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# Confocal microscopy 3D imaging and bioreactivity of La Palma volcanic ash particles

David Wertheim [a,*], Beverley Coldwell [b,c], Lisa Miyashita [d], Ian Gill [a], Simon Crust [a], Richard Giddens [a], Nemesio M. Pérez [c], Nick Petford [e], Jonathan Grigg [d]

[a] Faculty of Engineering, Computing and the Environment, Kingston University, Surrey KT1 2EE, UK
[b] Instituto Tecnológico y de Energías Renovables, Granadilla de Abona 38600, Canary Islands, Spain
[c] Instituto Volcanológico de Canarias (INVOLCAN), Tenerife, Canary Islands, Spain
[d] Centre for Genomics and Child Health, Blizard Institute, Queen Mary University of London E1 2AT, London, UK
[e] Department of Earth Sciences, Durham University, DH1 3LE, UK

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ABSTRACT

In September 2021 an eruption began of Cumbre Vieja, La Palma (Spain) that lasted 3 months. Previous studies have shown that volcanic ash particles can be associated with adverse effects on human health however, the reasons for this are unclear. Particle shape has been shown to contribute to cellular uptake in prostate cancer cells. Hence we aimed to study 3D structure, elemental composition and effects on cultured lung cells of particles collected from the La Palma volcanic eruption. 3D imaging of $PM_{10}$ sized and below particles was performed using a LEXT OLS4100 confocal microscope (Olympus Corporation, Japan). A Zeiss EVO 50 (Carl Zeiss AG, Germany) Scanning Electron Microscope (SEM) was used to assess elemental composition. In addition, volcanic particle concentration dose response for *pneumococcal adhesion* to A549 human alveolar epithelial cells was investigated. Confocal microscopy showed that some $PM_{10}$ and below sized particles had sharp or angular 3D appearance. SEM x-ray analysis indicated silicate particles with calcium, aluminium and iron. We observed increased colony forming units indicating increased Pneumococcal adhesion due to exposure of cells to volcanic particles. Thus in addition to the toxic nature of some volcanic particles, we suggest that the observed sharp surface particle features may help to explain adverse health effects associated with volcanic eruptions.

## 1. Introduction

Volcanic ash particles can cause widespread environmental disruption that has deleterious effects on for example vegetation, animal and human health as well as on infrastructure and aeroplanes (Hufford et al., 2000; Horwell and Baxter, 2006; Ayris and Delmelle, 2012; Lombardo et al., 2013; Clarkson et al., 2016; Tesone et al., 2018; Wygel et al., 2019; Stewart et al., 2022; Nogales et al., 2022). In addition to volcanic ash particles, volcanic gas emissions can also affect human health (Hansell and Oppenheimer, 2004). Volcanic particles are primarily aluminium and iron silicates, and silicate glass (Bukowiecki et al., 2011; Gislason et al., 2011; Jones and Bérubé, 2011; Horwell et al., 2012; Horwell et al., 2013; Damby et al., 2017; Wygel et al., 2019).

Particles of small size and volcanic gases such as $SO_2$ can travel hundreds of kilometres in the atmosphere thus being deposited far from the original eruption (Bukowiecki et al., 2011; Stevenson et al., 2013; Stevenson et al., 2015; Filonchyk et al., 2022; Milford et al., 2023). When breathing through the nose, it is particles that are of small size $PM_{10}$ and particularly $PM_{2.5}$ that are of most concern for health as it is thought that they are more likely to be absorbed in the airways and lungs (Buist et al., 1986; Brown et al., 2013; Chen et al., 2019); particles are also associated with other potential adverse health concerns such as eye conditions (Lombardo et al., 2013). Bacterial replication *in vitro* has been observed to increase with volcanic particles (Monick et al., 2013). In a cohort study of long-term health effects associated with the 2010 eruption of the Eyjafjallajökull volcano in Iceland, for example the prevalence of wheezing symptoms was increased in the group who had lived close to the volcano (Hlodversdottir et al., 2016).

