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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.

Keywords

immunoisolation; beta cell; alginate; nanoceria; free radical

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Supporting Information. Additional figures, as referenced in the manuscript, can be found in Supporting Information.

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, 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 have 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), although 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, anti-oxidant biomaterial via incorporation of cerium oxide nanoparticles within an encapsulating alginate hydrogel (see **Figure 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.

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 6mL 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 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 was 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 with 1.25 mM 3,3',5,5'-Tetramethylbenzidine (TMB, Merck) and oxidation read on the aforementioned spectrophotometer at 652 nm at the indicated timepoints. Superoxide radicals were generated through the reaction of Xanthine (XA, 100 μM) and Xanthine Oxidase (XO, 25 nM), whereby XA's conversion to urea results in the release of superoxide [49]. Superoxide generation was measured via the rate of oxidation of cytochrome C [50]. This reaction rate may be calculated using equation (1) below [25],

$$\left[\text{O}_2^- \right] = \frac{\Delta A \times v}{K \times l \times t} \quad (1)$$

Where ΔA is the change in absorbance, l is the pathlength, t is time, v is volume, and K is the extinction coefficient for the difference in absorption between reduced and oxidized cytochrome C ($K = 21 \times 10^3 \text{ cm}^{-1}/\text{M}$).

At the above concentrations of XA and XO, superoxide production peaked at 10 nmol/ min, with the majority of reaction completion within 10-15 min. The aforementioned XA/XO system was incubated without or with 1 mM CONP, with assessment of superoxide generation over the course of the experiment (15 min).

2.4 CONP MIN6 cytotoxicity and superoxide protection

MIN6 cells (subclone C3, courtesy of Dr. Valerie Lilla and Alejandra Tomas) were cultured as monolayers in T-flasks and fed every 2-3 days with fresh medium comprised of Dulbecco's modified Eagle's medium (DMEM, 1g/L D-Glucose, Mediatech) supplemented with 10% fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin (P/S, Gibco), and 1% (v/v) L-glutamine (Gibco). MIN6 cytotoxicity experiments were performed by exposure of 3×10^5 cells/well seeded within a 24-well plate to 0, 0.1, and 1.0 mM CONP-supplemented

DMEM for 48 h. Cell metabolic activity was assessed by Alamar Blue (Invitrogen). Superoxide protection was assessed after a 48 h pre-incubation with CONP-supplemented DMEM. Superoxide radicals were generated using the XA/XO system defined above for 2 h in CONP-supplemented PBS, and metabolic activity assessed by Alamar Blue and viability by Live/Dead staining (Calcein AM/Ethidium Homodimer, Invitrogen) on a Zeiss LSM510 confocal microscope. The percent of viable cells in each image was calculated using particle analysis in ImageJ. The ratio of Calcein-positive cells to total cells detected was normalized to untreated controls ($n = 3$ per group).

2.5 CONP-alginate fabrication

CONP-alginate capsules were formed by premixing a 1.6% (w/v) alginate (UP-MVG, $M_w = 300$ kDa, $M_w/M_n = 1.87$, DPn of 28, Batch # FP-504-03, Pronova Novamatrix, FMC) PBS solution with CONPs prior to extrusion through a capsule generator with parallel airflow (42 psi) into a crosslinking bath of 1.5% (w/v) barium chloride, supplemented with 10mM 3-(N-morpholino)propanesulfonic acid (MOPS), 140 mM DMannitol, and 0.025% Tween-20. After 10 min of crosslinking, CONP-alginate capsules were washed with PBS to remove excess crosslinker. Resulting alginate microbeads were 0.922 ± 0.149 mm in diameter.

2.6 CONP-biomaterial characterization

CONP-hydrogels were visually assessed for CONP retention by complete immersion of hydrogels in soluble TMB solution, and imaged on an Olympus SZX7 stereomicroscope. To further confirm visual observations, CONP-alginate beads were washed several times in excess PBS, and samples assessed in the DLS for free CONP detection. CONP-alginate hydrogel catalytic activity was further assessed quantitatively by immersing twenty CONP-alginate or pure alginate capsules in a $60 \mu\text{M}$ H_2O_2 solution for 1, 2, or 4 h. H_2O_2 levels of the bathing medium were assessed via colorimetric hydrogen peroxide detection kit (Enzo Life Sciences) at the above time points.

2.7 CONP-Alginate MIN6 encapsulation and superoxide protection

MIN6 were thoroughly mixed with alginate or CONP-alginate solutions at either 10×10^6 cells/mL (short-term study) or 25×10^6 cells/mL (long-term study), prior to capsule formation using the method described above. MIN6 were encapsulated at a higher cell density for the long-term study, as higher cell densities have been found to be more stable for long-term cultures [51]. This serves to minimize the contribution of long-term cell instability in this study. For both the short- and long-term study, encapsulated MIN6 were plated at 40 capsules/well (0.25×10^5 or 1×10^5 cells/well, respectively) in a 48-well plate. For the short-term CONP-alginate titration study, encapsulated MIN6 were cultured for 48 h prior to superoxide exposure ($200 \mu\text{M}$ XA and 50 nM XO in PBS) for 2 h, followed by metabolic activity assessment via Alamar Blue and correlation to viability via Live/Dead staining. For the long-term CONP-alginate encapsulation study, encapsulated MIN6 were cultured for 10 d, with superoxide exposure ($200 \mu\text{M}$ XA/ 50 nM XO) for 2 h on day 2 and 6 post-encapsulation, with metabolic activity and viability assessments (Alamar Blue and Live/Dead stain) performed 2, 4, 6, and 8 d post-encapsulation. Following Alamar Blue evaluation, DNA was extracted using DNA Extraction Buffer (0.2 % v/v Triton-X 100, 6.6% v/v 1N Ammonium Hydroxide) and PicoGreen DNA detection kit (Invitrogen). DNA

results were used to normalize Alamar Blue absorbance readings. Final metabolic activity results are expressed as fold change of control. The percent of viable cells in each image was calculated using particle analysis in ImageJ, and the ratio of Calcein-positive cells to total cells detected was normalized to untreated controls and day two untreated controls for the short and long-term studies, respectively ($n = 3$ per group).

