COLLEGE OF PHARMACY
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The Department of Pharmaceutical Sciences is pleased to announce the
Ph.D. Dissertation Defense Seminar of

Jason Albert
Pharmaceutical Sciences, Ph.D. Candidate
Mentor: Dr. Steven P. Schwendeman

Friday, January 21, 2022 at 8:30am
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"Metal-HisTag Coordination for Remote Loading of Biomacromolecules into PLGA Microspheres"

Abstract: Challenges to discovery and preclinical development of long-acting release systems for protein therapeutics include protein instability and use of organic solvents during encapsulation, specialized equipment and personnel, and high costs of proteins. Remote loading self-healing encapsulation has been used to gently and efficiently encapsulate proteins, primarily through protein-specific affinity for a trapping agent. To create a universal remote loading self-healing encapsulation platform, coordination bonds formed between polyhistidine tags (HisTags) and divalent transition metal cations (M^{2+}) were utilized, similar to immobilized metal affinity chromatography.

Porous, drug-free self-healing poly(lactic-co-glycolic acid) (PLGA) microspheres with high molecular weight dextran sulfate (HDS) and immobilized remotely-loaded M^{2+} ions were placed in the presence of proteins with HisTags to bind in the pores of the polymer before healing the surface with modest temperature. Using human serum albumin (HSA), insulin-like growth factor 1, and granulocyte-macrophage colony-stimulating factor (GM-CSF), encapsulation efficiencies (EE) of immunoreactive protein increased in a pH-dependent manner with the inclusion of HisTags and Zn^{2+}. Immunoreactive protein was continuously released over seven to ten weeks. GM-CSF showed bioactivity >95% relative to immunoreactive protein throughout. Increased EEs were found with other M^{2+} ions, but not with Ca^{2+}. Ethylenediaminetetraacetic acid interfered with this process, reverting EE to Zn^{2+}-free levels.

Following this promising proof-of-concept work, areas of potential improvement were identified: (1) reducing thermal stress, (2) decreasing the complexity and duration of the protocol, (3) increasing the loading capacity, (4) increasing the penetration depth of protein, and (5) improving the release profile. Directly encapsulating $ZnCO_3$, rather than remotely loading Zn^{2+}, increased the Zn content in the microspheres ~6-fold. Microspheres with directly encapsulated $ZnCO_3$ ($_{DE}ZnCO_3$) more efficiently encapsulated HSA at protein loading solutions concentrations ≥ 100 µg/mL than remotely loaded Zn^{2+} ($_{RL}Zn^{2+}$) microspheres. HisTag green fluorescent protein was more deeply encapsulated in $_{DE}ZnCO_3$ microspheres than in $_{RL}Zn^{2+}$ microspheres. Tributyl acetylcitrate was an effective plasticizer in terms of decreasing the glass transition temperature, but also led to a decrease in EE. The loading stage was reducible to 2 hours at 4°C and the healing stage to 6 hours at 37°C while maintaining strong EE for $_{DE}ZnCO_3$ microspheres, which slowly released immunoreactive protein for months, following a substantial burst release. Plasticization, though, decreased the initial burst release.

Next, the effects of various excipients on physiochemical properties of the formulations were studied. HDS was shown to function as a porosigen and encourage water uptake. While HDS did not significantly affect the Zn^{2+} loading of $_{DE}ZnCO_3$ microspheres, it was shown to play a critical role in remote loading of various M^{2+} cations. Though the erosion and degradation profiles of the microspheres were not affected by HDS, replacing $MgCO_3$ with $ZnCO_3$ accelerated erosion and degradation significantly, potentially owed to the superior pH-modulation of $MgCO_3$. HDS exhibited a high burst release followed by a plateau and seemingly degradation-dependent release. Zn^{2+} release appeared erosion-driven and was released more quickly from $ZnCO_3$-containing microspheres than from $MgCO_3$-containing microspheres. Release of HisTag HSA from HDS-free $_{DE}ZnCO_3$ microspheres showed higher burst and much faster release than HDS-containing formulations have shown previously.

This platform could be a valuable asset to drug discovery and early development scientists who seek to study the controlled release of delicate biologic candidates using very small quantities of the protein, allowing for improved translation to further development.