The structure of volcanic ash particles has mostly been investigated with 2D microscopic techniques, for example brightfield microscopy or more complex techniques such as scanning electron microscopy (SEM) (Veronesi et al., 2002; Bukowiecki et al., 2011; Damby et al., 2017). An

* Corresponding author.
*E-mail address:* D.Wertheim@kingston.ac.uk (D. Wertheim).

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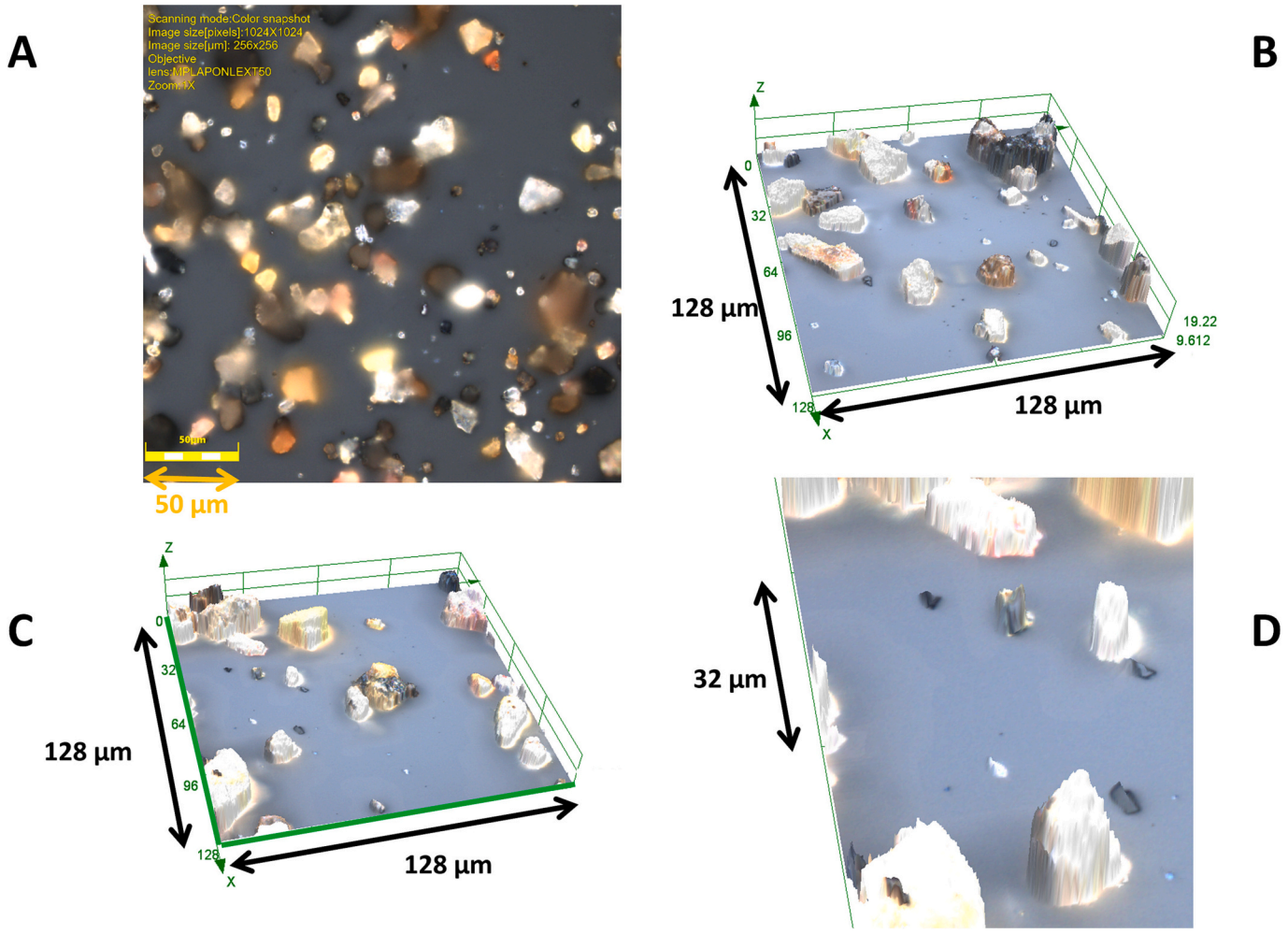



