MucilAir™, an *In Vitro* Model for Assessment of Human Airway Toxicity, Evaluated using Model Irritant Sodium Dodecyl Sulfate (SDS)

A Dickie[1,2,3], C Roper[1], S Constant[4], S Huang[4], J Welch[1]
[1] Charles River, Department of *In Vitro* Sciences, Tranent, Edinburgh, EH33 2NE, UK [2] Scottish Universities Life Sciences Alliance (SULSA), Mayfield Road, Edinburgh, EH9 3JR
[3] University of Edinburgh, School of Biological Sciences, Mayfield Road, Edinburgh, EH9 3JR [4] Epithelix Sàrl, 14 Chemin des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland

Introduction

MucilAir™ is an *in vitro* airway model with morphology and functions mirroring the tracheo-bronchial epithelium. It has potential applications in inhalation toxicity testing and pharmaceutical lead development, where it may be used to identify potential airway toxicants and facilitate *in vivo* dose range finding.

MucilAir™ was evaluated for use in predicting upper airway toxicity. Tissues were treated with increasing concentrations of sodium dodecyl sulphate (SDS). Monolayer integrity (trans-epithelial electrical resistance; TEER), membrane integrity (lactate dehydrogenase (LDH) release), metabolic competence (resazurin metabolism) and inflammatory mediator (IL-8) release were measured. Histology and scanning electron microscopy (SEM) were used to assess morphology.

Materials and Methods

MucilAir™ units comprise cells derived from human airway biopsies, cultured at the air interface on permeable membranes by Epithelix Sàrl. Units were obtained from 3 donors (1 unit per dose level from Donor 1; 2 units per dose level from Donors 2 and 3). SDS was obtained from Sigma-Aldrich, Dorset, UK.

MucilAir™ was treated with SDS in saline solution (0-10 mM) for 24 h (37°C; humidified 5% CO_2 atmosphere) and maintained in culture until 168 h post-dose. TEER was measured using a Millicell® ERS meter at 0 h (predose) and 24 h and 168 h post dose.

At 0, 24 and 168 h, LDH and IL-8 release was measured in culture media. LDH was measured using Promega CytoTox ONE™ Homogeneous Membrane Integrity Assays and IL-8 was measured using R&D Quantikine® ELISA Human CXCL8/IL-8 Immunoassays. At 168 h, units were incubated with resazurin solution for 1 h (37°C; humidified 5% CO_2 atmosphere) then sample aliquots were assessed for the presence of the metabolite, resorufin, by measuring fluorescence ($544_{em}/590_{em}$).

MucilAir™ was fixed at 168 h post-dose in glutaraldehyde buffer (pH 7.4) for 22 h at 5°C then rinsed for 30 min in sodium cacodylate buffer (pH 7.4) and for 5 min in ultrapure water. Samples were dehydrated through ethanol, submerged in hexamethyldisilazane for 10 min, air dried and stored in a dessicator until analysis. Samples were then mounted on aluminium stubs, gold sputter-coated and viewed using a Philips XL30CP Scanning Electron Microscope.