2.8 Statistical Analysis

All data analysis used Excel (Microsoft, USA), with statistical analysis and graphing via GraphPad Prism (GraphPad Software, Inc., USA). All cell experiments were performed using three to five independent samples with a minimum of three technical replicates. Data are presented as mean $\pm$ SEM. Results were analyzed via one or two-way ANOVA, with Bonferroni comparison post-hoc analysis. Observations were considered to be statistically significant with $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization of cerium oxide nanoparticles (CONP)

Cerium oxide nanoparticles (CONP) were synthesized and coated with dextran, similar to published reports [33, 48]. Dextran was selected as the coating agent due to its ease in fabrication and increased cytocompatibility compared to other stabilization coatings [48]. The resulting CONP solution was characterized via Fourier transform infrared (FT-IR) spectroscopy, whereby characteristic bands in the 800-1300 nm range confirmed dextran coating of the CONPs (**Figure 2A**). CONP particle size, characterized via dynamic light scattering (DLS), indicate a relatively monodisperse (23.8% polydispersity) distribution of nanoparticles with a radius of 2.7-9 nm (**Figure 2B**), which is similar to published reports [48]. X-ray photon spectroscopy (XPS) analysis of CONP solutions exhibits spectra for both cerium oxidative states; demonstrating characteristic cerium III (Ce_2O_3) and IV (CeO_2) peaks (**Figure 2C**).

To verify the capacity of CONPs to perform enzyme-mimetic electron transfer reactions with reactive oxygen intermediates (summarized in **Figure 3A**), CONP solutions were exposed to hydrogen peroxide, peroxidase substrate 3,3',5,5'-Tetramethylbenzidine (TMB), or superoxide. CONPs demonstrate catalase-mimetic behavior through their ability to reduce hydrogen peroxide (H_2O_2) to water and oxygen. Upon reaction with H_2O_2 , CONP solutions (1 mM) display a red spectral shift (**Figure 3B**), which is hypothesized to evolve from the shifting of cerium atoms from the +4 to +3 oxidative states [31]. The reversible nature of the particles was demonstrated via regression to pre-treatment spectrum over time. Incubation of TMB with CONPs resulted in rapid TMB oxidation (**Figure 3C**), demonstrated via characteristic color shift from clear to blue, with a catalyst concentration-dependent degree of oxidation. We further sought to investigate the capacity of CONP solutions to counteract the free radical superoxide (O_2^-), whereby superoxide was generated via XA/XO system (see Methods section). As shown in **Figure 3D**, the presence of 1 mM CONPs completely abrogated superoxide levels. Overall, these results illustrate the catalytic and reversible activity of dextran coated CONPs, correlating with previous reports using similar formulations [36].

3.2 Cytotoxic and protective effects of CONP solutions on beta cells

The cytocompatibility and protective effects of CONP was evaluated via co-incubation of CONPs, free in solution, with MIN6 cells, a murine beta cell line. For these studies, beta cells were incubated with a range of CONP concentration from 0.1 to 1.0 mM. To evaluate the capacity of CONPs in solution to scavenge superoxide radicals sufficiently in a cell culture system to prevent oxidative damage, the XA/XO system was used to generate superoxide in the bathing milieu. As shown in **Figure 4**, the generation of superoxide in the local media demonstrates a significant and detrimental impact on MIN6 beta cells, with approximately an 80% decrease in metabolic activity (0.185 ± 0.025 fold of control). The addition of CONPs to the media provides a significant protective effect, whereby MIN6 metabolic activity is preserved due to free radical neutralization by the nanoparticles ($P < 0.0001$ analysis of variance). The protective effect is dose-dependent, with 0.1 mM CONPs improving metabolic activity 1.5 fold over superoxide treated control cells (0.315 ± 0.104 fold of control), and 1.0 mM CONPs providing complete protection from superoxide (0.813 ± 0.093 fold of control), with metabolic activity levels not statistically different than pre-superoxide treatment levels ($P = 0.052$). Live/Dead imaging of MIN6 cells follow the same trend, demonstrating a 51% drop in superoxide-treated control cell viability ($P < 0.05$), and no statistical difference observed in CONP-only and CONP and superoxide-treated groups (see **Supplementary Figure S1**). These results illustrate the potent protective capacity of CONPs against free radical damage for beta cells.

While the protective effects of CONPs was elevated with increasing concentrations, a trend of decreased metabolic activity for beta cells was observed with increasing CONP concentration in solution, with a significant drop in metabolic activity detected for control beta cells exposed to 1 mM CONPs, with a fold change of 0.871 ± 0.038 ($P < 0.01$). The cytotoxicity of dextran coated CONPs has been evaluated for several cell lines, with most demonstrating no effect at concentrations at or below 1 mM [34, 38, 43]; however, cytotoxicity has been indicated at 1 mM when the CONPs are internalized within the lysosomes [48]. Transmission electron microscopy images (TEM) of MIN6 cells exposed to 1 mM CONPs revealed internalization of CONPs within cytoplasmic lysosomes (**Figure 4B**), indicating the mechanism of the observed CONP cytotoxicity is likely due to interactions with internal cellular structures after nanoparticle internalization. This mechanism of cytotoxicity is common when higher concentrations of nanoparticles are present in the bathing media [40, 44-46]. While lysosomal aggregation of dextran CONP was not observed in published studies using cardiac myocytes and kidney cells [48], this variation in internalization could be attributed to the cell type, as lysosomal localization of dextran coated nanoparticles has been reported for beta cell lines [52], or the duration of the experiment, as previously reported studies were for short-term incubations (< 3 hr).

