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Antioxidant cerium oxide nanoparticle hydrogels for cellular encapsulation

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ABSTRACT

Oxidative stress and the resulting radical by-products cause significant toxicity and graft loss in cellular transplantation. Here, the engineering of an auto-catalytic, antioxidant, self-renewing cerium oxide nanoparticle (CONP)-composite hydrogel is reported. This enzyme-mimetic material ubiquitously scavenges ambient free radicals, with the potential to provide indefinite antioxidant protection. Here, we evaluated the potential of this system to enhance the protection of encapsulated beta cells. Co-incubation of CONPs, free in solution with beta cells, demonstrated potent cytoprotection from superoxide exposure; however, phagocytosis of the CONPs by the beta cells resulted in cytotoxicity at concentrations as low as 1 mM. When CONPs were embedded within alginate hydrogels, the composite hydrogel provided cytoprotection to encapsulated beta cells from free radical attack without cytotoxicity, even up to 10 mM concentrations. This nanocomposite hydrogel has wide applicability in cellular transplantation, with the unique advantage of localization of these potent antioxidant CONPs and their capacity for sustained, long-term scavenging.

1. Introduction

Oxidative stress is defined as the imbalance between the production of oxidants or reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), and their elimination via antioxidants, such as superoxide dismutase (SOD) and catalase. Sustained oxidative stress results in significant destruction of cellular structures and functions and has been implicated in numerous pathological conditions, such as atherosclerosis, cancer, renal disease, and diabetes [1–3]. Given that a toxic oxidative milieu can be generated via hypoxia, cytokines, and inflammation, cellular transplants are particularly susceptible to oxidative damage, resulting in increased cell death and decreased efficacy of implants [4,5]. Protecting cellular grafts from oxidative damage due to this noxious environment is particularly challenging in the context of pancreatic islet transplantation for treatment of Type 1 diabetes mellitus, due to the inherently low gene expression and activity of important antioxidant enzymes in pancreatic islets [6–8]. Oxidative damage of islets following transplantation is one of the contributing factors resulting in graft destabilization and decreased long-term efficacy [9].

The encapsulation of transplanted donor islets within semi-permeable polymers is an appealing method for protecting allogeneic grafts from detrimental host responses [10–12]. The encapsulating polymer permselectivity permits passage of nutrients and release of secreting proteins or waste products, but blocks direct host cell interactions with graft cells. The most commonly used encapsulation material is alginate, due to its high biocompatibility, ease in encapsulation method, and demonstrated efficacy in small animal models [13–15]. While cellular encapsulation aids in reduction of generalized host cell responses via blocking direct cell–cell interactions, this strategy fails to protect donor cells from soluble by-products of the inflammatory response, in particular ROS [16–18].

Numerous anti-oxidant agents, such as edaravone and gliclazide, have been incorporated into islet transplants, either via systemic infusion, pre-culture treatment, or transgenic overexpression, with varying degrees of protective effects [19–23]; however,

Abbreviations: CONP, cerium oxide nanoparticle; SO, superoxide; SOD, superoxide dismutase; XA, xanthine; XO, xanthine oxidase; ROS, reactive oxygen species.

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these approaches are limited by the need for systemic delivery, the decreased duration of effect, and the complexities of transfection, respectively. Notable biomaterial strategies have sought to scavenge ambient free radicals via supplementation of encapsulation polymers with antioxidant enzymes such as SOD and catalase [24–28]. Inevitably, the catalytic reactivity of these free radical scavenging agents is exhausted, resulting in transient protection. A more potent approach to combat the continual inflammatory assault to the transplant would be in the development and application of a sustainable anti-oxidant mimetic.

The unique redox properties of selected metal oxides, e.g., yttrium and cerium, have been recently explored as scavenging agents for cellular oxidative stress [29]. The oxide form of the rare earth element cerium, found in the lanthanide series of elements, has the ability to cycle between its cerium(III) and cerium(IV) oxidation states, due to a lattice structure with a high tolerance for reversible oxidation/reduction [30]. Cerium oxide nanoparticles (CONPs) exhibit enhanced catalytic activity over bulk forms due to increased surface area, resulting in an amplified number of available oxygen vacancies [30,31]. The oxidative state of CONP appears related to its catalytic activity, whereby cerium(IV) correlates with catalase-like behavior and cerium(III) exhibits SOD mimetic responses [31,32]. The unique ability of CONPs to switch their oxidative states between III and IV lends itself to its desirable self-renewing property [33]. Further, CONPs have the potential to provide broad free radical protection, with demonstrated quenching of hydroxyl radicals, superoxide, peroxide, and nitric oxide [32,34–36].