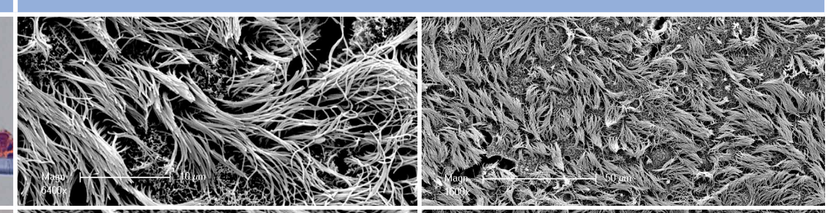
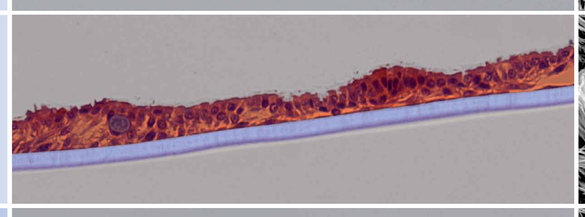
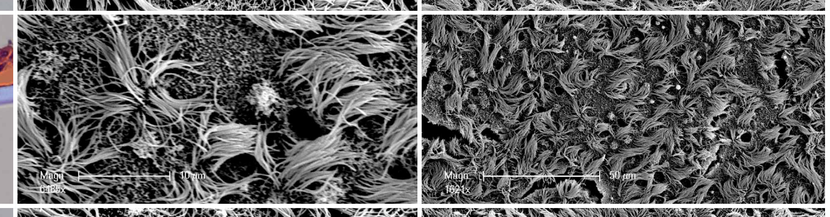
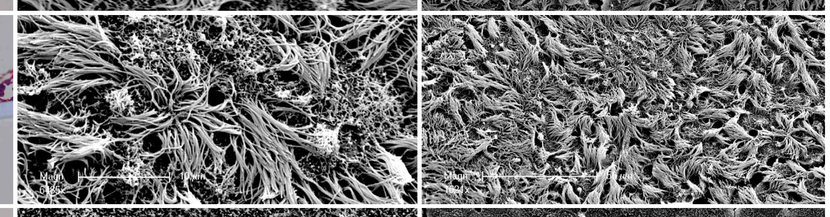
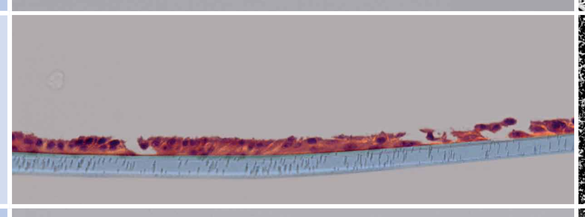
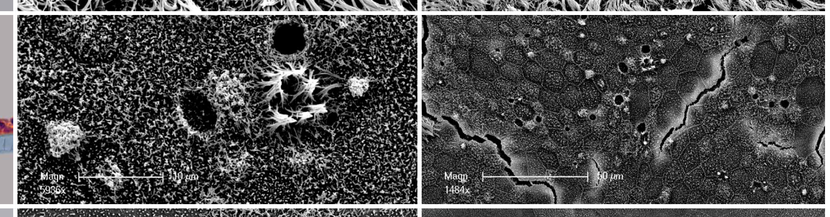
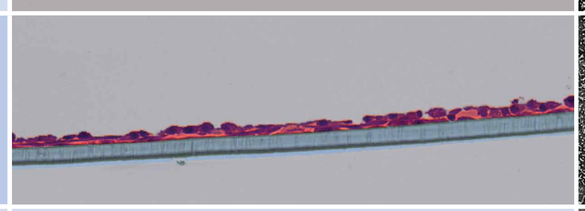
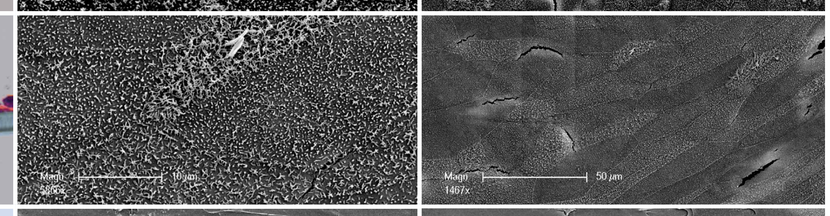
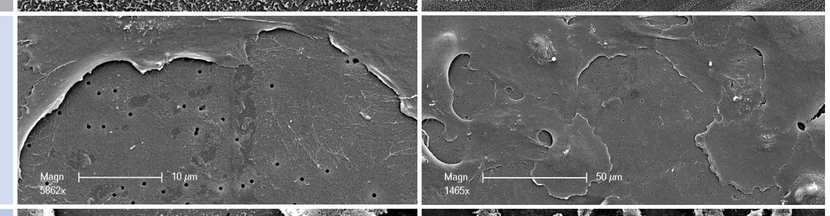
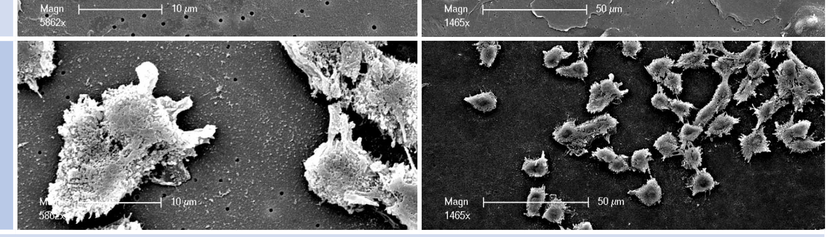
MucilAir™ was fixed at 168 h post-dose in neutral buffered formalin, embedded in paraffin, sectioned, mounted, stained with haematoxalin-eosin and visualised using a Leica DM-2500 light microscope.

