**ABSTRACT**

Introduction: Parkinson’s disease (PD) is chronic age-related neurodegenerative disorder, characterized by resting tremor, bradykinesia and rigidity. As current therapeutic strategies for PD are restricted to symptomatic treatment rather than disease-modifying effect, there is critical need to develop novel therapeutics that can stop progressive loss of dopamine neurons.

Methods: Plasmid DNA encoding for enhanced green fluorescent protein (EX-EGFP) or BDNF (pCMV6-BDNF) in transformed E. coli was purified and characterized by restriction enzyme digestion and gel electrophoresis. The plasmids were encapsulated in cationic liposomes and characterized for size, surface charge, encapsulation efficiency, and stability. Cellular uptake studies were performed in NIH-3T3 fibroblast cell line. EX-EGFP and pCMV6-BDNF in control and cationic liposomal formulation was incubated with cells at dose of 20 µg per 200,000 cells for periodic evaluation of transfection by PCR and ELISA.

Results: The isolated plasmids from E. coli were confirmed to have optimum quality and restriction enzyme digestion showed the cDNA corresponding to BDNF. The encapsulation efficiency of both EX-EGFP and pCMV6-BDNF in cationic liposomes was around 80%. After 3 hours of incubation with cells, there was significant uptake and nuclear availability of the EX-EGFP when administered in cationic liposomes. Lastly, Enhanced Green Fluorescent Protein and Brain Derived Neurotrophic Factor expression was maximum at 48 hours post-administration of the plasmids in cationic liposomes.

Conclusions: The preliminary results show that cationic liposomes encapsulating BDNF plasmid can effectively transfect mammalian cells in vitro. Further studies are in progress to evaluate transfection potential of these liposomes in N27 Rat Dopaminergic cell line.

**BACKGROUND**

In Parkinson’s disease, disease progression leads to degeneration and loss of dopamine secreting neurons, especially in the substantia nigra. The Center for Disease Control and Prevention (CDC) rated complications from PD as the 14th top cause of death in the United States. At present the Disease-modifying biological therapeutic molecules, however, have restricted CNS access due to the presence of the blood-brain barrier (BBB). Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that has shown significant activity in protecting dopaminergic neurons and even promoting growth and differentiation.

**GOAL:** To develop an invitro model which illustrates neurogenesis as a result of administration of liposomal formulation encapsulating plasmid DNA encoding for BDNF

**METHOD**

The bacterial culture of transformed E. coli was grown on agar plates and was upscaled to isolate the plasmid encoding for EX-EGFP or pCMV6-BDNF using Qiagen’s Giga kit. These isolated plasmids were characterized by employing restriction enzyme digestion and gel electrophoresis to verify the molecular weight and identity of the plasmid. Plasmid quantity corresponding to 5% of the total lipid were encapsulated in cationic liposomes using the lipids 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), Cholesterol and 1,2- dioleoyl-3-trimethylammonium-propane (DOTAP) in the ratio 5:3:2 and characterized for size and surface charge by using Malvern zetasizer. The encapsulation efficiency, and stability against DNAse was evaluated by using pico green assay. Cellular uptake studies were performed in NIH-3T3 fibroblasts at 15 mins, 30 mins, 60 mins, 3 hr and 6 hr time points. Z-stack analysis was done to understand the localization of plasmid within the cells at 15 mins, 30 mins and 3 hrs.

The EX-EGFP in control and cationic liposomal formulation was incubated with NIH-3T3 at a dose of 20 µg per 200,000 cells for periodic evaluation of transfection by confocal microscopy at 24 and 48 hrs. The pCMV6-BDNF in control and cationic liposomal formulation was incubated with NIH-3T3 at a dose of 20 µg and 40µg per 200,000 cells for periodic evaluation of transfection by Polymerase Chain Reaction (PCR) at 48 hours. The band intensity of sample and controls were compared to understand the level of gene expression.

**RESULTS**

**TABLE 1. Physical characterization of liposomal formulations (n=5) (All the values are expressed in the form of mean +/- S.D.)**

<table>
<thead>
<tr>
<th>Liposomes</th>
<th>Mean Particle Size (nm)</th>
<th>Zeta Potential (mV)</th>
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<tbody>
<tr>
<td>EX-EGFP</td>
<td>182.6 +/- 15.5</td>
<td>28.48 +/- 0.74</td>
</tr>
<tr>
<td>BDNF</td>
<td>203.0 +/- 66</td>
<td>28.3 +/- 1.41</td>
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**TABLE 2. Comparison of encapsulation efficiency of EGFP and BDNF containing liposomes (n=5) (All the values are expressed in the form of mean +/- S.D.)**

<table>
<thead>
<tr>
<th>Liposomes</th>
<th>Encapsulation Efficiency (%)</th>
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<tbody>
<tr>
<td>EX-EGFP</td>
<td>81.2 +/- 2.58</td>
</tr>
<tr>
<td>BDNF</td>
<td>78.4 +/- 1.21</td>
</tr>
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**Figure 3. EX-EGFP plasmid subjected to restriction enzyme digestion followed by Gel Electrophoresis**

**Figure 4. pCMV6 BDNF plasmid subjected to restriction enzyme digestion followed by Gel Electrophoresis**

**Figure 5. Z Stack in NIH3T3 fibroblasts at 15 mins**

**Figure 6. Z Stack in NIH3T3 fibroblasts at 30 mins**

**Figure 7. Z Stack in NIH3T3 fibroblasts at 60 mins**

**Figure 8. Z Stack in NIH3T3 fibroblasts at 3 hrs**

**Figure 9. Cellular uptake studies in NIH3T3 fibroblasts (Images taken on 3X)***

**Figure 10. Transfection studies in NIH3T3 fibroblasts treated with different controls and cationic liposomes encapsulating EGFP plasmid (Images taken 20X)**

**Figure 11. Gene expression profile in different controls and sample at 48 hours**

**Figure 12. Band intensity of different controls and sample at 48 hours**

**IMPACT**

The unique feature about my innovation/research is: This approach provides a novel gene therapy strategy for the treatment of Parkinson’s Disease. This addresses the problem of: Parkinson’s Disease

**REFERENCES**


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