CONP's potent scavenging capacity, with low loading volume and theorized unlimited auto-catalytic potential, inspired exploration of their pharmaceutical potential, with the aim of reducing oxidative damage in a variety of injury models. Co-culture of free CONPs with cells has resulted in radioprotective [34,37,38], cardioprotective [39], and neuroprotective effects [29] (for full review see [40]). Selected studies have also explored the potential of CONPs to protect beta cells and islets [41–43]. While highly promising, cytotoxic effects have been observed, particularly for particle sizes exceeding 100 nm or at concentrations higher than 1 mM (particle sizes 3–50 nm), broad assessment of CONP toxicity is complicated by the variable size, surface geometry, and zeta potential of the particles [31,34,44]. CONP cytotoxicity likely results from the cellular internalization and accumulation of the nanoparticles, as autophagy-induced apoptosis, a common outcome of nanoparticle phagocytosis, has been observed [40,44–46]. Additionally, many cellular processes are mediated by intracellular free radical signaling and internalization of CONPs may have unpredictable long-term consequences on these processes [47].

A means to mitigate the cytotoxicity of CONPs may be entrapment within an encapsulation hydrogel. This delivery strategy provides the means for localization of particles to the site of interest, minimizing their phagocytosis, while retaining their catalytic potential. Herein, we sought to engineer a nanocomposite, antioxidant biomaterial via incorporation of cerium oxide nanoparticles within an encapsulating alginate hydrogel (see Fig. 1). The potential of CONPs, embedded within a hydrogel, to retain their catalytic and self-renewal activity was examined. The capacity of CONPs and CONP-composite hydrogels to prevent ROS-induced beta cell death, as well as enhance cytocompatibility, was also evaluated. The benefits of this approach to provide the local presentation of potent CONPs at the transplant site, thereby reducing potential downstream or systemic effects, are discussed.

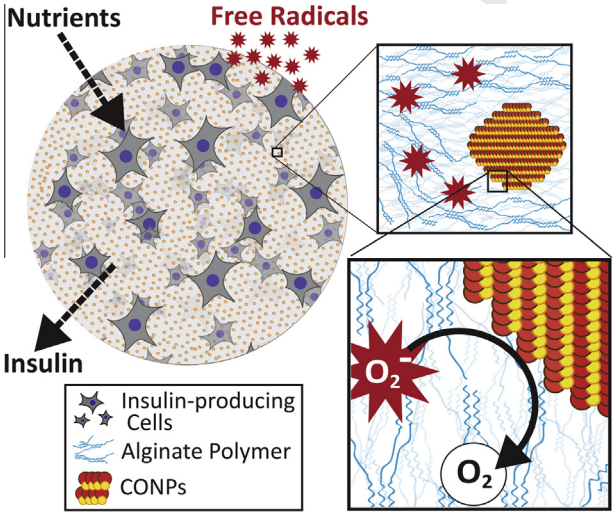

Fig. 1. Illustration of cerium oxide nanoparticle (CONP)-alginate composite hydrogel. Alginate microbead provides matrix for cellular encapsulation and permselectivity to permit nutrient diffusion in and insulin secretion out of the hydrogel. CONP, embedded within the alginate matrix, provides ubiquitous, renewable, antioxidant protection from external free radical damage.

2. Experimental section

2.1. Materials

All chemicals were obtained from Sigma–Aldrich unless otherwise noted.

2.2. CONP synthesis

Dextran coated, cerium oxide nanoparticles (CONP) were synthesized using a method similar to published reports [33]. Dextran coating was used to enhance stability of the CONPs in solution [48]. A 1 mL solution of 1 M cerium(III) nitrate was mixed evenly with a 2 mL solution of 100 mM dextran T-10, added drop-wise to 6 mL of ammonium hydroxide (30%), and stirred overnight. To remove excess dextran and reaction by-products, CONP solutions were dialyzed against PBS using 30 kDa MWCO centrifuge filters (Millipore) at 4000 rpm in 10 min intervals, until effluent pH was ~7.0. The CONP concentration is expressed in mM, per convention, and calculated as described elsewhere [33]. CONP solutions were further processed for analysis and cell culture by sonication, to prevent CONP aggregation, and sterile filtration (0.2 μm). Due to small size of the particles, no detectable particle loss was observed during filtration.

2.3. CONP solution characterization

All tests were performed at physiological pH. CONP size was characterized by dynamic light scattering (DLS) using a DynaPro Titan and Dynamics v6.0 software (Wyatt Technology), all samples diluted in PBS (Gibco), where percent polydispersity represents the standard deviation of detected peaks normalized to their mean intensity value. A polydispersity >30% indicates low homogeneity. CONP solution composition was characterized by Fourier transform infrared (FTIR) analysis on a Perkin-Elmer Spectrum 100 FTIR Spectrometer (average of four scans with a resolution of 4 cm^{-1}) using a lyophilized, processed sample. HR-TEM imaging was performed at 300 kV on a FEI Tecnai F30 TEM by the Advanced Materials Processing and Analysis Center (AMPAC) at the University of Central Florida (UCF). XPS analysis was performed on a Physical Electronics 5400 ESCA, also at AMPAC UCF, and peaks identified using PeakFit v4.12 (Systat Software, 8% Savitsky-Golay smoothing, linear two-point baseline subtraction). CONP catalytic activity and renewability were assessed by addition of CONPs (1 mM) to H_2O_2 (1 mM) and spectral analysis performed on a Molecular Devices SpectraMax M5 Microplate reader. CONP solutions were incubated