Antioxidant Hydrogels for Cellular Encapsulation using Cerium Oxide Nanoparticles

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Introduction

The encapsulation and subsequent immunoisolation of cells within semi-permeable membranes has many potential applications in tissue engineering. Of particular interest is the encapsulation of insulin-secreting cells for the long-term treatment of diabetes. While encapsulation of cells within perm-selective polymers has resulted in the dramatic reduction of immune cell activation and subsequent transplant destruction, it fails to prevent inflammation and indirect immune activation, which commonly results in oxidative stress. Increases in free radicals at the site of transplant represent a particular challenge for islets, as they are markedly low in antioxidant enzyme activity relative to other tissues. Supplementation of islet grafts with antioxidants may provide protection from free radical-induced apoptosis. Recently, cerium oxide nanoparticles, or nanoceria, have demonstrated behavior that mimics free-radical scavenging enzymes. Ubiquitous and self-renewing in their free radical scavenging capabilities, nanoceria also benefits from multiple catalytic sites in its nanoparticle form. Limited research is available on the effects of systemic exposure to free metal-oxide nanoparticles, therefore localization of nanoparticle dose to the transplant site is essential. Furthermore, localization of particles to the site of transplant may enhance their effect. In this study, we sought to localize nanoceria to microcapsule implant site by integrating these particles within the encapsulating hydrogel. Herein, we investigate the activity of a nanoceria-functionalized alginate hydrogel, as well as its capacity to protect co-encapsulated cells from free radical damage.

Materials and Methods

Nanoceria synthesis and activity: A dextran-complexed cerium oxide nanoparticle suspension was synthesized using methods similar to published reports[1] and confirmed via FT-IR. Nanoceria activity was confirmed via spectrophotometric measurements of colorimetric reactions produced by oxidation of 3,3',5,5'-tetramethylbenzidine (TMB, Sigma) and nanoceria oxidation by hydrogen peroxide (H_2O_2, Aldrich). Nanoceria diameter was quantified via dynamic light scattering (DLS) technique. *MIN6-Nanoceria Co-culture*: Mouse insulinoma (MIN6) cells ($3x10^5$) were incubated with varying concentrations of nanoceria (0.01 – 1mM) for 48 hours to evaluate cytotoxicity. *Nanoceria-Alginate microcapsule fabrication:* A nanoceria-alginate solution was prepared by mixing 1.6% w/v alginate (MVM, Novamatrix) with nanoceria (1.0mM). Capsules were formed by extruding droplets from a syringe with a 27 gauge needle into a bath of barium chloride (1.6% $BaCl_2$, Sigma) crosslinking solution. *MIN6-Nanoceria Alginate Encapsulation:* MIN6 were encapsulated at $25x10^6$ cells/mL in alginate, as described above, with or without nanoceria. Capsules were then cultured in full media during the course of the experiment. The capacity of nanoceria to protect the encapsulated cells from free radical damage was evaluated via a challenge with 300μM H_2O_2 for 6 hrs. Cell viability was assessed via MTT assay.

Results

Nanoceria exhibited nanoparticle stability in solution, with average diameter of 19.82 ± 0.81nm, and full scavenging capabilities, as demonstrated in oxidation of TMB and reduction of hydrogen peroxide. Co-incubation of nanoparticles with MIN6 cells in suspension for 48hrs demonstrated insignificant toxicity levels, at nanoceria concentrations below 1.0mM, as assessed via MTT. At 1.0mM nanoceria, MIN6 cells demonstrated a 20% decrease in metabolic activity, via MTT. Following encapsulation of nanoparticles within alginate microbeads, nanoceria activity was retained, as evaluated via visual assessment of TMB and hydrogen peroxide colorimetric reactions. Co-encapsulation of nanoceria with MIN6 resulted in undetectable toxicity levels at 1.0mM concentrations, demonstrating the enhanced cell compatibility of this system. Preliminary studies have found that challenging the encapsulated cells with hydrogen peroxide resulted in a substantial decrease in cellular viability, while 1.0mM nanoceria-functionalized hydrogels provided statistically significant protection ($p<0.005$) from hydrogen peroxide-mediated cell death, as assessed via MTT.

Conclusions

In this study, we have illustrated the ability to generate free-radical scavenging hydrogels through the encapsulation of cerium oxide nanoparticles within alginate microbeads. Incorporation of nanoceria within these hydrogels resulted in a decrease in cytotoxicity. In addition, nanoceria entrapped within alginate hydrogels provided protection to the co-encapsulated cells, by reducing hydrogen peroxide-mediated cell death. These results illustrate the potential of this system to improve the translation of these novel nanoparticles to tissue engineering, as well as provide a safe mechanism for the localization of these nanoparticles. Future studies will examine the potential of these hydrogels for the protection of islets in vitro and in vivo, as well as evaluate the conjugation of these particles to biomaterial surfaces.

References:

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

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