3.3 CONP-composite encapsulation hydrogel development and characterization

While catalytic CONP formulations demonstrated potent cytoprotection against free radical damage, the observed cytotoxicity of CONPs when internalized by beta cells impairs their potential. As such, we sought to engineer a nanocomposite hydrogel, which would provide localization of CONPs within an encapsulation polymer and dampen cellular internalization. We explored the utility of alginate hydrogel for entrapment of the dextran CONPs. Alginate

is composed of linear polysaccharide chains, with average mesh size of approximately 9 – 22 nm, depending on the ratio of 1,4•-linked -D-mannuronic acid and -L-guluronic acid residues [53-56]. For 1.6% UP-LVG alginate, we have observed restriction on the diffusion of agents • 10 nm (assuming the hydrodynamic radius for dextran using the formula published by Brussove et al [56]) [57]. CONP-alginate hydrogels were fabricated via thorough mixing of CONP (0, 1, or 10 mM) with alginate prior to gelation into microbeads via barium crosslinking. The catalytic reactivity of the CONPs within the alginate was visually assessed through the colorimetric reaction of TMB. Generation of oxidized TMB (blue color) was visualized for the CONP-alginate composites (**Figure 5A**), indicating reactivity of CONPs within the alginate nanocomposite. Incubation of TMB with alginate-only controls resulted in no color change. Of note, following reaction of the TMB with the entrapped CONP, oxidized TMB was retained within the hydrogel. Ionic interactions between the alginate and the oxidized TMB cation is the likely cause, as alginate is known to form coacervates with cationic agents [53, 58-60]. This was verified via subsequent studies illustrating binding of oxidized TMB within alginate hydrogels (see **Supplemental Figure S2**), which was observed only following oxidation of TMB and was independent of the oxidation agent (i.e. the observation was not unique to the presence of CONP).

The dextran cerium oxide nanoparticles were highly retained within the alginate hydrogel over extended time periods, as nanoparticle release in the surrounding milieu was not detected several weeks after fabrication and the reactivity of CONPs within the alginate hydrogel was observed over 1 year post-fabrication (**Supplemental Figure S3**). Conversely, high retention of the dextran CONPs within agarose gels was not stable (**Supplemental Figure S4**). Although the average mesh size for agarose is slightly larger than alginate (i.e. alginate ranges from 9 – 22 nm [53-56] and agarose ranges from 20 – 150 nm [61-63], both depending on type and percentage), it is unlikely that the dextran CONPs are retained due to complete physical entrapment within the material due to the scale of the CONPs. The long-term stability of the CONPs within the alginate hydrogel is likely to be due to dynamic exchange of the dextran coating of the CONPs with alginate, due to the instability of the physisorbed dextran [64]. This phenomenon has been reported for iron oxide nanoparticles, whereby the COO⁻ terminal of alginate macromolecules binds to the nanoparticle in a manner identical to dextran, even forming the classic “egg-box” structure as a result of interactions between alginate macromolecules and oxide vacancies of the nanoparticles [65-67]. While this study exhibits the utility of dextran coated CONPs for retention within alginate, it is envisioned that other nanocomposite materials could be engineered, such as through covalent tethering of coated CONPs to functionalized poly(ethylene glycol). These approaches are the focus of future studies.

To quantitatively confirm catalytic activity of the CONP-alginate composite hydrogels, microcapsules doped with varying CONP concentrations (i.e. 0, 0.1, 1.0, and 10 mM) were assessed for their capacity to scavenge ambient hydrogen peroxide. As shown in **Figure 5B**, ambient H₂O₂ was cleared from the incubating solution by the CONP-alginate composite hydrogels in a concentration-dependent manner, with 10 mM CONP-alginate microcapsules neutralizing nearly 100% of the H₂O₂ within 4 h. CONP reactivity may be further confirmed

via visualization of the characteristic red spectral shift within the capsules (**Figure 5B**, insets).

3.4 Cytotoxic effects of CONP-alginate encapsulation gels

Following evaluation of the CONP-alginate hydrogel composite, we assessed the potential of this system to decrease the cytotoxic effects observed for CONP nanoparticles free in solution. For these studies, MIN6 beta cells were encapsulated within alginate microbeads containing CONPs at concentrations of 0, 1, 5, and 10 mM. As shown in **Figure 6A**, no impairment of cellular metabolic activity was found for beta cells encapsulated within CONP-alginate composites, even at 10 mM CONP concentration (**Figure 6A**, black bars). In comparison, exposure of cells to only 1 mM CONPs free in solution induced statistically significant decreases in cell metabolic activity, with a fold decrease of 0.405 ± 0.097 ($P < 0.01$). Cellular internalization of CONPs for cells encapsulated in 10 mM CONP-alginate was not observed in TEM images (**Figure 6B**), contrary to their marked presence within cellular lysosomes when only 1 mM free CONPs was added to the culture media. This indicates that entrapment of nanoparticles within the hydrogel decreases the potential for cellular internalization of the nanoparticles, a trend observed for other nanoparticle-hydrogel composites [68, 69].

