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Sustained Release Micro-Encapsulates to Administer Peptides, Proteins and Nucleic Acids for Retinal Diseases

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Abstract

Retinal diseases warrants development of receptor-specific molecules targeting cellular mechanisms to arrest further disease progression, thus preventing ultimate blindness.

Proteins, peptides and nucleic acids demonstrate immense potential as treatment modalities. These therapies are administered via repeated injections, leading to issues such as endophthalmitis and retinal detachment. Delivery systems releasing predictable levels of intact drug into the vitreous for multiple months can transform the treatment of retinal diseases. One advantage of encapsulating drugs is the protection of biologics from rapid in-vivo degradation.

Development objectives for these delivery systems include achievement of high encapsulation efficiencies of intact drug, retention of biological potency of the encapsulated drug throughout delivery and precise release of the drug over time. One major issue encountered during the encapsulation process is the loss of the water-soluble drug, resulting in very low encapsulation efficiencies and typically, high "burst" release.

We encapsulated a highly water-soluble peptide and a water-soluble protein (MW 66,000 g/mole) as model compounds in poly(lactide-co-glycolide) (PLG), with encapsulation efficiencies greater than 90%, percent "burst" less than 1% and release profiles that can be modulated, based on polymer matrix composition and internal microstructure.

Concepts described in this work are part of a development program to develop a proprietary platform delivery system, EySite™. Small molecules, proteins and peptides can be delivered either singly or in combination formats.

Study Objectives

- Develop encapsulation methodology to achieve optimum size for long-term release of biologics
- Achieve optimum size for injectability.
- Develop encapsulation methods to minimize "burst".
- Demonstrate encapsulation of intact compounds.
- Achieve in-vitro sustained release of a water-soluble model peptide, MW 4200 Daltons.
- Achieve in-vitro sustained release of a model protein, M.W. 66,000 Daltons.

Materials & Methods

Preparation of Micro-encapsulates: PLG microencapsulates were prepared by a proprietary process, to achieve a narrow size distribution, optimum size and injectability. A model peptide of MW 4200 Da and a model protein of MW 66,000 Da were encapsulated.

Characterization:

- Particle Size Distribution (PSD): Samples were suspended, 20mg /mL in a carboxymethyl cellulose-based diluent, diluted with an equivalent volume of water. 500uL of the suspension was dispersed in a dispersal medium (a thirty fold dilution of the diluent in distilled water). Particle size was measured on a Horiba LA-950 Laser Diffraction Particle Analyzer.
- Imaging: Dry encapsulates were characterized by cryo-scanning electron microscopy.
- Encapsulation (mg/G): 20 mg of the microencapsulate was dissolved in 1mL of acetonitrile, and 4mL of 30mM acetate buffer, pH 4.4, added drop-wise while mixing. 1mL of the slurry was centrifuged (5min; 6000 RPM), and the supernatant was removed for HPLC analysis. HPLC analysis was performed on a RP C18 column.
- *In-Vitro* Burst (%): 30 mgs of the microencapsulates were reconstituted in PBS, pH 7.4 and rotated at 37C.
- *In-Vitro* Release: Samples were suspended 60 mg/mL in acetate buffer (100mM; pH 4.2) and placed in cellulose ester dialysis tubes (Spectrum; Spectra/Por; MWCO: 1000 kD; 5mL capacity). The tubes were suspended in 45mL and incubated at 50C. Time point samples were obtained after 1 hour and daily over the course of twenty days.
- Injectability of the microspheres suspended in the reconstituting fluid were tested both through 23G and 27G needles.

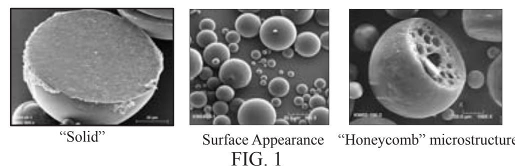
Results

Table 1 shows the PSD, encapsulation (mg/g), 1 hour burst and long term in-vitro release of both the peptide and the protein.

	Peptide	Protein (BSA)
PSD	D_{50}=56µ D_{90}=110µ	d_{50}: 49.2µ D_{90}: 79.5µ
Encapsulation (mg/g)	45 mg/G 47 mg/G	49.504 mg/G 46 mg/G
% burst	<2%	<2%

Results

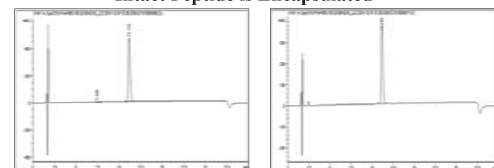
Proprietary Process Controls Microstructure and Size



"Solid" Surface Appearance "Honeycomb" microstructure

FIG. 1

Intact Peptide is Encapsulated



Standard, Peptide Encapsulated Peptide

FIG. 2

In-Vitro Release of Peptide as a Function of Microstructure, 37°C, pH 7.4

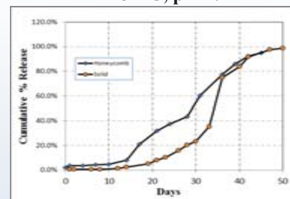


FIG. 3

Accelerated In-Vitro Release of Protein as a Function of Molecular Weight

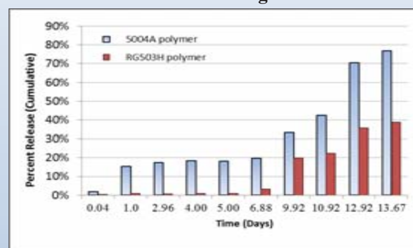


FIG. 4

Results

Process is Proprietary and Scaled-Up

FIG. 4 shows the average encapsulation efficiencies of 5-15 batches, at each scale. Encapsulation efficiency was utilized as a gauge to utilize scale-up feasibility.

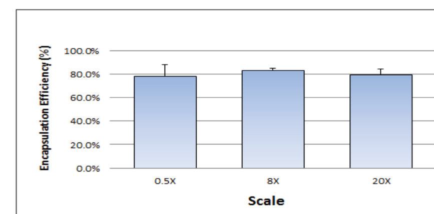


FIG. 4

Injectability

- The microspheres were injectable through a 23G needle, but not through a 27G needle. For future work, smaller PSD will be targeted.

Conclusions and Future Work

- Feasibility of delivering proteins and peptides via biodegradable micro-encapsulated was demonstrated.
- It was demonstrated that internal microstructure played a huge role in in-vitro release rates.
- Polymer composition and molecular weight plays a large role in the release rate.

Using concepts described in this work, a platform delivery system EySite™ is being developed for the delivery of proteins, peptides, nucleic acids.

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