**Fig. 1.** Example of confocal microscope images of volcanic particles. Fig. 1A, 2D image with x50 objective lens, field 256 × 256 µm. Fig. 1B, 3D visualisation of image field 128 × 128 µm, maximum height 19 µm from confocal microscopy using a x100 objective lens. A 3D visualisation of image field 128 × 128 µm, maximum height 19 µm from confocal microscopy using a x100 objective lens and close-ups of some $PM_{10}$ particles with sharp appearing surfaces in figs. C and D. For the image in D, the z axis is magnified by 2 to show the 3D surface appearance more clearly.

angular appearance in some volcanic particles has been noted from SEM imaging (Damby et al., 2017). We have previously described a method using confocal microscopy to assess the structure of such particles in 3D as this helps to better elucidate the nature and colour of the surface in which some particles have a sharp or jagged appearance (Wertheim et al., 2017). The confocal microscope approach has also recently been applied to study the 3D structure of Diesel Particulate Matter (DPM), as well as roadside and underground railway train station Particulate Matter (Miyashita et al., 2021; Wertheim et al., 2023); using this confocal microscopy technique a true colour image can be superimposed on the 3D surface which can help in understanding the visualisation of the particle's 3D shape. The structure and shape of particles is likely to be important as it has been shown to for example affect surfactant film spreading in lungs (Gerber et al., 2006), drug delivery (Minchinton and Tannock, 2006; Champion et al., 2007; Zellnitz et al., 2019; Shachar-Berman et al., 2020) and influence their ability to attach to cancer cells (He and Park, 2016).

Inhalation of volcanic ash particles is considered to be associated with increased incidence of adverse health conditions affecting for example respiratory and cardiac function (Buist et al., 1986; Bernstein et al., 1986; Gudmundsson, 2011; Lombardo et al., 2013; Carlsen et al., 2015; Tam et al., 2016; Mueller et al., 2020; Michellier et al., 2020; Carlsen et al., 2021). For example, the prevalence of exercise-induced bronchoconstriction has been reported to be four times higher in children exposed to high levels of volcanic particles compared with those exposed to low levels (Forbes et al., 2003). However, the mechanism of action of volcanic particles on health is poorly understood.

The September 2021 eruption of Cumbre Vieja, La Palma (Spain), occurred in a populated area and lasted 3 months. As well as constant lava output from at least one main vent, 2 or more vents at the top of the cone continually emitted tephra, ash and gas to form a plume. The average plume height was 3 km, varying between 1.2 and 6 km (PEVOLCA, 2021). The prevailing wind direction caused the thickest tephra and ash build-up on the southwestern side of the cone, and after 2 months of the eruption around 6630 ha of the Aridane valley (population 20,000) was covered by deposits 0.1-3 m thick (Copernicus EMSR546, 2021). Ash was deposited across the island, including in the capital city Santa Cruz de La Palma, and regularly caused the closure of the island airport. In Los Llanos, a large town that became a centre for those displaced by lava flows and the emergency services, air quality levels of $PM_{10}$ were Unfavourable´ or worse (Índice de Calidad del Aire [ICA], 2019) for 33 days of the eruption, while $PM_{2.5}$ levels were Unfavourable´ or worse for 16 days of the eruption (GOBCAN, 2021); these levels signify risk for all sections of the population. The first week of November 2021 saw four days of the highest warning Extremely Unfavourable´ (grave risk) level, which together with sulphur dioxide levels, led local authorities to close schools and businesses (PEVOLCA, 2021). Ash reached neighbouring islands including La Gomera, Tenerife

