Shape-Specific Nanoceria Alleviate Oxidative Stress In Patient-Derived Valvular Interstitial Cells

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Statement of Purpose: Valvular heart diseases (VHD) such as valvular fibrosis and calcification remain significant clinical problems due to the increasing patient population and the lack of effective pharmacological treatments [1]. Elevated levels of reactive oxygen species (ROS) in valve tissue have been identified as a prominent hallmark and driving factor for a series of VHD [2]. Thus, ROS is a promising therapeutic target to halt VHD progression. The goals of this study are two-fold. We aim to 1) develop and characterize a disease model that can induce transdifferentiation of valvular interstitial cells into osteoblastic phenotype, found to be present in VHD; 2) develop ROS-modulating nanoparticles to halt VHD progression. Cerium oxide nanoparticles (CNPs) were chosen due to their unique ability to reversibly switch between the Ce^{3+} and Ce^{4+} oxidation states in response to the oxidative/reductive microenvironment which endows CNPs with self-regenerative ROS-modulating property [3]. Here, we demonstrated that 1) biophysical parameters like CNP shape can affect their ROS-modulating properties; and 2) such CNPs can reduce intracellular ROS level upon ROS assault and inhibit the transdifferentiation of hVIC to osteoblastic phenotype.

Methods: Diseased valve interstitial cells (hVICs) were obtained and isolated from the aortic valves of two aortic valve stenosis patients with Institutional Review Board approval (denoted as 03-0110 and 01-0067). Two hVIC populations were characterized for their phenotypic (mesenchymal, myofibroblast and osteoblast) marker expression by qRT-PCR and inherent antioxidant enzyme (superoxide dismutase and catalase) activity at baseline level as well as after response to ROS assault by hydrogen peroxide. Cerium oxide nanoparticles (CNPs) with ROS-modulating properties in four different shapes, namely, sphere, cube, short rod and long rod, were synthesized using ultra-sonication and hydrothermal methods. The shape and size of CNPs were examined by transmission electron microscopy (TEM). CNP oxidation state was examined by UV-vis spectrum. Cytotoxicity of CNPs to hVICs were measured using alamarBlue® assay. The abilities of CNPs to mitigate hydrogen peroxide induced ROS assault were measured via intercellular ROS detection by DCFDA assay and phenotypic (mesenchymal, myofibroblast and osteoblast) marker expression by qRT-PCR.

Results: Both hVICs exhibited similar mRNA expression of *vimentin* (mesenchymal), *alpha-SMA* (myofibroblast) and *osteopontin* (osteoblast). However, intracellular ROS level upon hydrogen peroxide induction was markedly different, which may be attributed to the significant differences observed in their inherent catalase activity. When treated with hydrogen peroxide (ROS assault), both hVICs exhibited increased intracellular ROS level and *osteopontin* expression, suggesting the transdifferentiation of hVIC into osteoblastic phenotype.

The shapes of four shape-specific CNPs were confirmed by TEM (Figure 1a). UV-vis spectrum revealed Ce^{4+} is the dominating oxidation state of cerium in CNPs. Both CNP-treated hVICs maintained more than 80% viability at doses lower than 100 µg/mL. CNPs possessed dose-dependent ROS-scavenging properties in both hVICs which significantly reduced hydrogen peroxide induced intracellular ROS level at 25 µg/mL (Figure 1b). It was also observed that there were shape-dependent differences in ROS-scavenging properties of different CNPs. Specifically, cube shaped CNPs demonstrated the least ROS-scavenging effect while sphere, short rod and long rod shaped CNPs exhibited similar antioxidant properties. More importantly, CNPs were able to mitigate the effect of ROS assault as demonstrated by reduced intracellular ROS level, and *osteopontin* expression at mRNA level.

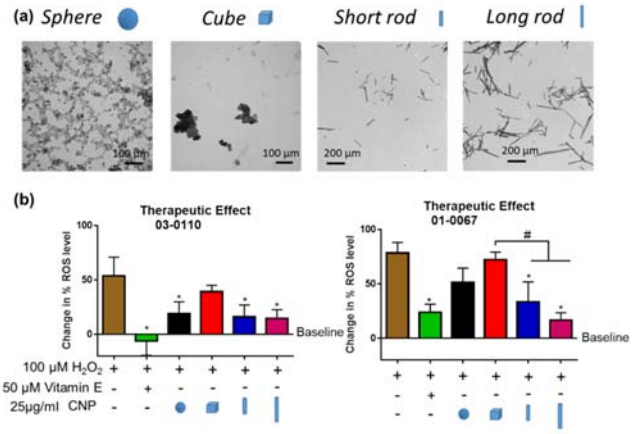

Figure 1 (a). Shape-specific CNPs as demonstrated by TEM observation; (b) CNPs demonstrated shape-dependent antioxidant effect in both hVICs (denoted as 03-0110 and 01-0067) induced by hydrogen peroxide as demonstrated by reduced intracellular ROS level.

Conclusions: In this study, we developed a diseased cell model for probing ROS-mediated valvular diseases using patient-derived VICs. Additionally, we synthesized CNPs with defined shapes and demonstrated their antioxidant properties for treating ROS-mediated valvular diseases. Future studies will investigate detailed mechanisms behind shape-specific CNPs as potential therapeutics for VHD.

References:

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