**Toxicity Evaluation of Electrostatically Charged Aerosols for Chronic Rhinosinusitis Therapy: An *In-Vitro* Study**

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**Background and aims.** Chronic rhinosinusitis (CRS) is a persistent inflammatory condition affecting the nasal passages and paranasal sinuses, typically lasting for at least 12 weeks [1]. Recent studies have highlighted the potential of electrostatics to enhance drug delivery within the nasal cavity [2-5]. However, there are currently no reports evaluating the biological effects of electrostatically charged aerosols on nasal epithelial cells, which is essential to support the safety of this approach. This study addresses this gap by investigating the cytotoxic effects of charged aerosols containing clinically relevant drugs on human nasal epithelial cells.

**Methods.** Charged aerosols were generated from aqueous drug solutions using a vibrating mesh nebuliser (Tekceleo, France), equipped with a ring electrode and a grounded electrode, mounted via a 3D-printed holder. To assess safety, 5 mL of isotonic saline was nebulised with and without applying a 5 kV charge to the ring electrode. The aerosol was delivered onto human nasal epithelial cells (HNECs), obtained from three CRS patients. Cells were then incubated with the treatments for 30 minutes, reflecting the typical mucociliary clearance time *in vivo*, before being washed and incubated overnight in fresh media. Cytotoxicity was assessed using an LDH assay, comparing charged and non-charged saline with negative controls. Tobramycin and mupirocin were selected as two commonly used medications for the treatment of chronic rhinosinusitis (CRS). The concentrations evaluated were based on the minimum biofilm eradication concentration (MBEC) against Staphylococcus aureus.

**Results.** As shown in Figure 1, the results indicate no significant damage or reduction in cell viability associated with the charging mechanism, supporting the safety of the applied electrostatic approach. This trend is supported by LDH assay analysis results.



Figure 1. LDH assay analysis results for charged and uncharged nebulized saline, mupirocin and tobramycin.

**Conclusion/Discussion.** This study demonstrates the biological safety of an electrostatic method for enhancing drug delivery to the paranasal sinuses in the treatment of CRS.

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