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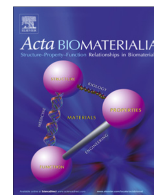

Full length article

# Manganese oxide particles as cytoprotective, oxygen generating agents

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ABSTRACT

Cell culture and cellular transplant therapies are adversely affected by oxidative species and radicals. Herein, we present the production of bioactive manganese oxide nanoparticles for the purpose of radical scavenging and cytoprotection. Manganese comprises the core active structure of somatic enzymes that perform the same function, *in vivo*. Formulated nanoparticles were characterized structurally and surveyed for maximal activity (superoxide scavenging, hydrogen peroxide scavenging with resultant oxygen generation) and minimal cytotoxicity (48-h direct exposure to titrated manganese oxide concentrations). Cytoprotective capacity was tested using cell exposure to hydrogen peroxide in the presence or absence of the nanoparticles. Several ideal compounds were manufactured and utilized that showed complete disproportionation of superoxide produced by the xanthine/xanthine oxidase reaction. Further, the nanoparticles showed catalase–like activity by completely converting hydrogen peroxide into the corresponding concentration of oxygen. Finally, the particles protected cells (murine β-cell insulinoma) against insult from hydrogen peroxide exposure. Based on these observed properties, these particles could be utilized to combat oxidative stress and inflammatory response in a variety of cell therapy applications.

**Statement of Significance**

Maintaining viability once cells have been removed from their physiological niche, e.g. culture and transplant, demands proper control of critical variables such as oxygenation and removal of harmful substances e.g. reactive oxygen species. Limited catalysts can transform reactive oxygen species into molecular oxygen and, thereby, have the potential to maintain cell viability and function. Among these are manganese oxide particles which are the subject of this study.

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## 1. Introduction

Reactive oxygen species (ROS) are derivatives of molecular oxygen (e.g.·$H_2O_2$, $O_2^-$) which are produced by living organisms and play an important role in cell metabolism and signaling, etc [1–9]. At high concentrations they can cause damage to different cellular macromolecules leading to cell apoptosis and necrosis [10]. Phagocytic cells of the immune system produce toxic doses of ROS to kill pathogens or attack other foreign entities (including transplanted cells and tissues) [11]. Other cell types also produce high levels of ROS when they experience abnormal levels of oxygen, i.e. in hypoxic or hyperoxic conditions [12–14]. It is, therefore, advantageous to employ “agents” which can control the level of ROS through different chemical reactions.

Biological systems address this need by producing sacrificial scavengers and enzymes. Among these, SOD (superoxide dismutase) and catalase (both metalloenzymes) have become an area of focused research and a large body of work has been devoted to synthesizing mimetic, small molecule antioxidants [15]. These small molecules, many of which are manganese or iron complexes, are typically designed to be used as systemic pharmaceuticals that would be administered when the body's capacity to ameliorate oxidative stress is overwhelmed.

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Of increased interest is the concept of localized protection against oxidative stress that is characteristic of the early phase of encapsulated cell transplants. In encapsulation, certain cells (e.g. insulin-producing pancreatic islets of Langerhans) are covered in a semi-permeable hydrogel to prevent direct contact with the host immune response; particularly, cell/cell contact or binding by host immunoglobulins [16–24]. These immune-isolating gels, however, provide minimal protection against ROS and could benefit from anti-oxidant strategies. Using this reasoning, K. Anseth et al. [25,26] utilized a SOD mimetic molecule, a manganese metalloporphyrin which was photopolymerized with PEG diacrylate, to create functional gels [26]. The murine β-cell insulinoma (MIN6 cells) encapsulated in these gels showed resilience to multiple peroxide exposures. These SOD mimetics, however, still suffer from instability [15], hindering their utility as antioxidant biomaterials. Alternatively, nanoparticles with catalytic activity toward ROS can be used to provide a different set of properties compared to small molecules. C. Stabler et al. showed that cerium oxide nanoparticles stabilized with dextran could provide cytoprotection for MIN6 β-cells when the particles were included within immunoisolating alginate hydrogels, thereby reducing the observed toxicity of direct contact with these bioactive materials [27].

Currently, there are preclinical cell-based therapies in development to treat Type 1 Diabetes Mellitus using islets of Langerhans or stem cell aggregates with the capacity to secrete insulin in glucose responsive fashion [28–31]. What is lacking for these cell therapies is a delivery device that will remove the need for ongoing systemic immunosuppression, making these therapies limited in their clinical scope. As a result, many research groups are working to develop immune-isolating devices to contain these therapeutic cells. One significant obstacle to the success of these therapies is the unique oxygen/metabolic demands of islets and insulin-producing cell aggregates that exceed other somatic cell types often by several orders of magnitude. Additionally, these tissues are incredibly susceptible to oxidative stress mechanisms and by-products, given their characteristically low content of constitutive cellular free-radical scavengers such as superoxide dismutase (SOD) and catalase [32,33]. Slight deviations in the local concentration of oxidative species can dramatically affect their viability.

