Incorporation of Protein-based Therapeutic Agents into Coaxial Electrosprayed Particles for Cardiac Regeneration

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Statement of Purpose: Controlled delivery of protein-based therapeutic agents such as growth factors and cytokines into infarcted myocardium is an emerging regenerative approach for MI treatment. However, because of susceptible 3D structure of protein-based regenerative cues, their incorporation into polymeric carriers, is associated with several challenges e.g. protein denaturation/inactivation, protein aggregation and burst release, due to exposure of protein to organic-aqueous interfaces during the common fabrication processes such as emulsification. In this study, we explored incorporation of protein structures into core-shell PLGA particles using coaxial electrospraying. This technique enables production of protein loaded particles with separate perfusion of polymer-contained organic phase (shell) and protein-contained aqueous phase (core), which minimizes the organic-aqueous interfaces during the fabrication process. Thus, the risk of protein damage and subsequent inactivation is significantly lowered. It also provides the possibility of tailoring drug release profile via physical changes in the core-shell structure (e.g. shell thickness), which may positively affect the efficacy of the regenerative cues, resulting in improved functionality of the infarcted heart.

Methods: Fig. 1a illustrates the schematic setup of the coaxial electrospray process in this study. Initial experiments were conducted using PLGA dissolved in different solvents (e.g. dichloromethane (DCM), N,N-dimethylformamide (DMF)) as the shell, while aqueous solution of the model protein, bovine serum albumin (BSA), was used as the core. SEM, XPS and TEM was used to characterize the morphology and core-shell structure of the particles. Next, the optimized shell/core solution parameters and processing variables were employed to incorporate stromal derived factor-1 alpha (SDF-1α), the chemotactic factor with proven effectiveness for cardiac regeneration, into the particles. In vitro release of SDF-1α from core-shell particles was investigated using ELISA kit. Furthermore, cell migration assay was conducted using transwell, to explore the stimulatory effect of released SDF-1α on migration of mesenchymal stem cells (MSC).

Results:

The results of first part of the study using BSA as the model protein showed that, formation of core-shell structures is dependent on solution properties (e.g. concentration, viscosity and electrical conductivity) as well as processing parameters (e.g. shell:core feeding ratio). Spherical core-shell particles were obtained using pure DCM as the shell solvent, while shell:core feeding ratio was between 1.0:0.2 to 1.0:0.1 ml/h. According to XPS results, a higher atomic concentration of N was detected on the surface of particles, when the inner flow rate was increased from 0.1 to 0.2 ml/h, which is an indication of higher amounts of BSA near/on the surface. Further, the optimized condition (6% w/v PLGA dissolved in DCM as the shell, aqueous solution of 1% w/v BSA as the core, at shell:core feeding ratio of 1.0:0.1 ml/h) was employed to encapsulate SDF-1α (SDF-1α was added to the core). SDF-1α is a susceptible low molecular weight cytokine capable of stimulation of homing and engraftment of stem cells into the site of action. The electropsrayed particles exhibited distinct core–shell structure with mean diameter of 4.30±0.75 μm (Fig. 1b). A sustained release of SDF-1α from core-shell particles was obtained for 40 days with about 27% of burst release within the first day. Moreover, cell migration assay confirmed the bioactivity of the released SDF-1α, with more that 50% increase in MSC migration in response to release samples of BSA/SDF-1α incorporated particles.

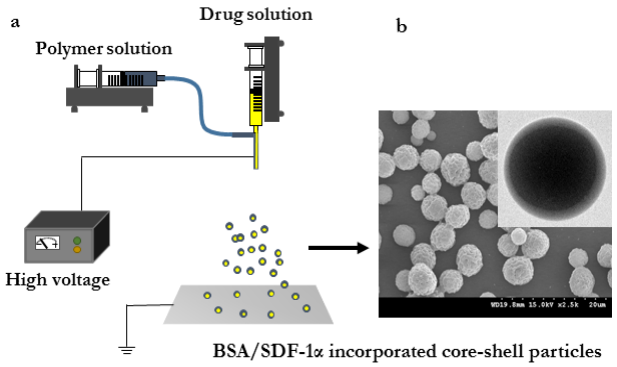

Figure 1. a) Schematic setup of the coaxial electrospraying process b) SEM and TEM images of BSA/ SDF-1α core-shell particles

Conclusions:

The developed technique of coaxial electrospraying was successfully employed to incorporate protein structures into the core-shell particles. The material and processing variables, effective on encapsulation and release of SDF-1α were identified. Prolonged release of SDF-1α was obtained for 40 days, which might be long enough to elicit considerable regeneration, induced by recruited stem cells into the infarcted myocardium. More importantly, released SDF-1α from the particles could drastically stimulate migration of stem cells, which is an indication of the capability of the process to preserve the bioactivity of SDF-1α, due to very limited contact of organic phase with aqueous SDF-1α solution during the process.