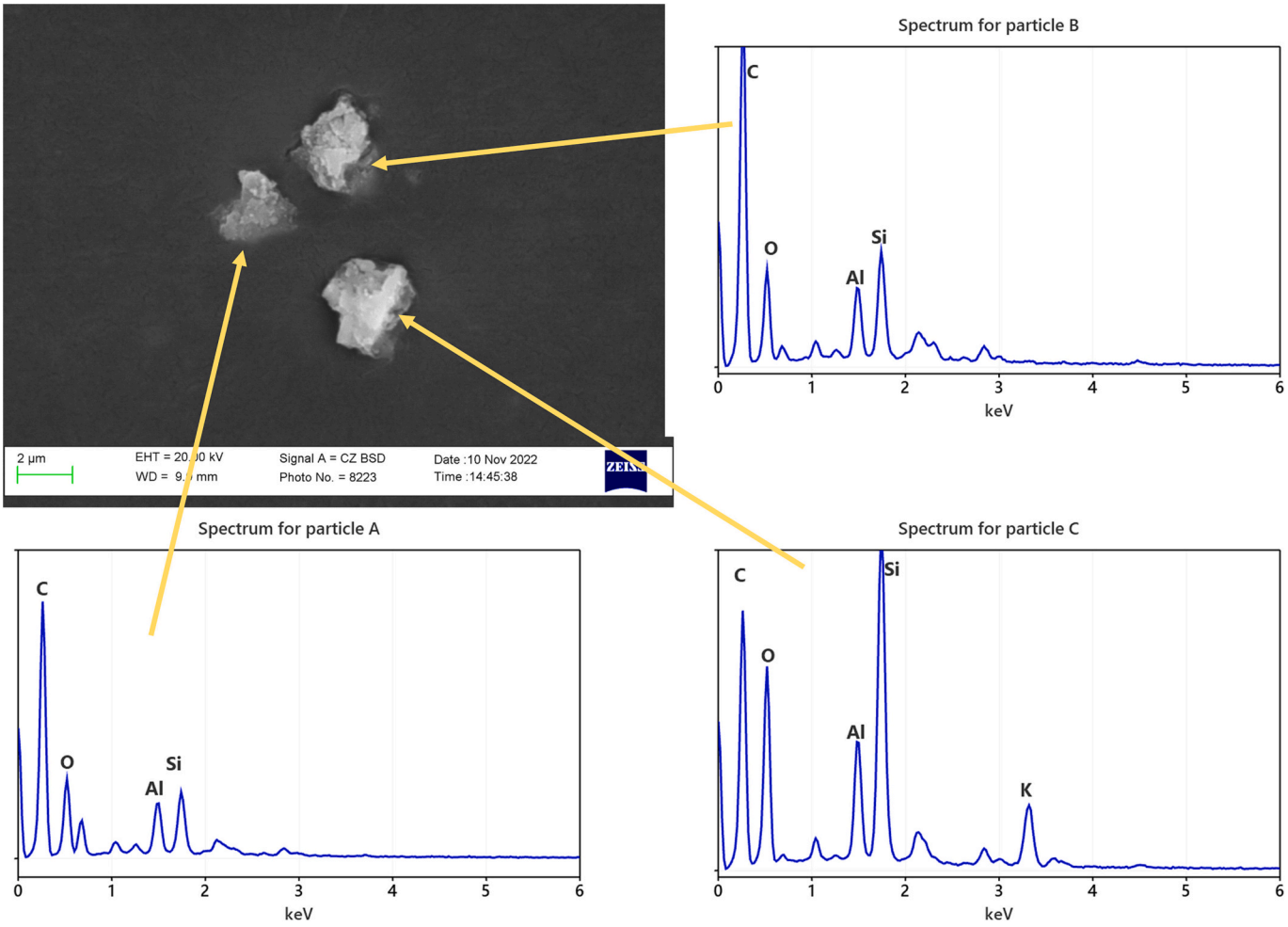



**Fig. 2.** Example of SEM backscattered electron image of 3 $PM_{10}$ particles and corresponding energy dispersive x-ray (EDX) microanalysis spectra.

and Gran Canaria. The quantity of ash and tephra emitted classified the eruption at VEI 3 (Nogales et al., 2022) (10 million $m^3$ of tephra released), with ash particles now regularly remobilised by strong winds.

The aims of this study were thus to examine the 3D structure, elemental composition and effects on cultured lung cells of La Palma volcanic ash particles.