3.5 Cytoprotective effects of CONP-alginate encapsulation gels

To evaluate the capacity of CONP-alginate composites to provide protection to embedded cells from external oxidative stress, encapsulated cells were exposed to superoxide radicals using the XA/XO system. A two-fold increase in concentration of XA/XO was required for these studies to induce significant cell death, as the instability of the radical and the time delay imparted by the alginate diffusional barrier reduces its potency [16]. This resulting dose of superoxide (~100 nanomole total exposure) correlates to that released by approximately 1×10^6 activated macrophages [70]. Significant protective effects from free radical damage were observed for CONP-alginate composites in a dose-dependent manner. While superoxide treated controls exhibited a fold decrease of 0.495 ± 0.05 , CONP-alginate groups demonstrated dose-dependent protection from superoxide exposure, with a fold change of 0.751 ± 0.032 , 0.885 ± 0.05 , and 0.954 ± 0.034 for 1, 5, and 10 mM CONP, respectively. At 5 and 10 mM CONP, complete abrogation of free radical damage was observed, with metabolic activity not statistically variant from untreated controls (**Figure 7A**). This trend was further confirmed by Live/Dead imaging of encapsulated cells, where substantial cell death was visualized in superoxide treated controls, while CONP-alginate composites demonstrated a concentration dependent protective effect (**Figure 7B**). Quantification of percent viable cells in these images exhibited a similar trend, with a significant drop in viability of 60% ($P < 0.001$) only observed in superoxide treated controls (**Supplemental Figure S5A**). These studies illustrate the capacity of CONP-alginate composite hydrogels to provide significant protection to beta cells from oxidative stress.

3.6 Long-term protective effects of self-renewing CONP-alginate encapsulation gels

A notable advantage of CONPs is their capacity to self-renew. To investigate the continuous protective effects of novel CONP-alginate encapsulation hydrogels, MIN6 beta cells,

entrapped within control or 10 mM CONP-alginate composite hydrogels, were exposed to repeated hits of substantial oxidative stress, specifically on day 2 and 6 post-encapsulation. **Figure 8** summarizes the effects of superoxide treatment on encapsulated beta cell metabolic activity. Following the first exposure to superoxide on day 2, significant impairment of metabolic activity was observed within control alginate hydrogels, with a fold decrease of 0.547 ± 0.073 , compared to untreated controls. No recovery was observed on day 4 ($P = 0.662$ when comparing day 2 and day 4). Cells were exposed again to superoxide on day 6, which resulted in a continued impairment of metabolic activity for alginate-only hydrogel controls, with a fold decrease of 0.548 ± 0.084 compared to untreated controls. By contrast, CONP-alginate groups exhibited metabolic activity at 0.955 ± 0.144 and at 0.847 ± 0.10 of untreated CONP-alginate controls for days 2 and 6, respectively. Overall, for all time points, the superoxide treated alginate-only group was significantly impaired in metabolic activity when compared to untreated controls, while the CONP-alginate group was never statistically different from its untreated controls. This is further supported by visualization of confocal microscopy images of live/dead cells within these microbeads (**Figure 8**), as well as image analysis (**Supplemental Figures 5B & C**), which found a statistically significant decrease in viable cells for alginate-only, superoxide treated, groups. These results demonstrate the capacity of these gels to neutralize repeated exposure to superoxide radicals. Of note, the proliferative nature of the beta cell line provides a means for cellular recovery following free radical damage, while primary islets would exhibit limited ability to recuperate, given their low capacity for proliferation.

Of note, while the second superoxide treatment in the alginate-only controls did not impart an additional decrease in metabolic activity on day 6, this is likely due, in part, to the limited penetration of the superoxide into the alginate microbead. The exceptionally short half-life of superoxide, combined with the fact that the passive diffusion of small molecules through alginate capsules is typically limited to $150 \mu\text{m}$ [71], results in the cells at the periphery of the sphere experiencing the greatest impact from superoxide exposures. Following superoxide exposure of the outer cell layer on day 2, the repeated exposure on day 6 to the same area likely minimizes the impact. This is supported by examining live/dead images of sections that highlight the periphery of the bead. In these sections, elevated cell death in the periphery of the bead on day 2, when compared to untreated or CONP loaded groups, was evident (see **Supplementary Figure S6**). Further, recovery of the beta cells to control levels was never achieved, even after 8 d of culture, demonstrating the impact of the double exposure to superoxide. Each dose of superoxide administered is on the range of approximately 100 nanomole total exposure, which correlates approximately to that released by 1×10^6 activated macrophages [70]; this data reflects CONP-alginate's potent protection in a highly inflammatory environment. While it is unclear whether this observed effect is due to CONPs' self-renewal or lack of CONP depletion during the first superoxide dose, it is evident that CONP-alginate gels at 10mM concentration convey significant, continuous protection to encapsulated cells from ambient free radicals.

While these studies clearly demonstrate the potential of this system for providing potent antioxidant protection, additional studies screening long-term protective capacity of CONP-alginate on primary islets, as well as the biocompatibility and effectiveness of these coatings

in vivo, are necessary to fully validate this system. Further, while this platform demonstrates the potential of CONP-hydrogel systems for cytoprotection, a distinct advantage of this approach is the flexibility of CONP synthesis, which allows for coating chemistry modifications that could facilitate generation of physically-linked CONP-hydrogel coatings. Future studies will also seek to examine the capacity of CONP nanocomposites to provide long-term protection against oxidative stress in a transplant model.

