Electrosprayed Dry Powder Inhaler (DPI) Formulations of Bevacizumab for the Treatment of Non-small Cell Lung Cancer (NSCLC)

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Background: Cancer is now the second leading cause of death worldwide, with lung cancer having the highest incidence and mortality rates. Non-small cell lung cancer (NSCLC) accounts for 85% of cases, making it a major research focus. Pulmonary delivery via DPIs provides rapid onset, targeted therapy, and better compliance. Electrospray technology ensures uniform, small particles suitable for pulmonary delivery. Bevacizumab is a first-line FDA-approved monoclonal antibody for NSCLC, targeting vascular endothelial growth factor A (VEGF-A) to inhibit angiogenesis and reduce tumour blood supply. In this work, we sought to prepare inhalable bevacizumab-loaded particles by electrospraying.

Methods: We optimised the formulation by assessing electrospray stability and analysing particle shape and dispersion in scanning electron microscopy images. Promising candidates were further evaluated for pulmonary delivery potential using Mastersizer (volumetric size distribution) and Next Generation Impactor (NGI, aerodynamic size distribution). Bevacizumab distribution was visualised via FITC conjugation under fluorescence microscopy, while its encapsulation efficiency and activity were confirmed using BCA (Bicinchoninic Acid) assays, SDS-PAGE, and ELISA (Enzyme-Linked Immunosorbent Assay). Cellular uptake and cytotoxicity were evaluated in relevant cell lines, and Bevacizumab's anticancer mechanism (angiogenesis inhibition) was investigated through a scratch assay and chorioallantoic membrane (CAM) model.

Results: Eight formulations were obtained, four via coaxial and four via triaxial electrospraying. The optimised formulations showed stability during the process, with particles that were smooth and spherical. Furthermore, six out of the eight formulations demonstrated potential for pulmonary drug delivery, with a D_{50} of <5 μm in the Mastersizer and a fine particle fraction (FPF) of around 50% in the NGI. The encapsulation efficiency of bevacizumab varied across formulations, ranging from 56.97 ± 3.65% to 67.77 ± 8.72%, and the protein could be recovered from the particles without observable aggregation or degradation, as confirmed by SDS-PAGE and ELISA results. The low/non-toxicity of the formulation for the A549 cell line was confirmed by the CellTiter-Glo® 3D Cell Viability Assay and the LIVE/DEAD™ Viability/Cytotoxicity Kit (for mammalian cells). The uptake of the formulation is being investigated in the A549 cell line, while the anti-angiogenic mechanism of the antibody is being evaluated through a scratch assay with HUVEC cells and a chicken fertilised egg-based CAM model.

Conclusions: The potential of optimised dry powder inhaler formulations based on coaxial/triaxial electrospray technology, for the pulmonary delivery of bevacizumab was demonstrated. In *vitro* analysis confirmed the volumetric and aerosol properties of the formulations, alongside their functional performance.