## 2. Materials and methods

### 2.1. Volcanic ash particle acquisition

Volcanic ash particles were collected from the opening phase of the Cumbre Vieja 2021 eruption, on 19th September 2021. Particles were collected by airfall using a wide-mouth (10 cm) high-density polyethylene (HDPE) bottle over a period of 1 h. Samples were collected at a distance of 3 km from the initial volcanic vents, in the former town of Todoque. Todoque was under the prevailing wind direction for La Palma and was receiving steady tephra-fall, yet was outside the zone of larger particles falling (>1 cm, which dominantly occurred within 2 km of the vent at that time). Therefore samples could be considered representative of steady tephra flux to the local population at that time.

### 2.2. Confocal microscope slide preparation and imaging

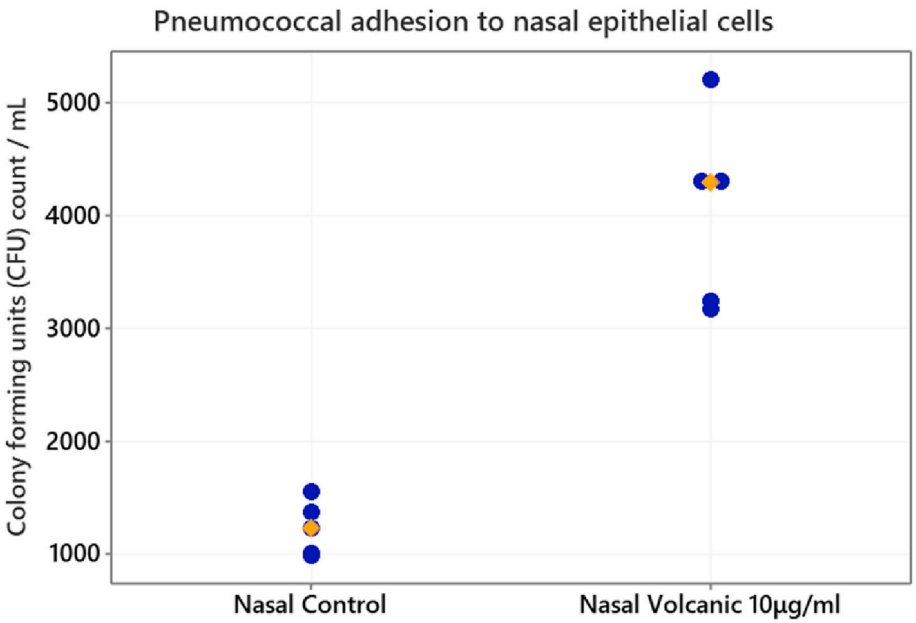
A drop of propan-2-ol was placed on a glass slide in a petri-dish. Grains of the collected aggregated volcanic particles were sprinkled through a 20 µm gauze filter onto the propan-2-ol droplet on the slide. The droplet containing the particles in suspension dispersed across the surface of the glass slide and the propan-2-ol was allowed to evaporate naturally for 20 min at room temperature thus leaving just the particles on the glass slide.

A LEXT OLS4100 confocal microscope (Olympus Corporation, Japan) was used to image volcanic particles on the glass slide using a similar approach to that previously described (Wertheim et al., 2017; Miyashita et al., 2021; Wertheim et al., 2023); for 3D imaging a x50 or x100 dry objective lens was used both of which have a numerical aperture of 0.95. The scanning acquisition range took account of the working distance of the lens and was set at just below the slide surface to just above the highest particle level in order to obtain the full height of the particles in the image field. The scanning was performed in fine mode setting with images acquired with a resolution of 1024 × 1024. Particle size measurements were made with the Olympus microscope software.

### 2.3. Scanning electron microscope slide preparation and imaging and spectroscopy

Volcanic particles in propan-2-ol were separated using an ultrasound bath for 5 min. One drop of the solution on a glass cover slip, mounted on a specimen stub, was allowed to air dry overnight and subsequently coated with a gold / palladium alloy.