4. CONCLUSIONS

Herein, we designed a catalytically antioxidant hydrogel capable of efficiently scavenging free radicals, a major contributor to cell graft death post-transplantation. This hydrogel can serve as a cellular encapsulation system to provide a bioactive barrier between transplanted cells and ambient radicals. This novel, nanocomposite CONP-alginate hydrogel provides significant protection to encapsulated cells, with the additional advantage of negligible cytotoxicity at nanoparticle concentrations ten-fold higher than free CONPs. For translational studies, this approach should provide enhanced cytocompatibility and safety over bolus injection methods. Consequently, incorporation of CONPs within the encapsulation material retains the particles to the site of transplantation, enabling the localized delivery of potent doses of nanoparticles with reduced concern for down-stream effects. As such, this platform has great potential for applications and pathologies (e.g. chronic inflammation) which necessitate the localization of potent antioxidants to a specific site.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CONP	cerium oxide nanoparticle
SO	superoxide
SOD	superoxide dismutase
XA	xanthine
XO	xanthine oxidase
ROS	reactive oxygen species

REFERENCES

1. Griending KK, FitzGerald GA. Oxidative Stress and Cardiovascular Injury: Part I: Basic Mechanisms and In Vivo Monitoring of ROS. *Circulation*. 2003; 108:1912–6. [PubMed: 14568884]
2. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radic Biol Med*. 2010; 49:1603–16. [PubMed: 20840865]
3. Muhammad S, Bierhaus A, Schwaninger M. Reactive oxygen species in diabetes-induced vascular damage, stroke, and Alzheimer's disease. *J Alzheimer's Dis*. 2009; 16:775–85. [PubMed: 19387112]
4. Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes*. 1997; 46:1733–42. [PubMed: 9356019]
5. Tiedge M, Lortz S, Munday R, Lenzen S. Complementary action of antioxidant enzymes in the protection of bioengineered insulin-producing RINm5F cells against the toxicity of reactive oxygen species. *Diabetes*. 1998; 47:1578–85. [PubMed: 9753295]
6. Ho E, Bray TM. Antioxidants, NFkappaB activation, and diabetogenesis. *Proc Soc Exp Biol Med*. 1999; 222:205–13. [PubMed: 10601879]
7. Oberley LW. Free radicals and diabetes. *Free Radic Biol Med*. 1988; 5:113–24. [PubMed: 3075947]
8. Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic Biol Med*. 1996; 20:463–6. [PubMed: 8720919]
9. Drews G, Krippeit-Drews P, Düfer M. Oxidative stress and beta-cell dysfunction. *Pflügers Archiv - European Journal of Physiology*. 2010; 460:703–18. [PubMed: 20652307]
10. Sefton MV, May MH, Lahooti S, Babensee JE. Making microencapsulation work: conformal coating, immobilization gels and in vivo performance. *Journal of controlled release : official journal of the Controlled Release Society*. 2000; 65:173–86. [PubMed: 10699279]
11. Kizilel S, Scavone A, Liu X, Nothias JM, Ostrega D, Witkowski P, et al. Encapsulation of pancreatic islets within nano-thin functional polyethylene glycol coatings for enhanced insulin secretion. *Tissue engineering Part A*. 2010; 16:2217–28. [PubMed: 20163204]
12. Wilson JT, Cui W, Chaikof EL. Layer-by-layer assembly of a conformal nanothin PEG coating for intraportal islet transplantation. *Nano Lett*. 2008; 8:1940–8. [PubMed: 18547122]
13. Sun AM. Microencapsulation of pancreatic islet cells: a bioartificial endocrine pancreas. *Methods Enzymol*. 1988; 137:575–80. [PubMed: 3131633]
14. Zimmermann H, Shirley SG, Zimmermann U. Alginate-based encapsulation of cells: past, present, and future. *Curr Diab Rep*. 2007; 7:314–20. [PubMed: 17686410]
15. Cui H, Tucker-Burden C, Cauffiel SM, Barry AK, Iwakoshi NN, Weber CJ, et al. Long-term metabolic control of autoimmune diabetes in spontaneously diabetic nonobese diabetic mice by nonvascularized microencapsulated adult porcine islets. *Transplantation*. 2009; 88:160–9. [PubMed: 19623010]
16. Kavdia M, Lewis RS. Free radical profiles in an encapsulated pancreatic cell matrix model. *Ann Biomed Eng*. 2002; 30:721–30. [PubMed: 12108846]
17. Wiegand F, Kroncke KD, Kolb-Bachofen V. Macrophage-generated nitric oxide as cytotoxic factor in destruction of alginate-encapsulated islets. Protection by arginine analogs and/or coencapsulated erythrocytes. *Transplantation*. 1993; 56:1206–12. [PubMed: 8249124]
18. Chen B, Keshive M, Deen WM. Diffusion and reaction of nitric oxide in suspension cell cultures. *Biophys J*. 1998; 75:745–54. [PubMed: 9675176]
19. Mancarella R, Del Guerra S, Masini M, Bugliani M, Valgimigli L, Pedulli GF, et al. Beneficial effect of the nonpeptidyl low molecular weight radical scavenger IAC on cultured human islet function. *Cell Transplant*. 2008; 17:1271–6. [PubMed: 19181220]
20. Fukudome D, Matsuda M, Kawasaki T, Ago Y, Matsuda T. The radical scavenger edaravone counteracts diabetes in multiple low-dose streptozotocin-treated mice. *Eur J Pharmacol*. 2008; 583:164–9. [PubMed: 18291360]

