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Enzymatic protection and biocompatibility screening of enzyme-loaded polymeric nanoparticles for neurotherapeutic applications

Rick Liao [a], Jessica Pon [a], Michael Chungyoun [a], Elizabeth Nance [a,b,c,*]

[a] Department of Chemical Engineering, University of Washington, Seattle, WA, USA
[b] Department of Radiology, University of Washington, Seattle, WA, USA
[c] Center on Human Development and Disability, University of Washington, Seattle, WA, USA

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ABSTRACT

Polymeric nanoparticles provide a non-invasive strategy for enhancing the delivery of labile hydrophilic enzymatic cargo for neurological disease applications. One of the most common polymeric materials, poly(lactic-co-glycolic acid) (PLGA) copolymerized with poly(ethylene glycol) (PEG) is widely studied due to its biocompatible and biodegradable nature. Although PLGA-PEG nanoparticles are generally known to be non-toxic and protect enzymatic cargo from degradative proteases, different formulation parameters including surfactant, organic solvent, sonication times, and formulation method can all impact the final nanoparticle characteristics. We show that 30s sonication double emulsion (DE)-formulated nanoparticles achieved the highest enzymatic activity and provided the greatest enzymatic activity protection in degradative conditions, while nanoprecipitation (NPPT)-formulated nanoparticles exhibited no protection compared to free catalase. However, the same DE nanoparticles also caused significant toxicity on excitotoxicity-induced brain tissue slices, but not on healthy or neuroinflammation-induced tissue. We narrowed the culprit of toxicity to specifically sonication of PLGA-PEG polymer with dichloromethane (DCM) as the organic solvent, independent of surfactant type. We also discovered that toxicity was oxidative stress-dependent, but that increased toxicity was not enacted through increasing oxidative stress. Furthermore, no PEG degradation or aldehyde, alcohol, or carboxylic acid functional groups were detected after sonication. We identified that inclusion of free PEG along with PLGA-PEG polymer during the emulsification phases or replacing DCM with trichloromethane (chloroform) produced biocompatible polymeric nanoparticle formulations that still provided enzymatic protection. This work encourages thorough screening of nanoparticle toxicity and cargo-protective capabilities for the development of enzyme-loaded polymeric nanoparticles for the treatment of disease.

1. Introduction

Polymeric nanoparticles have been extensively researched for drug delivery applications to the central nervous system due to their ability to overcome physiological barriers, exhibit controlled release, and alter drug metabolism and clearance kinetics [1–3]. Therapeutic-encapsulating polymeric nanoparticles can provide stability and protection for enzymatic cargo from proteolytic conditions and can traverse the blood-brain barrier (BBB) [4,5]. One of the most common polymeric materials is the copolymer poly(lactic-*co*-glycolic acid) (PLGA) due to its biocompatibility and biodegradability [6–8]. PLGA nanoparticles are capable of carrying a variety of hydrophobic or hydrophilic therapeutic cargo within their polymer matrix core, including small molecule drugs and macromolecules such as proteins or DNA [9]. Formulating PLGA nanoparticles with a dense poly(ethylene glycol) (PEG) coating can imbue stealth-like properties for the avoidance of reticuloendothelial system detection and enhance brain tissue penetration while retaining biocompatibility [6,10]. For PLGA-PEG block copolymer, the most common nanoparticle formulation approaches are the emulsion solvent evaporation (double emulsion (DE) for hydrophilic cargo), nanoprecipitation (NPPT), also known as solvent displacement, and salting out methods [6,9].

Therapeutic enzymes are of special interest due to their precise catalytic functions, but oral and intravenous administration of free enzymes have had limited success due to proteolytic degradation and poor brain biodistribution [11]. Attempts to overcome these issues include

* Corresponding author. Department of Chemical Engineering, University of Washington, Seattle, WA, USA.
E-mail address: eanance@uw.edu (E. Nance).

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