# The barrier-forming abilities of a sodium hyaluronate formulation delivered using the PillHaler® DPI device for protection against urban dust

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## INTRODUCTION

- The direct exposure of the airway epithelium to environmental hazards (smoke, dust, silica, and other pathogens) can result in infection and inflammation and the development of chronic respiratory diseases [1,2].
- Sodium hyaluronate is a well characterised ingredient known to form hydrogels in aqueous solutions [3].
- An inhalable formulation (PolmonYDEFENCE / DYFESA) targeting the upper respiratory tract, with sodium hyaluronate as the key ingredient (KI) has been developed which has the potential to form a psychical hydrogel barrier to protect the airway epithelium from direct exposure to environmental hazards [4].

## AIM & OBJECTIVE

To conduct biological characterisation of the sodium hyaluronate formulation to assess its potential barrier protective abilities.

## METHODS

- **Cell viability:** To determine if the formulation causes any cytotoxicity using calorimetric MTS assay to measure metabolic activity of the cells in 96-well plate format using Calu-3 cells.
- **Reactive oxygen species (ROS) assays:** To evaluate the anti-oxidant properties of the sodium hyaluronate formulation using fluorescent H2DCFDA assay with Calu-3 cells in a 96-well plate format with urban dust (UD) as the inducer of oxidative stress.
- **ELISA assay:** To assess the anti-inflammatory properties of the formulation, ELISA assay measuring levels of interleukin 6 (IL-6) was used in the air-liquid interface (ALI) culture using Transwell plate format with UD as the inducer of inflammation.
- **Sodium** Fluoresceine permeability assay: To determine if the formulation has any effect on functionality of tight junctions, paracellular permeability of sodium fluoresceine was used in ALI culture using Transwell plate format.
- **Alcian blue stain:** To assess mucus production, epithelial layers (ALI culture) were fixed and glycoproteins in mucus was stained with Alcian blue dye.

## RESULTS

- Oxidative stress on Calu-3 cells induced by UD is reduced by co-incubation for 2 hours with the sodium hyaluronate formulation.
- No significant changes in cell viability was observed, indicating that the formulation was not cytotoxic to Calu-3 for up to 2h exposure (Fig.1).

### ROS ASSAY ON CALU-3 CELLS

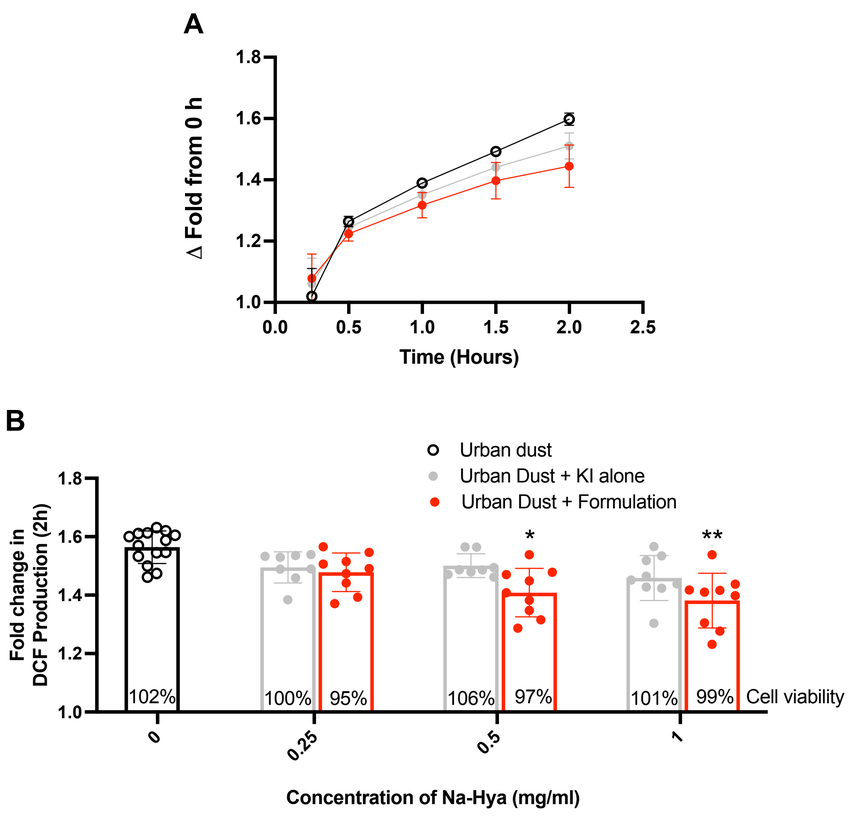


**Figure 1**. Co-incubation of oxidative stress with UD and sodium hyaluronate on Calu-3 cells (A) Changes in oxidative stress over a 2h time course 0.5mg/ml concentration of the key ingredient (KI). (B) Concentration-dependent effect on oxidative stress at 2h co-exposure. Statistical significance was calculated by comparing urban dust only to every other condition. An ordinary One-Way ANOVA with Sidak's multiple comparisons test was used (*p<0.033; **p<0.0021; ***p<0.0002; ****p<0.0001

- Inflammation of the Calu-3 epithelia was induced by exposure to UD (Table 1) and detected by measuring levels of IL-6 and was found to be 2.2-fold higher than media alone.
- Increased amounts of the key ingredient alone as well as in formulation was shown to reduce the secreted IL6.
- The barrier layer of the formulation compared to the KI alone was shown to be more effective in reducing UD induced inflammation on Calu-3 epithelia.

