From Laboratory to Pilot Scale: Advanced Dosage Forms of Protein/Peptide Medicines

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ABSTRACT SUMMARY
We report two formulation processes to produce sustained-release microspheres of uniform sizes and 90%+ biologic encapsulation efficiency (named EasySphere), and phase-transition microneedle (PTM) patches capable for efficient and accurate transdermal delivery of proteins/peptides at pilot scale.

The microsphere process comprises a stir-free unit operation to form and solidify embryonic microspheres from a drug-loaded polymer solution efficiently. This process causes no breaking or fusion of the dispersed polymer droplets, the two factors causing protein/peptide leaking and uneven sizes. For proteins having tertiary structures, a pre-formulation step involving aqueous-aqueous emulsification was used to pre-load the drug into solvent resistant dextran fine particles without contacting water-oil interfaces prior to microencapsulation into EasySpheres. For proteins having highly delicate conformations, microencapsulation was achieved by organic solvent-free partition into pre-made porous polylactic-co-glylic acid (PLGA) microspheres, followed by a low temperature pore-sealing step.

The PTM patches were produced using a unique Teflon mold which is permeable to air due to its porous matrix but impermeable to water due to its hydrophobicity. A polyvinyl alcohol (PVA) solution loaded with proteins/peptides was casted on the mold mounted on a vacuum chamber, soaked into the micro-holes by vacuum, and subjected to a freeze-thaw treatment to form nano-crystalline domains as the cross-linking junctions of the hydrogel network. The gelled microneedle sheet was detached from the mold, dried, and punched to designed sizes.

The EasySphere and PTM technologies were examined by applying them in formulating a series of proteins/peptides (erythropoietin [EPO], insulin and exenatide) into respective dosage forms. Physical characterization and animal trials for PK, efficacy and immunogenicity using monkey and pig models confirmed that all the design criteria of the two systems are met.

INTRODUCTION
While the numbers and market of biologic medicines are rapidly increasing in recent years, their dosage regimes are limited to frequent injections. Many of the biologic therapeutics are applied for chronic treatment for which sustained release injections and non-invasive dosing represent the two greatest unmet demands for advanced dosage forms of native biologic drugs. Successful formulation technologies must meet the criteria for both therapeutic performance and manufacture efficiency.

For sustained-release microspheres (the only feasible dosage form to achieve monthly dosing to date), although some microsphere dosage forms for peptides are seen in the market, none of such a product is currently available for proteins due to the conformation instability, and none of the marketed peptide microspheres meets the two criteria mentioned above. For non-injection delivery of proteins and peptides, bioavailability, dosing accuracy and production efficiency are the critical challenges in developing practical dosage forms.

To meet these demands in advanced dosage forms of proteins/peptides, we developed two respective engineering processes to produce microspheres of uniform size and 90%+ encapsulation efficiency and microneedle patches offering efficient and accurate transdermal delivery without depositing the needle-tip materials to the skin. Exenatide, erythropoietin (EPO), and insulin were used as the model drugs to examine the druggability of the two formulation strategies by efficacy, pharmacokinetics and immunology assays in pig and monkey models. The rationale for the engineering and detailed results of the bioassays will be presented on the CRS meeting.

METHODS AND RATIONALES
The unit operation to produce microspheres of uniform size and high encapsulation efficiency comprises 1) pressing the drug loaded polymer solution through a membrane possessing uniform pores to form embryonic microspheres and 2) hardening the formed microspheres by settling along a column filled with an aqueous medium to extract solvent (dichloromethane) of the polymer. The hardened microspheres were collected at the bottom of the settling column and transferred to a rinsing
tank through a tube under the hydraulic pressure within the column.

Proteins having tertiary structures were pre-formulated into dextran fine particles via aqueous-aqueous emulsification, followed by lyophilization. Once loaded in the fine particles, the protein conformation is immobilized in the glassy matrix of the polysaccharide and can therefore be loaded in microspheres through EasySphere process without denaturing.

For highly delicate proteins having complicated conformations, microencapsulation was achieved by suspending pre-made porous PLGA microspheres loaded with dextran particles in a solution of high molecular weight polyglycolethylene (PEG-20KD, 15%). The protein was driven thermodynamically into the hydrated dextran phase dispersed inside of the porus PLGA microspheres by a preferential partition favoring the dextran phase. The pores of the PLGA microspheres were sealed by low temperature annealing which was achieved by re-suspending the harvested microspheres in a concentrated solution of low molecular weight PEG (PEG-400, ~90%). Partial swelling by PEG-400 lowered the phase transition temperature (Tg) of PLGA.

In order to produce phase-transition (PTM) patches with accurate drug loading, air permeable Teflon molds of defined and uniform dimensions were prepared first. A fine Teflon powder of given mass was loaded in a stainless steel (primary) mold and compressed to an air-permeable Teflon plate of designed dimension, followed by annealing at 380 °C for 2 hours. This Teflon plate was then pressed against another stainless steel mold with a microneedle array on the surface at 180 °C to form the microneedle patch casting mold.

To produce the PTM patch, a PVA solution loaded with protein/peptide drugs was casted on the Teflon mold mounted on a vacuum trough, sucked into the micro-holes by vacuum applied on the back of the mold, and attached with a pre-made drug-free PVA sheet. The casted workpiece was then treated by two freeze-thaw cycles, detached from the mold, and dehydrated in dry air under guidance. The dried microneedle sheet was finally punched to patches of designed size (determined dose), and attached to an adhesive back-membrane prior to packaging.

RESULTS AND DISCUSSION

Our exenatide microspheres showed a fairly steady blood concentration of the drug over 30 days after a SC injection in monkeys (n=5) with a dose equivalent or less than the weekly product. The PK profiles are shown in the figure below.

Fig. 1. Blood concentration of exenatide in monkeys

EPO EasySpheres showed a prolonged (2 weeks) efficacy by a single injection in monkeys (n=5) without evoking anti-EPO antibodies as compared with saline and solution formulation. The microspheres made by conventional double emulsion method resulted in an antibody level 40 times higher (see the figure below).

Fig. 2. Antibodies evoked in monkeys after EPO dosing.

Applying insulin PTM patch on diabetic pigs resulted in a PK profile comparable to that of the injection pen. Its $T_{max}$ was 18 min behind and the AUC was 20% as compared with the injection pen (see the Figure below, left). As long-term efficacy, the glycated hemoglobin in the pigs was lower than those treated with injection pen (see below, right).

Fig. 3. PK profiles (L) and efficacy (R) of insulin patch.

CONCLUSION

Easy Sphere and PTM patch offer nearly ideal performance in sustained-release and transdermal delivery of biologics, respectively, as well as scalable engineering for high quality production.