Unexpected interactions of nanoparticles with endothelial and epithelial cells

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ABSTRACT

Nanoparticles regardless as subjects of nanotoxicity or nanomedicine studies will inevitably interact with endothelial and epithelial cells. In the case of nanomedicine, while these cells are usually not the intended target, nanoparticles introduced via circulation would interact with them before even beginning to act on the target cells or tissues. It is therefore important to understand the effects of these nanoparticles on these cells. Similarly, for nanomaterials that are exposed to skin or via inhalation or ingestion, the cell types would be epithelial in nature. Using generic and commonly found non-decorated nanomaterials like TiO_2, silica, silver, hydroxyapatite nanoparticles, we discovered interesting new effects and solved their mechanisms. We discovered that nanoparticles can cause endothelial leakiness (NanoEL) in vitro and in vivo by finding its way into the spaces (adherens junctions) between endothelial cells, disrupts the homophillic interactions of VE-Cadherin and trigger the signaling pathway that leads to micron sized gaps between endothelial cells. We also discovered that nanoparticles can inhibit epithelial cell migration by targeting and disrupting microtubules. This increases focal adhesion at the interface of surfaces and transmit to increased cell traction.

Keywords: nanosafety, nanobiology, endothelial cells, epithelial cells.

1 INTRODUCTION

Nanotechnology is gaining more prominence and prevalence in our modern lives; touching on various facets of life close to heart; from healthcare to food, from personal hygiene products to immediate living environment. Therefore it is important to study the effects of these nanoparticles on biological systems.

Here we have investigated two biological systems which would represent most of the contextual scenario that nanoparticles will interact with. Firstly, the endothelial cell system and secondly, the epithelial cell system.

2 RESULTS AND DISCUSSION

2.1 Endothelial system

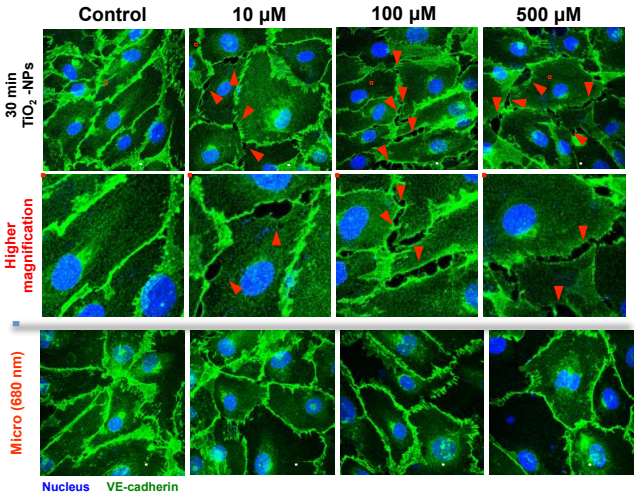

Figure 1: TiO_2 nanoparticles treatment resulted in micron-sized gaps to form between enodthelial cells and at very short time exposures of 30 minutes. No gaps were observed when treated instead with larger TiO_2 particles (680nm). Images were taken from immunostained (for VE-cadherin) samples. Nucleus was stained with DAPI.

We first allowed the endothelial cells to form a confluent layer to establish good cell-cell contact (Fig 1 control). The cells were treated with TiO_2 nanoparticles (primary size 20-25nm) (P25 Evonik Degussa) for various time points. After 30 minutes, we observed the formation of microsized gaps between the cells in the TiO_2 nanoparticles treated group (Fig 1) while no such gaps were observed with the larger TiO_2 particles group even after several hours. We coined these gap formation phenomenon "Nanomaterials induced endothelial cell leakiness" (NanoEL for short). Size of the nanoparticles seemed to play a role. NanoEL were initially thought to be linked to induced oxidative stress but measuring oxidative stress only showed significant oxidative stress after 24hours of exposure to TiO_2 nanoparticles. This suggested that this is a different mode of increasing endothelial permeability from iron oxide nanoparticles induced oxidative stress' mechanism (Apopa et al. 2009). We also tested whether apoptosis was a cause of NanoEL as shrinkage of the cells is an obvious outcome of apoptosis and this shrinkage would naturally create gaps. We found that there was no activation of the apoptotic pathway. We also tested whether

cellular uptake was necessary for the NanoEL effect to occur. We blocked the major endocytosis pathways and expected to also block NanoEL from occuring if endocytosis was necessary. NanoEL still occurred even with blocking. Therefore in light of the preliminary "negative" results, it suggested that the event has to occur before cellular uptake of the nanoparticles further supported by the short time point occurrence for NanoEL. We hypothesized that the nanoparticles may have directly act on the cell-cell junctions, namely the adherens junction between endothelial cells.

