Cerium Oxide Nanoparticles: A Promising Outlook into Ophthalmic Applications

Mir Patel, John Burn, Dr. Vladimir Reukov

University of Georgia

Statement of Purpose: Cerium oxide nanoparticles (nanoceria) are being studied in the biomedical field for their ability to combat oxidative stress and unique auto-regenerative properties. Nanoceria acts as free radical scavengers and can be effective in reducing damage caused by reactive oxygen species. Cerium oxide is observed to be auto-regenerative due to oxygen vacancies and the ability to switch between two oxidation states. At the nanoscale, nanoceria has an extensive surface area in relation to their volume, increasing the concentration of oxygen vacancies. Increased oxygen vacancies allow for more effective free-radical scavenging. Reactive oxygen species (e.g. superoxide radicals) form as a result of acute inflammation which can cause damage to the cells and healthy tissues. The buildup of reactive oxygen species can ultimately lead to chronic inflammation and various other complications. Nanoceria has shown to be biocompatible with cellular tissues among their other properties which could make them a potential new treatment for ophthalmic diseases including cataracts, chronic inflammation, Dry Eye syndrome, retinal degeneration, and other diseases. This study aims to encapsulate nanoceria into liposomes so they can be used in eye drops. Nanoceria is poorly miscible in aqueous solutions and does not effectively penetrate the ocular surface. Encapsulating the nanoceria in liposomes has been shown to make nanoceria miscible and could effectively help them penetrate the ocular surface. Incorporating the nanoceria into eye drops could lead to the development of non-invasive alternatives to commonly used intravitreal injections.

Methods: A precipitation method was used to synthesize the nanoceria. Cerium nitrate hexahydrate and citric acid were used as precursors in an aqueous solution, such as ammonium hydroxide. A variety of assays were used to test our synthetic nanoceria. An MTT assay is used to measure the cell viability of the cerium oxide nanoparticles. The main reagents in this assay are MTT dye and PBS. A DCF assay is used to measure the ROS scavenging ability of the nanoceria in a cell environment. The main reagents used are the H2DCFDA dye, DMSO, and LPS. LPS is used to simulate ROS in the cell culture. Liposomes are synthesized from lecithin in a rotary evaporator. The lecithin was rotovapped with an organic solvent, such as chloroform, which produced a lipid film. The nanoceria is encapsulated through sonication.

Results: To measure the validity of the cerium oxide nanoparticles, MTT and DCF assays were used. In order to determine how to effectively use nanoceria to reduce the ROS, the reactive oxygen species, which is shown to cause chronic inflammation and other ophthalmic diseases, a DCF assay was used. In Figure 1, the results are shown on how using Lipopolysaccharides, LPS, in conjunction with the DCF assay, causing a stimulated inflammation, similar to that which would be seen occurring in the eyes, can have an effect on the ROS scavenging. The ability to scavenge ROS increases as the dosage of nanoceria increases, as seen below in the figure. Column 1 and Column 2, a blank and no nanoceria added, respectively, are presented as the control of the experiment, to show the comparison of the relatively low scavenging ability both have as opposed to the other columns. It is seen that as the dosages seem to increase to 90μL, the scavenging ability does as well, it can be inferred that higher dosages would most likely have an even higher scavenging ability. However, with higher dosages of nanoceria, there will be the risk of toxicity remaining, which is why the MTT assay was done. The MTT assay measures the viability of the nanoparticles with the macrophages, and whether or not the cells are able to withstand the toxicity of the cerium oxide particles. Overall, this experiment proved that despite there being a decline in cell viability when nanoceria dosages were increased, the cells remained relatively stable. This experiment is still ongoing, and are working on optimizing the experiment conditions, such as trying to encapsulate the nanoceria in liposomes to be able to more efficiently and effectively penetrate through the ocular surface. With the use of rotary evaporation, liposomes will be produced by lecithin, in which nanoceria will be encapsulated through sonication. Lastly, the experiment's focus is to be able to incorporate the encapsulated nanoceria into a form of eye drops, in order for a simpler method of application, rather than the intrusiveness the intravitreal injections result in.

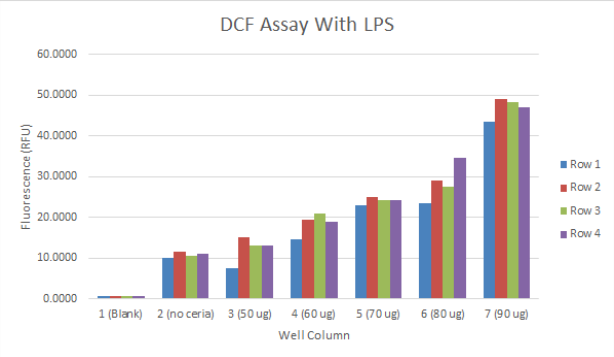

Figure 1. Increased nanoceria dosages effect on ROS scavenging ability.

References: Agar R., Tisi A. Pass M., & Cian, M., Opth app of Cerium oxide nan. https://www.tandfonline.com/doi/full/10.3109/10717544.2014.943336