Antioxidant Cerium Oxide Nanoparticle Composite Hydrogels for Islet Encapsulation and Protection

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Statement of Purpose: The transplantation of allogeneic islet cell clusters has demonstrated promise and feasibility for treating Type 1 Diabetes; however, the current technique is limited by immediate graft losses due to immune and inflammatory responses upon transplantation. Encapsulation of islets within hydrogels has been shown to partially camouflage transplanted cells from the host immune system; yet benign materials do little to ameliorate the localized inflammatory response. This has a significant impact on islets, which possess markedly low levels of antioxidant enzymes. Recently, studies have highlighted potent antioxidant cerium oxide nanoparticles (CONP) due to their highly catalytic and theoretically infinite radical scavenging capacity. While CONPs demonstrate promise for alleviating inflammatory byproducts, the potential toxicity of systemic nanoparticle exposure is not well understood. Therefore, entrapment and localization of CONPs to an encapsulating hydrogel may confer protection while limiting systemic nanoparticle exposure. Within this study, we sought to develop CONP-functionalized encapsulation hydrogels with the capacity to enhance cell viability by locally neutralizing free radicals at the transplant site.

Methods: *CONP synthesis and activity*: A dextran-complexed cerium oxide nanoparticle suspension was synthesized using methods similar to published reports[1] and confirmed via FT-IR, XPS, DLS, and TEM. CONP activity was confirmed via spectrophotometric measurements of colorimetric reactions produced by TMB oxidation or reduction of hydrogen peroxide (H_2O_2,) and superoxide (O_2^-, xanthine/xanthine oxidase reaction) radicals. *MIN6-CONP Co-culture*: Mouse insulinoma (MIN6) cells ($3x10^5$) were incubated with varying concentrations of CONP (0.01 – 1mM) for 48 hours to evaluate cytotoxicity. *CONP-Hydrogel fabrication:* CONP-hydrogel prototypes were prepared by embedding CONP (0-10mM) within alginate, agarose, or matrigel hydrogels. *Cytoprotection by MIN6-CONP Alginate Capsules:* MIN6 were encapsulated ($25x10^6$ cells/mL) in alginate microbeads, with or without CONPs. The capacity of CONPs to protect the encapsulated cells from free radical damage was evaluated via challenge with O_2^- for 2 hrs, and cell viability was assessed via Alamar Blue assay (Invitrogen) and Live/Dead fluorescent imaging (Invitrogen).

Results: CONPs exhibit stability in solution, with an average diameter of 19.82 ± 0.81 nm and full scavenging capabilities, as determined by colorimetric reaction with TMB, H_2O_2, and reduction of O_2^-. Co-incubation of nanoparticles with MIN6 cells in suspension for 48 hrs demonstrated insignificant toxicity levels at CONP concentrations < 1.0mM. At 1.0mM CONP, MIN6 cells demonstrated a 20% decrease in metabolic activity. Incorporation of CONPs within hydrogels of varying pore size resulted in varying degrees of physical entrapment, with alginate exhibiting the most efficient nanoparticle retention. CONP activity was retained within alginate, as evaluated via visual assessment of TMB and H_2O_2 colorimetric reactions, and quantitative assessment of ambient H_2O_2 reduction. This reactivity was long-term, with multiple trials exhibiting scavenging abilities of the CONP hydrogels. Encapsulation of MIN6 within CONP-alginate hydrogels resulted in undetectable toxicity levels at 1.0-10.0mM, demonstrating the enhanced cyto-compatibility of this system. Preliminary studies challenging free and encapsulated cells with superoxide radicals found substantial and significant ($P < 0.05$) protection of the cells from superoxide radical induced cell loss when CONP, either in solution or embedded within CONP-functionalized hydrogels was present (Fig 1).

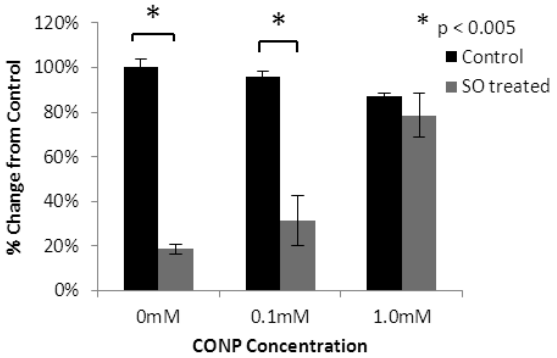

Figure 1. MIN6 exposed to CONP and superoxide (SO) radicals demonstrate significant improvement in viability at 1.0mM CONP concentrations.

Conclusions: Within this study, we have demonstrated the potential of CONPs to catalytically scavenge damaging free radicals, and convey antioxidant protection to MIN6. In addition, we have demonstrated the ability to incorporate and retain CONPs within encapsulation hydrogels while preserving their catalytic activity. Finally, the biocompatibility and protective capacity of CONPs is enhanced through incorporation within encapsulation hydrogels by the potential to localize high doses of the nanoparticles with negligible toxicity. Of note, this antioxidant material platform has widespread application to fields outside of islet transplantation, whereby this ubiquitous, self-renewing, free radical scavenging biomaterial could provide substantial protection.

References:

1. Asati, A., et al. *Angew. Chem. Int. Ed.* (48:2308-2312, 2009)

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