Results

MucilAir™ displayed dose-dependent responses to SDS treatment as assessed by morphological appearance and membrane integrity (LDH release), monolayer integrity (TEER) and cytokine (IL-8) release.

After 24 h SDS exposure, the most sensitive toxicity indicator was histology (Figure 1), revealing damage at ≥0.6 mM SDS. SEM imaging, TEER and IL-8 release measurements (Figures 1-3, respectively) indicated toxicity at ≥1.25 mM SDS and LDH release at ≥ 2.5 mM (Figure 4). Extensive cell damage may explain the reduced IL-8 and LDH release at 5-10 mM SDS. At 168 h metabolic activity was compromised in units dosed at ≥5 mM SDS (Figure 5).

Although histology demonstrated that pseudo-stratified morphology did not recover, recovery of basic cellular functions was observed in moderately damaged units (1.25-2.5 mM SDS) at 168 h post dose. Predose levels of TEER and LDH and IL-8 release were consistent within and between donors. Dose-response patterns at 24 h and 168 h were broadly comparable, with IL-8 release patterns the most consistent.

SDS (mM)	Cross Sectional Histology (400x)	Surface Morphology
0.00		
0.31		
0.63		
1.25		
2.50		
5.00	N/A	
10.00	N/A	

N/A: Samples too damaged from test item treatment therefore sections could not be mounted.

Figure 1. Effect of [SDS] on MucilAir™ Morphology as Assessed by Histology and SEM