A Zeiss EVO 50 (Carl Zeiss AG, Germany) Scanning Electron Microscope (SEM) in both the secondary and backscattered mode was used to examine the slide. Bright phases seen on Backscattered Electron (BSE)



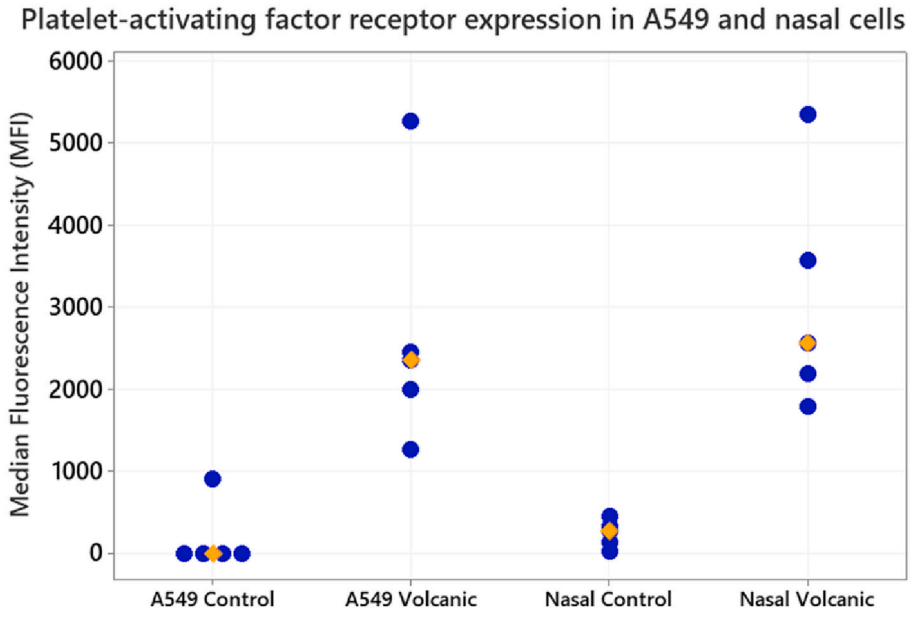




**Fig. 3.** The upper graphs shows individual value plots of Pneumococcal adhesion in human primary nasal epithelial cells with volcanic particles added compared with cells without volcanic particles expressed as Colony forming units (CFU) count / mL . The lower graphs shows Median Fluorescence Intensity (MFI) of platelet-activating factor receptor expression in the A549 and nasal epithelial cells with volcanic particles added compared with cells without volcanic particles. Each round blue dot shows one measurement with the median being shown with an orange diamond symbol.

imaging were then assessed qualitatively with energy dispersive x-ray (EDX) microanalysis.

### 2.4. Cell investigations

Volcanic particles were filtered using a mesh (10 μm) and suspended in Dulbecco's phosphate-buffered saline (DPBS). Aliquots of volcanic particles were diluted in DPBS to a final concentration of 1 mg/mL and stored as a master stock at −20 °C.

### 2.5. Airway cells

Cells from an A549, human alveolar type II epithelial cell line (Sigma- Aldrich, Poole, UK) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and penicillin-streptomycin (Lonza, Basel, Switzerland). Human primary nasal epithelial cells (HPNEpC) from PromoCell® (Heidelberg, Germany) were maintained in airway epithelial cell growth medium, with supplement kit, Primocin (InvivoGen, San Diego, USA), and 10 % FBS; the Passage number was <5.

### 2.6. Platelet-activating factor receptor (PAFR) expression

Airway cells were seeded overnight into adherent cell culture plates ($2 \times 10^5$ cells per well) and cultured with volcanic particles for 2 h before washing and detaching with trypsin. Cells were stained with an anti-PAFR primary antibody (1:200; ab104162 Abcam, Cambridge, UK) for 1 h with shaking at room temperature. A PAFR isotype control (1:200; ab172730, Abcam, Cambridge, UK was included to control for nonspecific staining. The epithelial marker E-cadherin was included in all assays (1:100; ab1416, Abcam, Cambridge, UK). Cells were subsequently washed and stained with secondary antibodies conjugated to

**Table 1**
PAFR and pneumococcal adhesion for A549 human alveolar cells and human primary nasal epithelial cells with and without addition of volcanic particles.