We showed that there was binding of TiO_2 nanoparticles on the key junctional protein, VE-cadherin. This binding (Fig. 2) led to the disruption of the interaction between paired VE-cadherin that is essential in maintaining the junctions (Fig. 3). We also showed that this loss in interaction brought about intracellular signaling pathways that results in the endocytosis and lysosomal degradation of VE-cadherin (data not shown but will be in the talk). The signaling pathway activated essentially depletes the junction of any VE-cadherin which would then prevent immediate re-formation of the junction. We also showed that intrinsic cellular tension after relief of the junctional connections, tore the cells away from one another, therefore resulting in the macroscopic gaps between the cells. (Fig. 4; more details and data in Setyawati 2013).

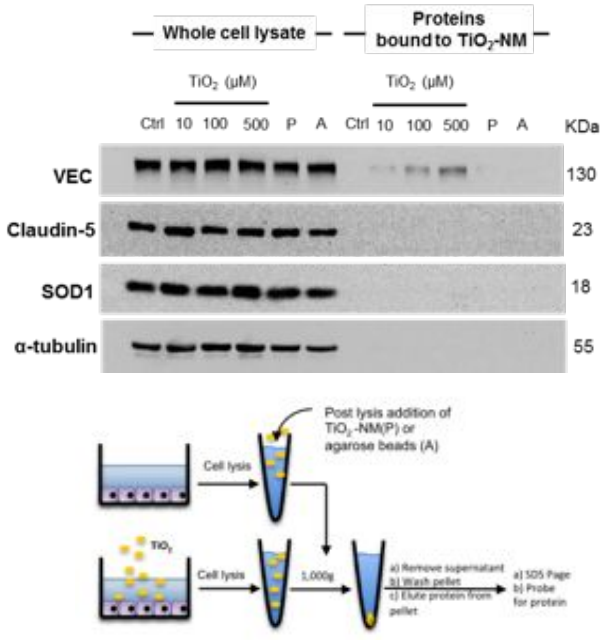

Figure 2: TiO_2 nanoparticles bind to VE-cadherin. VE-cadherin were likewise pulled down together with TiO_2 nanoparticles after centrifugation. Proteins pulled down were stripped off and subjected to electrophoresis separation and subsequently detected with specific antibodies against stated proteins.

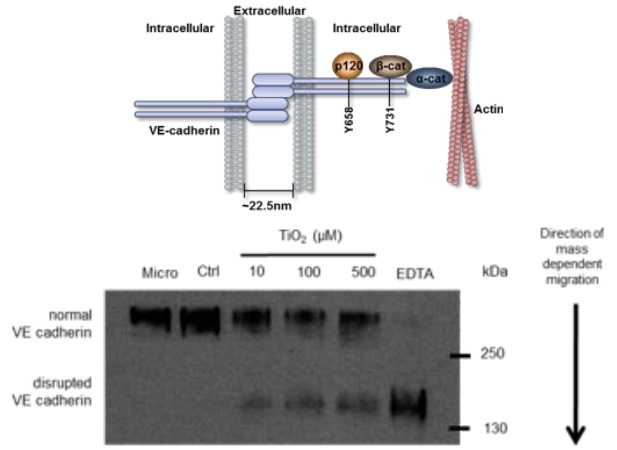

Figure 3: TiO_2 nanoparticles disrupted natively interacted VE-Cadherin. Confluent monolayer of endothelial cells were subjected to the various stated treatments. Total proteins were isolated and electrophoretically separated under non-denaturing conditions. EDTA group results in total disruption of any interacting VE-cadherin and controls for the electrophoretically separated size for disrupted VE-cadherin.

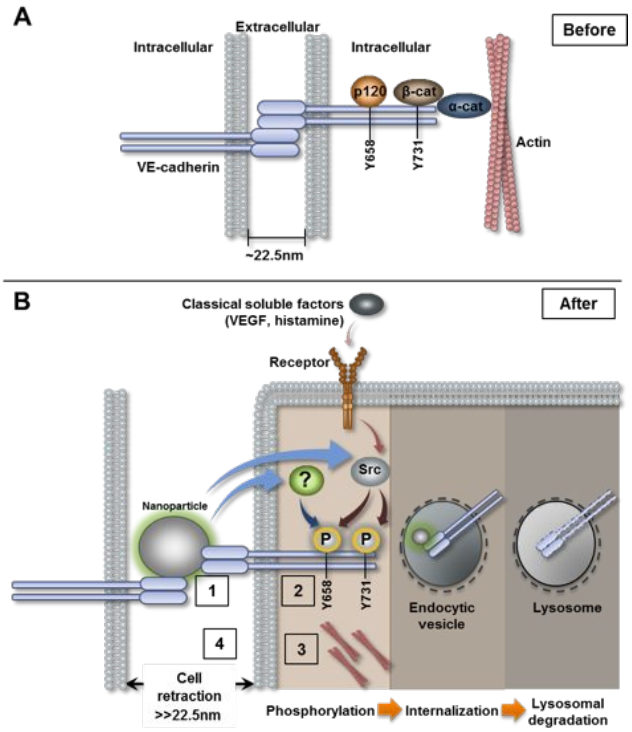

Figure 4: Our model of NanoEL. Nanoparticles by virtue of its size "fall" into the space between endothelial cells. Binds to VE-cadherin and disrupts the interaction holding adjacent VE-cadherin together. This triggers the pathway that leads to intracellular events that lead to the

neighbouring cells by a few tens of microns which is about 1000-fold wider than the original distance.