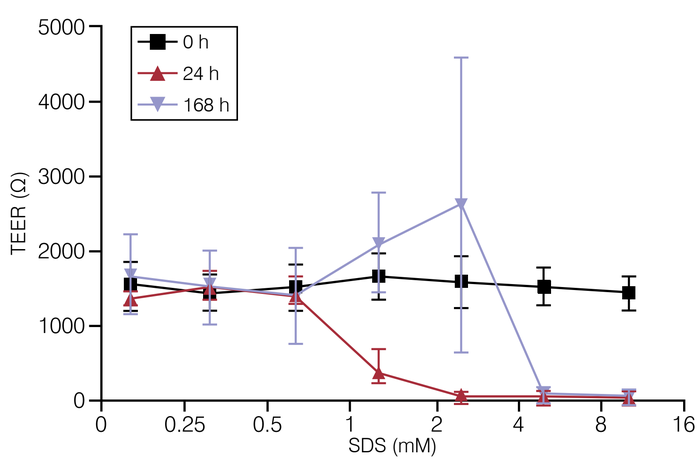


Figure 2. Effect of [SDS] on MucilAir™ Monolayer Integrity

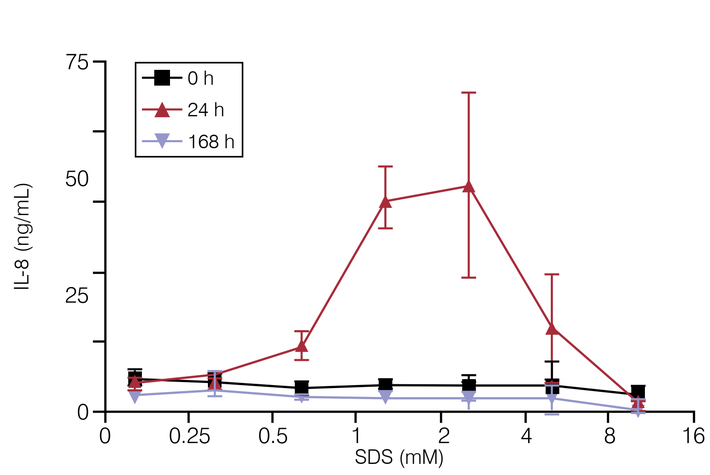


Figure 3. Effect of [SDS] on MucilAir™ IL-8 Release

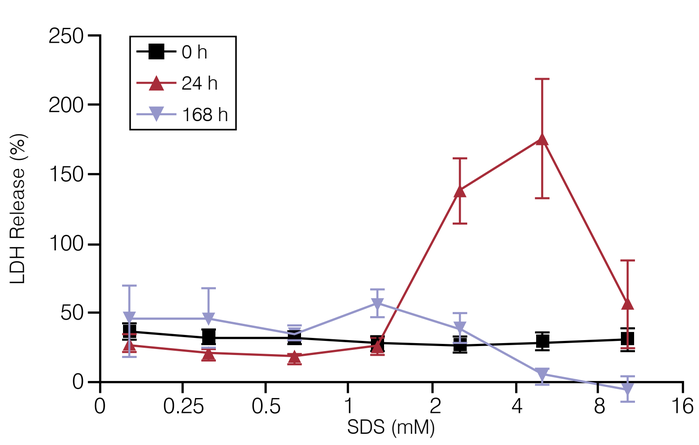


Figure 4. Effect of [SDS] on MucilAir™ Membrane Integrity (% Maximal Damage)

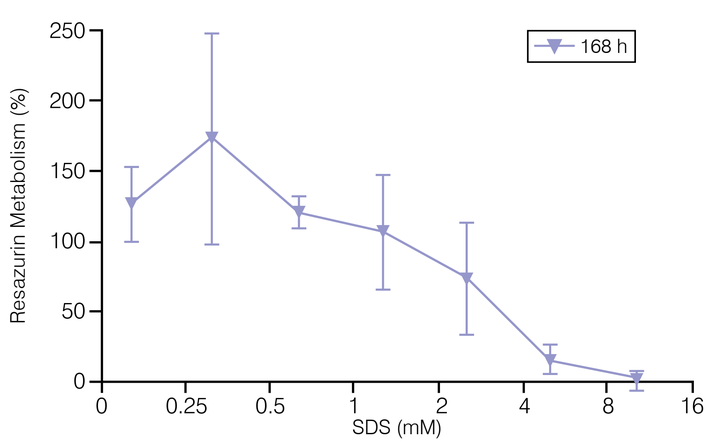


Figure 5. Effect of [SDS] on MucilAir™ Metabolic Activity (% Metabolism vs Untreated Units)

Conclusion

In conclusion, MucilAir™ displayed dose-dependent responses when treated with SDS, with cytotoxicity visualised by histology at ≥0.6 mM and measured chemically at ≥1.25 mM. Monolayer integrity (TEER) and marker release (LDH and IL-8) patterns were comparable between equivalently dosed units. These data support the use of MucilAir™ as a relevant model for airway toxicity studies.

Acknowledgements

The authors acknowledge the assistance of Dr. Nicola Cayzer (University of Edinburgh) with SEM analysis.