Development and *in vitro* biocompatibility evaluation of a microparticle type drug delivery device for controlled delivery of therapeutic proteins using a novel L-tyrosine based polyphosphate

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Statement of Purpose: Proteins are an integral part of the body as they perform important physiological and biological processes such as signaling, biotransformations, transcription, translation, etc[i]. Several disorders are associated with the complete absence of specific proteins within the body or their presence at sub-optimal levels. Under such circumstances, the delivery of proteins may help alleviate disease conditions. Unfortunately, the use of proteins as therapeutic agents is severely restricted by problems associated with their delivery and retention of activity upon delivery. The goal of our research is to develop a microparticle type device capable of providing sustained delivery of therapeutic proteins while preserving their bioactivity. A model protein, FITC-bovine serum albumin was initially investigated for feasibility studies. Microparticles were produced using a water-in-oil-in-water (w/o/w) emulsion system and characterized for surface morphology, size distribution, protein loading and distribution, *in vitro* degradation and protein release studies. Further, microparticles loaded with vasoactive intestinal peptide and aprotinin have been fabricated and characterized successfully. Finally, the biocompatibility of the polymer was evaluated using a live/dead cell assay. Based on our research results we conclude that a device for the controlled release of proteins was successfully fabricated and found to be non-cytotoxic.

Methods, Materials and Analytical Procedures:

L-tyrosine, n-hexanol, thionyl chloride, diethyl ether, phloretic acid, tetrahydrofuran, methylene chloride , 1-ethyl-1'-dimethylaminopropylcarbodiimidehydrochloride (EDC.HCl), phosphate buffer saline (PBS), FITC-bovine serum albumin, aprotinin, vasoactive intestinal peptide, polyvinylpyrrolidone, human dermal fibroblasts, DMEM feeding media, Live/Dead cell assay kit, hydrogen peroxide.

Microparticle fabrication: Blank and protein loaded microparticles were prepared using a w/o/w emulsion technique. Proteins were dissolved at an appropriate concentration and emulsified with a polymer solution (100mg/ml) at 2500 rpm for 1 minute.5% PVP solution was added to this emulsion and the secondary emulsion was prepared by stirring at 1000 rpm for 3 minutes.

Subsequently, the organic solvent evaporation was carried out, the microparticles collected by centrifugation and washed thrice with distilled-deionized water, shell frozen and lyophilized.

Microparticle Characterization: Scanning electron microscopy was used to determine the surface morphology of polyphosphate microparticles. Laser light scattering studies were performed for evaluation of blank and protein loaded particle sizes. Protein loading experiments were performed by dissolving protein loaded

microparticles in chloroform and extracting the protein with distilled deionized water. The protein concentration was determined using fluorescence spectrometry for FITC-BSA loaded microparticles. ELISA will be used to determine the loading of VIP or aprotinin within the microparticles. The protein distribution within the FITC-BSA loaded polymeric microparticles was studied using confocal microscopy. *In vitro* release studies were performed in 0.5% BSA solution in PBS to determine the kinetics of protein release. Finally, human dermal fibroblasts at approximately 90% confluency were used for live dead cell assay. The cells were incubated with poly (DTH-EP) and PLGA microparticles for 1, 3 and 7 days for this study and PBS was used as a positive control.

Results/Discussion: Microparticles show a rough surface morphology along with the presence of some surface defects such as bumps and nodes. Both SEM and laser light scattering results show that protein loaded microparticles (mean size: 3.6μm) are significantly smaller than blank microparticles (mean size: 10.9μm) show a significantly smaller particle. Confocal microscopy results showed the presence of pockets within the core of microparticles which is a characteristic of microparticles fabricated using w/o/w emulsion technique. However, the uniform distribution of proteins within the particles could not be determined conclusively. Results obtained from FITC-BSA loading studies show a high loading efficiency (~90%) for the proteins within the microparticles. The release studies were performed over a period of 7 days. Approximately 70% of the release was observed to occur over a period of 4 days in case of the FITC-BSA release studies. Microparticle degradation studies showed that the particles progressively shrunk with increasing incubation periods and completely degraded over a period of 7 days. This is suggestive of combination of diffusion and degradation as a mechanism of protein release from the microparticles. Finally, live dead cell assay showed no significant differences in human dermal fibroblast cell viability between positive control (PBS alone), PLGA and poly (DTH-EP) microparticles over a period of 7 days.

Conclusions

The experimental results show that a microparticle type device for the controlled release of proteins upto 7 days using poly (DTH-EP) was successfully fabricated.

References

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