21. Kimoto K, Suzuki K, Kizaki T, Hitomi Y, Ishida H, Katsuta H, et al. Gliclazide protects pancreatic beta-cells from damage by hydrogen peroxide. *Biochem Biophys Res Commun*. 2003; 303:112–9. [PubMed: 12646174]
22. Li X, Chen H, Epstein PN. Metallothionein protects islets from hypoxia and extends islet graft survival by scavenging most kinds of reactive oxygen species. *J Biol Chem*. 2004; 279:765–71. [PubMed: 14576162]
23. Padmasekar M, Lingwal N, Samikannu B, Chen C, Sauer H, Linn T. Exendin-4 Protects Hypoxic Islets From Oxidative Stress and Improves Islet Transplantation Outcome. *Endocrinology*. 2013; 154:1424–33. [PubMed: 23471218]
24. Cheung CY, McCartney SJ, Anseth KS. Synthesis of Polymerizable Superoxide Dismutase Mimetics to Reduce Reactive Oxygen Species Damage in Transplanted Biomedical Devices. *Adv Funct Mater*. 2008; 18:3119–26.
25. Hume PS, Bowman CN, Anseth KS. Functionalized PEG hydrogels through reactive dip-coating for the formation of immunoactive barriers. *Biomaterials*. 2011; 32:6204–12. [PubMed: 21658759]
26. Nakaoka R, Tabata Y, Yamaoka T, Ikada Y. Prolongation of the serum half-life period of superoxide dismutase by poly(ethylene glycol) modification. *J Controlled Release*. 1997; 46:253–61.
27. Kojima Y, Akaie T, Sato K, Maeda H, Hirano T. Polymer conjugation to Cu,Zn-SOD and suppression of hydroxyl radical generation on exposure to H₂O₂: Improved stability of SOD in vitro and in vivo. *J Bioact Compatible Polym*. 1996; 11:169–90.
28. Li Z, Wang F, Roy S, Sen CK, Guan J. Injectable, highly flexible, and thermosensitive hydrogels capable of delivering superoxide dismutase. *Biomacromolecules*. 2009; 10:3306–16. [PubMed: 19919046]
29. Schubert D, Dargusch R, Raitano J, Chan S-W. Cerium and yttrium oxide nanoparticles are neuroprotective. *Biochem Biophys Res Commun*. 2006; 342:86–91. [PubMed: 16480682]
30. Bumajdad A, Eastoe J, Mathew A. Cerium oxide nanoparticles prepared in self-assembled systems. *Adv Colloid Interface Sci*. 2009; 147-148:56–66. [PubMed: 19027889]
31. Pirmohamed T, Dowding JM, Singh S, Wasserman B, Heckert E, Karakoti AS, et al. Nanoceria exhibit redox state-dependent catalase mimetic activity. *Chem Commun (Camb)*. 2010; 46:2736–8. [PubMed: 20369166]
32. Heckert EG, Karakoti AS, Seal S, Self WT. The role of cerium redox state in the SOD mimetic activity of nanoceria. *Biomaterials*. 2008; 29:2705–9. [PubMed: 18395249]
33. Perez JM, Asati A, Nath S, Kaittanis C. Synthesis of biocompatible dextran-coated nanoceria with pH-dependent antioxidant properties. *Small*. 2008; 4:552–6. [PubMed: 18433077]
34. Das M, Patil S, Bhargava N, Kang JF, Riedel LM, Seal S, et al. Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. *Biomaterials*. 2007; 28:1918–25. [PubMed: 17222903]
35. Korsvik C, Patil S, Seal S, Self WT. Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. *Chem Commun (Camb)*. 2007:1056–8. [PubMed: 17325804]
36. Asati A, Santra S, Kaittanis C, Nath S, Perez JM. Oxidase-like activity of polymer-coated cerium oxide nanoparticles. *Angew Chem Int Ed Engl*. 2009; 48:2308–12. [PubMed: 19130532]
37. Colon J, Herrera L, Smith J, Patil S, Komanski C, Kupelian P, et al. Protection from radiation-induced pneumonitis using cerium oxide nanoparticles. *Nanomedicine*. 2009; 5:225–31. [PubMed: 19285453]
38. Tarnuzzer R, Colon J, Patil S, Seal S. Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. *Nano Lett*. 2005; 5:2573–7. [PubMed: 16351218]
39. Niu J, Azfer A, Rogers LM, Wang X, Kolattukudy PE. Cardioprotective effects of cerium oxide nanoparticles in a transgenic murine model of cardiomyopathy. *Cardiovasc Res*. 2007; 73:549–59. [PubMed: 17207782]
40. Celardo I, Pedersen JZ, Traversa E, Ghibelli L. Pharmacological potential of cerium oxide nanoparticles. *Nanoscale*. 2011; 3:1411–20. [PubMed: 21369578]