### ELISA IL-6 ASSAY WITH CALU-3 CELLS

| | Secreted Il-6 (pg/ml) | SD |
|---|---|---|
| Media | 57.05 | 13.15 |
| Vehicle Control | 60.87 | 8.42 |
| Urban Dust | 135.97 | 16.14 |
| Urban Dust + 6.25 ug KI | 127.76 | 18.92 |
| Urban Dust + 12.5 ug KI | 115.95 | 20.34 |
| Urban Dust + 25 ug KI | 105.34 | 12.42 |
| Urban Dust + 6.25 ug Formulation | 112.17 | 11.37 |
| Urban Dust + 12.5 ug Formulation | 108.73 | 15.89 |
| Urban Dust + 25 ug Formulation | 87.82 | 24.75 |

**Table 1**. Quantitation of secreted IL-6 from Calu-3 epithelium (n=3, blue – red = low – high).

- No changes in the mucus production of the Calu-3 epithelium was observed up to 24h exposure to the formulation
- The permeability of the epithelial layer was unaffected for up to 6h with the formulation and was significantly lower at 24h compared to epithelia without any treatment (Fig. 2).

### SODIUM FLOURESCINE ASSAY AND MUCUS STAINING OF CALU-3 CELLS

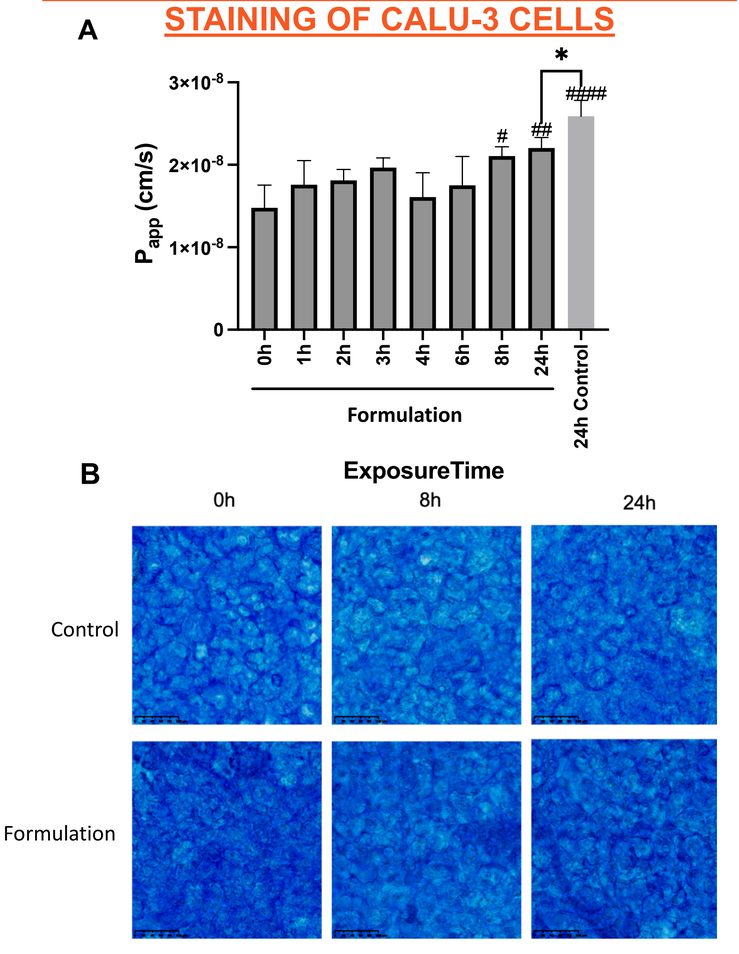


**Figure 2**. (A) Permeability coefficient (Papp) measurements epithelium exposed to the formulation over a 24h time course. Statistical significance was calculated using One-Way ANOVA with Dunnett's multiple comparisons test (#p<0.033; ##p<0.0021; ####p<0.0001) and student t-test (* p<0.05). (B) Images of Alcian blue mucus staining of Calu-3 cells grown in ALI model ± formulation.

## CONCLUSIONS

- The sodium hyaluronate formulation reduced direct contact of the epithelial layer to UD, decreasing oxidative stress and inflammation.
- The formulation was not cytotoxic in the in the airway epithelial model cells used during the 2h exposure.
- Epithelial permeability and mucus production was unaffected by the barrier layer for up to 24h exposure.
- The formulation can be used as a localized physical barrier for the upper respiratory tract to protect against external harmful pollutants.

## REFERENCES

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