| | | | Median | Min | Max | N |
|---|---|---|---|---|---|---|
| PAFR (MFI) | A549 | Control | 0 | 0 | 907 | 5 |
| | | Volcanic | 2356 | 1270 | 5255 | 5 |
| | Nasal | Control | 276 | 25 | 457 | 5 |
| | | Volcanic | 2557 | 1789 | 5337 | 5 |
| Adhesion (CFU / mL) | A549 | Control | 1233 | 500 | 2233 | 6 |
| | | Volcanic | 4050 | 3000 | 5167 | 6 |
| | Nasal | Control | 1227 | 989 | 1554 | 5 |
| | | Volcanic | 4300 | 3166 | 5200 | 5 |

Median, minimum and maximum of PAFR expression (median fluorescence intensity, MFI) in both A549 and nasal epithelial cells (HPNEpC) when comparing with cells with and without addition of volcanic particles. Lower part of table has median, minimum and maximum pneumococcal adhesion (CFU count / mL) to both A549 human alveolar cells and human primary nasal epithelial cells when comparing with cells with and without volcanic particles. The concentration of volcanic particles was 10 μg/mL for the data in this table.

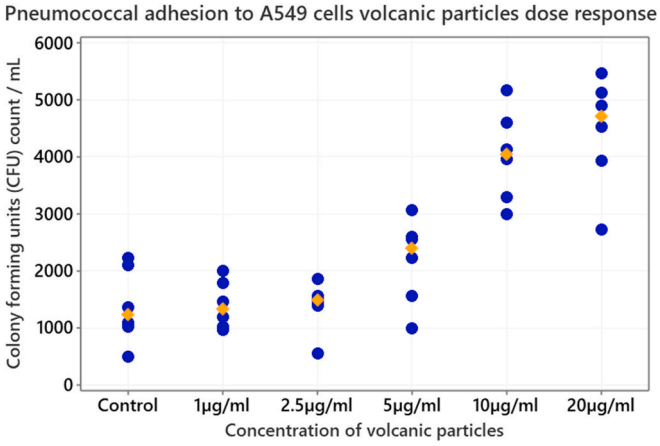



**Fig. 4.** Dose dependent individual value plots of Pneumococcal adhesion in A549 human alveolar cells with volcanic particles added compared with cells without volcanic particles expressed as Colony forming units (CFU) count / mL . Each round blue dot shows one measurement with the median being shown with an orange diamond symbol.

either Alexa Fluor 488 (1:3000; ab150077, Abcam, Cambridge, UK) for detection of PAFR/isotype expression, or allophycocyanin (1:1500; ab130786, Abcam, Cambridge, UK) for detection of E-cadherin. Analysis was carried out on the BD Fluorescence-Activated Cell Sorting (FACS) Canto II system using BD FACSDiva software (BD Biosciences, Oxford, UK). PAFR expression was compared to an isotopic control for median fluorescent intensity (MFI).

### 2.7. Pneumococcal adhesion

The *Streptococcus pneumoniae* type 2 encapsulated strain D39 (NCTC 7466) was from the National Collection of Type Cultures (Central Public Health Laboratory, London, UK), grown to mid-logarithmic phase ($OD_{600}$ = 0.4 to 0.6) in brain–heart infusion broth (BHI) (Oxoid, Basingstoke, UK) and stored at −80 °C. Airway epithelial cells were seeded overnight into adherent cell culture plates ($2 \times 10^5$ cells per well) and exposed to volcanic particles for 2 h. Cells were subsequently washed to remove particles before adding *S. pneumoniae* D39 for a further 2 h to allow adhesion. Cells were finally washed to remove non-adherent bacteria and lysed before plating on blood agar plates for colony forming unit count (CFU/mL).

### 2.8. Data analysis

Data were analysed using Excel (Microsoft Corporation, USA) and Minitab v19 (Minitab LLC, USA); graphs were prepared with Minitab. The Ryan-Joiner test in Minitab was used to assess normality and data analysed accordingly.