41. Hosseini A, Baeeri M, Rahimifard M, Navaei-Nigjeh M, Mohammadirad A, Pourkhalili N, et al. Antiapoptotic effects of cerium oxide and yttrium oxide nanoparticles in isolated rat pancreatic islets. *Hum Exp Toxicol*. 2013; 32:544–53. [PubMed: 23696423]
42. Pourkhalili N, Hosseini A, Nili-Ahmadabadi A, Rahimifard M, Navaei-Nigjeh M, Hassani S, et al. Improvement of isolated rat pancreatic islets function by combination of cerium oxide nanoparticles/sodium selenite through reduction of oxidative stress. *Toxicol Mech Methods*. 2012; 22:476–82. [PubMed: 22409398]
43. Tsai YY, Oca-Cossio J, Agering K, Simpson NE, Atkinson MA, Wasserfall CH, et al. Novel synthesis of cerium oxide nanoparticles for free radical scavenging. *Nanomed*. 2007; 2:325–32.
44. Hussain S, Al-Nsour F, Rice AB, Marshburn J, Yingling B, Ji Z, et al. Cerium Dioxide Nanoparticles Induce Apoptosis and Autophagy in Human Peripheral Blood Monocytes. *ACS Nano*. 2012; 6:5820–9. [PubMed: 22717232]
45. Stern ST, Zolnik BS, McLeland CB, Clogston J, Zheng J, McNeil SE. Induction of Autophagy in Porcine Kidney Cells by Quantum Dots: A Common Cellular Response to Nanomaterials? *Toxicol Sci*. 2008; 106:140–52. [PubMed: 18632727]
46. Patil S, Sandberg A, Heckert E, Self W, Seal S. Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. *Biomaterials*. 2007; 28:4600–7. [PubMed: 17675227]
47. Dröge W. Free Radicals in the Physiological Control of Cell Function. *Physiol Rev*. 2002; 82:47–95. [PubMed: 11773609]
48. Asati A, Santra S, Kaittanis C, Perez JM. Surface-charge-dependent cell localization and cytotoxicity of cerium oxide nanoparticles. *ACS Nano*. 2010; 4:5321–31. [PubMed: 20690607]
49. Fridovich I. Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. *J Biol Chem*. 1970; 245:4053–7. [PubMed: 5496991]
50. Cohen HJ, Chovanec ME. Superoxide production by digitonin-stimulated guinea pig granulocytes. The effects of N-ethyl maleimide, divalent cations; and glycolytic and mitochondrial inhibitors on the activation of the superoxide generating system. *J Clin Invest*. 1978; 61:1088–96. [PubMed: 207722]
51. Lin CC, Anseth KS. Cell-cell communication mimicry with poly(ethylene glycol) hydrogels for enhancing beta-cell function. *Proc Natl Acad Sci U S A*. 2011; 108:6380–5. [PubMed: 21464290]
52. Oishi K, Miyamoto Y, Saito H, Murase K, Ono K, Sawada M, et al. In Vivo Imaging of Transplanted Islets Labeled with a Novel Cationic Nanoparticle. *PloS one*. 2013; 8:e57046. [PubMed: 23451139]
53. Gombotz WR, Wee S. Protein release from alginate matrices. *Adv Drug Del Rev*. 1998; 31:267–85.
54. Decho AW. Imaging an alginate polymer gel matrix using atomic force microscopy. *Carbohydr Res*. 1999; 315:330–3.
55. Baumberger T, Ronsin O. From thermally activated to viscosity controlled fracture of biopolymer hydrogels. *The Journal of Chemical Physics*. 2009:130.
56. Briššová M, Petro M, Lacík I, Powers AC, Wang T. Evaluation of Microcapsule Permeability via Inverse Size Exclusion Chromatography. *Anal Biochem*. 1996; 242:104–11. [PubMed: 8923972]
57. Gattas-Asfura KM, Fraker CA, Stabler CL. Covalent stabilization of alginate hydrogel beads via Staudinger ligation: assessment of poly(ethylene glycol) and alginate cross-linkers. *Journal of biomedical materials research Part A*. 2011; 99:47–57. [PubMed: 21793196]
58. Josephy PD, Mason RP, Eling T. Cooxidation of the Clinical Reagent 3,5,3'-5'-Tetramethylbenzidine by Prostaglandin Synthase. *Cancer Res*. 1982; 42:2567–70. [PubMed: 6805942]
59. Stockwell AF, Davis SS, Walker SE. In vitro evaluation of alginate gel systems as sustained release drug delivery systems. *J Controlled Release*. 1986; 3:167–75.
60. Bouhadir KH, Alsberg E, Mooney DJ. Hydrogels for combination delivery of antineoplastic agents. *Biomaterials*. 2001; 22:2625–33. [PubMed: 11519782]
61. Pluen A, Netti PA, Jain RK, Berk DA. Diffusion of Macromolecules in Agarose Gels: Comparison of Linear and Globular Configurations. *Biophys J*. 1999; 77:542–52. [PubMed: 10388779]

62. Fatin-Rouge N, Starchev K, Buffle J. Size Effects on Diffusion Processes within Agarose Gels. *Biophys J*. 86:2710–9. [PubMed: 15111390]
63. Ramzi M, Rochas C, Guenet J-M. Structure–Properties Relation for Agarose Thermoreversible Gels in Binary Solvents. *Macromolecules*. 1998; 31:6106–11.
64. Creixell M, Herrera AP, Latorre-Esteves M, Ayala V, Torres-Lugo M, Rinaldi C. The effect of grafting method on the colloidal stability and in vitro cytotoxicity of carboxymethyl dextran coated magnetic nanoparticles. *J Mater Chem*. 2010; 20:8539–47.
65. Ma, H-l; Qi, X-r; Maitani, Y.; Nagai, T. Preparation and characterization of superparamagnetic iron oxide nanoparticles stabilized by alginate. *Int J Pharm*. 2007; 333:177–86. [PubMed: 17074454]
66. Shen F, Poncet-Legrand C, Somers S, Slade A, Yip C, Duft AM, et al. Properties of a novel magnetized alginate for magnetic resonance imaging. *Biotechnol Bioeng*. 2003; 83:282–92. [PubMed: 12783484]
67. Kawaguchi T, Hanaichi T, Hasegawa M, Maruno S. Dextran-magnetite complex: conformation of dextran chains and stability of solution. *J Mater Sci Mater Med*. 2001; 12:121–7. [PubMed: 15348317]
68. Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, Scarpa T, et al. Non-cytotoxic Silver Nanoparticle-Polysaccharide Nanocomposites with Antimicrobial Activity. *Biomacromolecules*. 2009; 10:1429–35. [PubMed: 19405545]
69. Meenach SA, Anderson AA, Suthar M, Anderson KW, Hilt JZ. Biocompatibility analysis of magnetic hydrogel nanocomposites based on poly(N-isopropylacrylamide) and iron oxide. *Journal of Biomedical Materials Research Part A*. 2009; 91A:903–9. [PubMed: 19090484]
70. Drath DB, Karnovsky ML. Superoxide production by phagocytic leukocytes. *The Journal of Experimental Medicine*. 1975; 141:257–62. [PubMed: 804030]
71. Buchwald P. A local glucose-and oxygen concentration-based insulin secretion model for pancreatic islets. *Theoretical Biology and Medical Modelling*. 2011; 8:20. [PubMed: 21693022]

Figure 1.

