

PLGA ENCAPSULATION OF NANOCERIA AND SUPEROXIDE DISMUTASE (SOD) YIELDS A DELIVERABLE, ANTIOXIDATIVE THERAPY

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ABSTRACT

Cerium oxide (nanoceria/ceria/CeO_2) exhibits antioxidative characteristics reminiscent of metabolic enzymes such as superoxide dismutase (SOD), suggesting its therapeutic application for reducing reactive oxygen species (ROS) that result from environmental stressors. Our previous work demonstrated that the combination of SOD and nanoceria increased the antioxidative activity of SOD by 22.9% over SOD, alone. Additionally, circular dichroism (CD) indicated no change in the molecular structure of SOD in the presence of nanoceria. This symbiotic activity motivated our search for a means of delivering both nanoceria and SOD, simultaneously. Herein, we report the successful encapsulation of nanoceria and SOD in PLGA—selected for its tunable degradation rate and biocompatibility—via a standard double-emulsification synthesis. Particle size and stability was evaluated via direct light scattering (DLS). A 40% drug loading efficiency was achieved for nanoceria as determined by spectroscopic measurements at 240 nm. Our BCA results indicated a loading efficiency of 18.9% for SOD. Additionally, cellular uptake studies were performed using PLGA tagged with Rhodamine-B. Fluorescent images of macrophage cultures indicated widespread endocytosis of the microparticles. These promising results support the use of PLGA as a vehicle for delivering nanoceria and SOD. Additional studies should aim to increase the drug-loading efficiency of SOD, and our own future work will explore the biocompatibility of PLGA-Ce-SOD particles and ensure that the antioxidative activity of nanoceria and SOD are maintained after encapsulation.

Keywords: Nanoceria, Cerium, PLGA, Antioxidant, Inflammation, ROS, Oxidative Stress

INTRODUCTION

Oxidative stress is associated with a number of undesirable conditions, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, diabetes, and cardiovascular disease [1, 2, 3]. Oxidative stress is characterized by the presence of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$), and superoxide radicals ($\cdot O_2^-$) [4]. ROS are often integral to cell-to-cell communication networks, and so proper regulation of their concentration is crucial for maintaining homeostasis and resolving inflammatory environments. The importance of ROS regulation, particularly for reducing inflammation and resolving ischemic stress, has motivated research into therapies aimed at reducing localized concentrations of ROS.

In one approach, several groups have followed the path of least resistance by delivering naturally occurring, antioxidative enzymes such as catalase and superoxide dismutase (SOD). Though this enzymatic therapy has been successful in a number of animal models [5,6,7,8,9], a significant and opposing body of research claims that SOD is ineffective and that the lifetime of SOD, *in vivo,* is too short for practical implementation of SOD as a therapeutic [10].

Another approach to antioxidative therapy utilizes nanocrystalline cerium oxide (nanoceria/CeO_2). Nanoceria, already a popular compound for a variety of industrial applications, has also demonstrated a unique capacity for attenuating redox reactions. Nanoceria is a non-stoichiometric compound, meaning that it has dual, interchangeable valence states which leave oxygen vacancies in the material. These vacancies enable the reduction of ROS by the oxidation of Ce^{+3} to a Ce^{+4} state [11]. The catalytic activity of cerium oxide is further enhanced by using its microcrystalline form, nanoceria, to maximize the ratio of surface area to volume. When introduced to a biological environment, nanoceria is capable of mimicking the activity of SOD or catalase by converting superoxide anions into hydrogen peroxide or reducing hydrogen peroxide, respectively [12].