channel, not release from intracellular stores. A representative selection of data is shown in Figure 1 depicting a single concentration of particles tested in flow. On exposure of HUVEC in 6 dynes/cm^2 to 20 nm Au nanoparticles, the intracellular level of Ca^{2+} reduced from 151.08 ± 6.44% (no EGTA) to 17.84 ± 2.1% (EGTA), with percentage indicating change compared to untreated HUVEC. A similar but less sizeable trend was shown by cells treated with 5 and 50 nm Au NPs. Antagonists were used to investigate channel/receptor activation. On addition of TRPV4 antagonist, the level of Ca^{2+} dropped drastically, whereas samples treated with Piezo1 and GqPCR antagonists matched untreated controls.

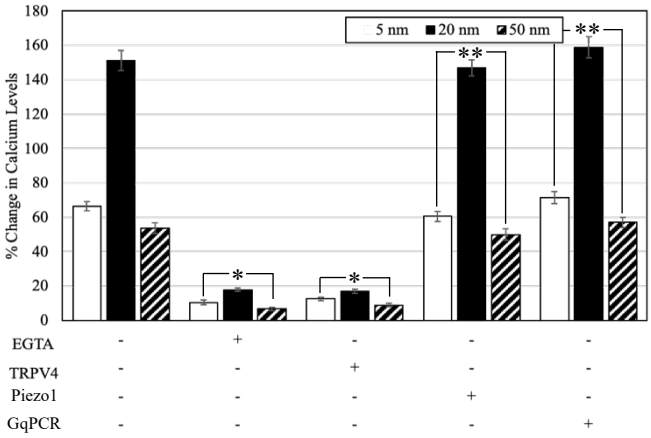

Figure 1. Percentage difference of intracellular Ca^{2+} produced as compared to the positive control (cells only) after HUVEC were exposed to 5, 20, and 50 nm Au nanoparticles (100 µg/mL) with (+)/without (-) EGTA and Ca^{2+} ion channel inhibitors TRP4, Piezo1, and GqPCR in Flow. Data shown are mean +/- standard deviation. Data with '*' represent a statistically significant difference between the cells treated with and without EGTA & the channel antagonists, n = 4, $p < 0.05$, data with '**' represent a statistically significant difference between the cells treated with EGTA and the channel antagonists, n = 4, $p < 0.05$.

Conclusions: Intracellular Ca^{2+} was significantly reduced when HUVEC exposed to 20 nm Au NPs were treated with EGTA in media and after addition of TRV4 antagonist. This reduction in the level of Ca^{2+} indicated that the intracellular Ca^{2+} elevation caused by NPs was due to an increased influx of Ca^{2+} through the TRPV4 channel. This new result coupled with our previous work showing nanoparticle exposure induced endothelial barrier dysfunction and transient permeability. This is a significant finding to understand the future potential of NP delivery technologies while limiting toxicity concerns. Future studies will involve investigating this mechanism across particle materials and endothelial cell lines as well as confirmation in animal models of endothelial permeability.

References: (Yizhong L. Nanoimpact. 2018;82-91.), (Matteo O. Compr Physiol. 2021; 11:1831-1869)