Illustration of cerium oxide nanoparticle (CONP)-alginate composite hydrogel. Alginate microbead provides matrix for cellular encapsulation and perm-selectivity 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.

Figure 2.

Dextran-coated cerium oxide nanoparticle (CONP) characterization. A) FTIR of cerium dioxide without (CeO₂; dashed grey line) and with (CONP; black line) dextran coating; dextran spectra (Dextran T-10; dotted grey line) shown for comparison. B) Representative DLS analysis of CONP particles exhibiting moderate polydispersity. C) X-ray photon spectroscopy (XPS) analysis of CONP solutions demonstrating both characteristic cerium +3 (Ce₂O₃) and +4 (CeO₂) peaks.

Figure 3.

CONPs in solution exhibit strong catalytic reactivity. A) Hypothesized reactions of CONPs with ROS superoxide and hydrogen peroxide. B) Reactivity of CONP (1 mM) with H_2O_2 (1 mM) in solution via spectroscopic assessment of color shift. Return to initial state is tracked over 14 d. C) Effect of CONP concentration (0 – 13 mM) on TMB oxidation, measured via absorbance shift over time.*Error bars too small to visualize D) Neutralization of superoxide, generated via the XA/XO system, as measured through the oxidation of cytochrome C without (“XA+XO”, black circles) and with CONPs (1 mM, “XA+XO +CONP”, purple circles). Additional controls of XA with no XO added (XA, open circles) and CONP only (“CONP”, purple triangles) are also shown.

Figure 4.

CONPs in solution provide cytoprotective effects for beta cells following exposure to superoxide. MIN6 beta cells were co-incubated with CONPs (0, 0.1, or 1.0 mM), free in solution. A) Beta cell metabolic activity was evaluated after exposure to superoxide ((+)SO; black bars) via Alamar Blue. Controls were not exposed to superoxide ((-)SO; grey bars). Results were normalized to day 0 controls (no CONPs, no superoxide). *** $P < 0.0001$ between designated groups; # $P < 0.01$ from control (no CONP) group. B) TEM imaging of MIN6 cells for both control and 1 mM CONP-treated groups. Inset: Higher magnification images of cell lysosomes demonstrate localized concentration of nanoparticles in CONP-treated cell lysosomes (white arrows). Scale bar = 1 μm .

Figure 5.

CONP-alginate hydrogel catalytic activity verified via TMB oxidation (color shift to blue) and H_2O_2 scavenging. A) Visualization of TMB oxidation via embedded CONP after 15 min incubation. B) Detection of ambient H_2O_2 via absorbance assay for CONP-alginate composites loaded with 0, 0.1, 1, 10 mM CONPs. Results were normalized to initial ambient H_2O_2 concentration (60 μM) and assessed at 1, 2, and 4 h; CONP-alginate groups were significantly different from control gels ($P < 0.05$) at all time points except 0.1 mM CONP-Alg at 4 hours.

Figure 6.

CONP-alginate composite hydrogels provide enhanced beta cell cytocompatibility. CONPs (1, 5, or 10 mM) were co-cultured with beta cells, either free in solution or embedded within alginate hydrogels. A) Metabolic activity (via Alamar Blue) for beta cells incubated with CONPs in solution (white bars) or within alginate microbeads doped with CONPs (black bars). Results were normalized to CONP-free controls. ** $P < 0.01$; *** $P < 0.0001$ between designated groups; ND – not determined. B) TEM imaging of MIN6 cells within control alginate or 10 mM CONP-alginate hydrogels. Inset: Higher magnification images of cell lysosomes exhibit no localization of nanoparticles in cytoplasmic lysosomes. *denotes alginate. Scale bar = 1 μ m

Figure 7.

CONP-alginate composite hydrogels provide cytoprotection from oxidative stress. MIN6 beta cells were encapsulated within CONP-alginate hydrogels with varying CONP concentrations (0, 1, 5, 10 mM) and cultured for 48 hr. A) Metabolic activity (via Alamar Blue) was evaluated after exposure to superoxide ((+)SO; black bars). Controls were not exposed to superoxide ((-)SO; white bars). Results were normalized to untreated controls. B) Multi-plane Live/Dead (live, green; dead, red; merged) confocal imaging of beta cells within alginate microbeads doped with 0, 1, 5, and 10 mM CONPs. Scale bars = 200 μ m. *P < 0.05

Figure 8.

CONP-alginate composite hydrogels provide extended beta cell cytoprotection from multiple exposures to oxidative stress. Beta cells encapsulated within CONP-alginate hydrogels (10 mM CONP) or control alginate were treated with superoxide on days 2 and 6 post-encapsulation (indicated by $\uparrow$). A) Beta cell metabolic activity for control alginate beads untreated (white bars) or treated with superoxide (black bars). B) Beta cell metabolic activity for CONP-alginate beads (10mM CONP) untreated (grey bars) or treated with superoxide (black bars). Results were normalized to day 2 untreated controls. C) Multi-plane

Live/Dead (live, green; dead, red; merged) confocal imaging of beta cells within alginate microbeads without and with CONPs 2 and 6 day post-encapsulation. Scale bars = 200 μm .
P < 0.01; *P < 0.001

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