2.2 Epithelial system

We were interested in epithelial cell migration as migration is critical in one of the key functions of the epithelium; wound healing. We created a migration front by first placing a PDMS strip and then plating down the cells and after establishment of a monolayer, the PDMS strip was removed. We then measured the displacement of the migration front over a time course. At the same time, we measured cell traction stress (Fig 5). We were surprised that migration was inhibited with a corresponding increase in the final cell traction stress (Fig 5).

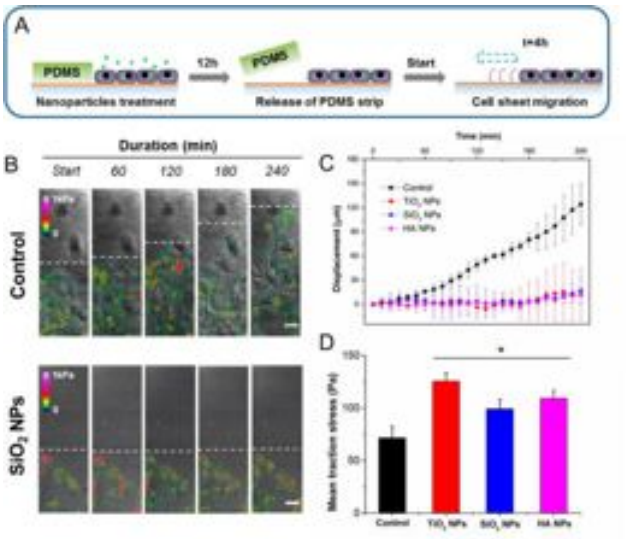

Figure 5. TiO_2, silica and hydroxyapatite nanoparticles treatment of epithelial cells impaired their migration. Cell traction stress increases were also observed after treatment.

We found that the various TiO_2, silica and hydroxyapatite nanoparticles were endocytosized by these cells and disrupted the microtubules network (Fig 6). This affected the dynamic force equilibrium between focal adhesions, microtubules and actin; resulting in increased cell traction (Fig 7 and more details in Tay 2014).

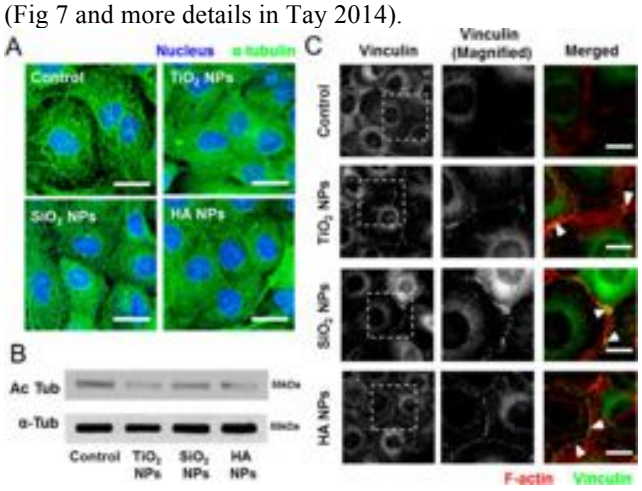

Figure 6: Microtubules appeared disrupted and destabilized with a corresponding increased activity of focal adhesion vinculin.

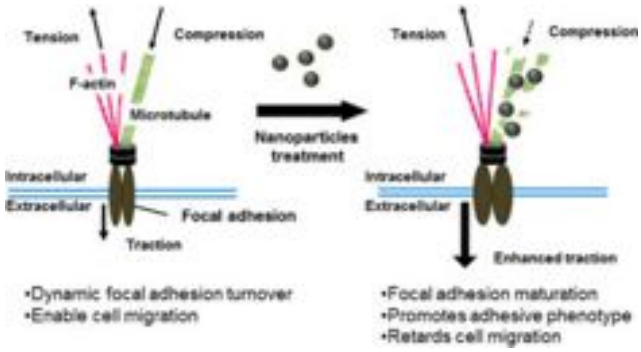

Figure 7. Nanoparticles after destabilizing microtubules, also offset the dynamic equlibrium force balance between actin, microtubules and focal adhesion-substrate force. These resulted in an increased traction to compensate for the loss in compressive force by the microtubules at the focal adhesion nexus.

3 Conclusions

Nanoparticles due to their small size and other unique properties may have profound effects on cells and biological systems. We have showed that nanoparticles have the ability to cause a high degree of permeability due to the formation of gaps between endothelial cells. There are implications to human health since many of these nanoparticles if entered into the body will spend an significant amount of time in the bloodstream. We have

also showed that nanoparticles could bring about a decreased in cell migration. This opens up the option that in the future, one can control metstastic cancer cells.

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