In hypoxic conditions, increases in free radical concentration occur due to local intra-aggregate passenger leukocytes, including macrophages These cells initiate cytokine secretion and signaling cascades that lead to inflammatory processes regulated by hypoxia inducible factors [34]. This increase overwhelms the scavenging system of the islets and can result in pronounced cell death. In their native environment, this hypoxia-induced radical production is generally countered by the rich, vascular network of islets that provides them adequate oxygenation and nutrient supply. When isolated from this network, cultured and then transplanted, survival and function are greatly reduced. This is further exacerbated by encapsulation in polymer matrices. Antioxidant manganese nanoparticle preparations could aid in the survival and continued function of these cells when isolated from their native environment by reducing free radical load and improving oxygenation.

The subjects of this study, manganese oxides, occur naturally as more than 30 minerals [35] and have been synthesized and used for a wide range of applications from water splitting [36,37] to pseudo-capacitors [38,39] and contrast agents for magnetic resonance imaging [40–42]. Wu et. al. [43,44] used manganese oxide nanoparticles to catalytically remove $H_2O_2$ produced through oxidation of glucose in their self-regulating insulin release device. They have also used different manganese oxide preparations to alleviate hypoxia and glycolytic acidosis in a tumor environment and, as a result, enhanced tumor destruction through radiation therapy [45,46]. Herein we synthesized and characterized four manganese oxide particles and evaluated their catalytic ability to remove $O_2^-$ and $H_2O_2$. We then assessed their behavior *in vitro* by measuring direct cytotoxicity and cytoprotective capacity against $H_2O_2$ exposure in a beta cell line.

## 2. Materials and methods

### 2.1. X-ray diffraction (XRD)

The crystal structure of the particles was analyzed with a PANalytical X'PERT powder X-ray diffractometer. The diffractometer was operated using Cu $K_\alpha$ radiation at 45 kV beam voltage and 40 mA beam current. The data was collected between 8° and 70° (2θ), using 0.05° steps. A 14 s counting time was used at each step.

### 2.2. Scanning Electron Microscopy (SEM)/energy dispersive Spectroscopy (EDS)

A sample of each synthesized powder was put on an aluminum stub covered with a carbon tab. The preparations were coated with a thin layer of Pd. The preparations were then examined in an FEI Field Emission XL-30 SEM at the University of Miami Center for Advanced Microscopy (UMCAM). Manganese to potassium ratios were measured at three randomly chosen spots.

### 2.3. Transmission Electron Microscopy (TEM)

A small sample of each synthesized powder was suspended in deionized water using sonication. A drop of the resulting suspension was placed on a 200 mesh copper grid covered with a layer of carbon and Formvar. The solution on the grid was air and subsequently oven dried at 50 °C. The grid preparations were examined in a Philips CM-10 TEM fitted with a Gatan digital camera at the UM EM Core Lab at the University of Miami Miller School of Medicine.

### 2.4. Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES)

~10 mg of each nanoparticle preparation was added to 100 ml of nitric acid (4%). To this was added ~200 mg of sodium sulfite. The mixture was sonicated until all particles were fully dissolved. The solution was then diluted to 1/10 of the original concentration in nitric acid (4%). The samples were analyzed using Varian Vista Pro axial ICP-OES.

### 2.5. Thermal Gravimetric Analysis (TGA)

~10 mg of each nanoparticle preparation was placed in an alumina crucible. TGA measurements were performed under airflow (60 ml/min) and 5 °C/min heating rate using the Netzsch TG 209 F3 Tarsus® instrument. Weight loss between 30 and 500 °C was used to calculate amount of water in each sample.

### 2.6. X-ray Photoelectron Spectroscopy (XPS)

XPS was carried out by using an AXIS Nova (Kratos Analytical) equipped with monochromatic Al Kα (hν = 1486.69 eV) X-ray source. The charge neutralizer system was used for all analyses. Sample preparation for XPS comprised of forming a micrometer-thick layer of powders on a double-sided adhesive carbon tape, mounted on a 1 × 1 cm Si wafer. XPS low resolution spectra (surveys) were acquired through 6 sweeps with pass energy of 180 eV, scan steps of 1000.0 meV and step dwell time of 10 ms. XPS high resolution spectra (narrow scans) were acquired through

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