## 3. Results and discussion

### 3.1. 3D microscopy imaging

Confocal microscope imaging demonstrated a range of volcanic particle sizes including $PM_{2.5}$, $PM_{10}$ and above; the particles were of different shapes and colours as seen in the example in Fig. 1. The true colour images demonstrated particles corresponding to $PM_{2.5}$ and $PM_{10}$ frequently had edges with a sharp or jagged appearance as shown in the examples in Fig. 1b, c and d. The sharp appearing particles were seen particularly in the $PM_{10}$ and below particles. Other particles had a crystalline surface appearance.

### 3.2. Scanning electron microscopy

Scanning electron microscopy (SEM) showed image shapes that were consistent with that seen from the confocal microscope images. SEM x-ray analysis indicated the presence primarily of silicon, calcium, oxygen, aluminium and iron as shown in the 2D spectrum of a $PM_{10}$ particle in Fig. 2. These particles are thus likely to be silcate glasses, amphiboles and pyroxenes.

### 3.3. Cell investigations

For each set of experiments at least five separate measurements were taken as shown in the individual value plots in Fig. 3. Volcanic particles increased pneumococcal adhesion to human primary nasal epithelial cells when compared with cells without volcanic particles as seen in Fig. 3 (upper graph) and Table 1 as well as dose response to A549 human alveolar cells in Fig. 4, where each set of experiments had six separate measurements. There was a significant difference in colony forming unit (CFU) values for volcanic particle concentrations of 10 μg/mL and 20 μg/mL compared with the control results ($p < 0.001$, one-way ANOVA using Dunnett's method); when comparing with the control data there was a mean (95 % confidence interval) difference of 2639 (1551 to 3727) count / mL at 10 μg/mL and 3061 (1973 to 4149) count / mL at 20 μg/mL. Volcanic particles also increased PAFR expression in both A549 and the nasal epithelial cells (HPNEpC) when compared with cells without addition of volcanic particles as seen in Fig. 3 (lower graphs) and Table 1.

$PM_{2.5}$ and $PM_{10}$ particles are thought to be of particular interest in understanding possible associations with respiratory health (Buist et al., 1986). As we have previously seen in particles from Eyjafjallajökull and Grímsvötn volcanic eruptions in 2010 and 2011 respectively, the 3D appearance indicated that some particles had sharp or jagged appearing edges (Wertheim et al., 2017) which could be hard to discern from 2D imaging. Previous studies have identified possible associations between volcanic eruptions and adverse health effects (Lombardo et al., 2013; Carlsen et al., 2021) however, the underlying reasons are not well understood.

The presence of silicon and oxygen in particles we examined using energy dispersive x-ray (EDX) microanalysis, suggests some of the particles are likely to be silicates as expected. Studies have highlighted the issue of silica particles being emitted in volcanic eruptions as well as those involving other sources and possible adverse health effects (Cook et al., 2005, Horwell et al., 2012, Horwell et al., 2013, Damby et al., 2017). A study of bronchoalveolar lavage (BAL) samples suggest adverse health effects associated with volcanic eruptions may also be related to the presence of toxic metals in people exposed to the 2001 Mount Etna

particulate fallout (Censi et al., 2011).

## 4. Conclusions

We observed that volcanic particles increased pneumococcal adherence and PAFR expression in A549 lung epithelial cells and human primary nasal epithelial cells *in vitro*; for *Pneumococcal adhesion* we observed an increase in colony forming units which was dependent on the concentration of volcanic particles. Furthermore, the La Palma volcanic particles can have angular and sharp appearing surface features which may affect cellular uptake. Thus in addition to the toxic nature of some particles we suggest that the observed sharp surface particulate features may help to explain the adverse health effects associated with volcanic eruptions.

## CRediT authorship contribution statement

All authors contributed to devising the study as well as the design. Beverley Coldwell collected the volcanic particles samples. Ian Gill, Simon Crust, Richard Giddens and Lisa Miyashita prepared slides used in this study. Lisa Miyashita, Simon Crust, Richard Giddens, Beverley Coldwell and David Wertheim contributed to the microscope image data acquisition in this study. Lisa Miyashita conducted the cell experiments. All authors read and approved the manuscript.

## Declaration of competing interest

DW, BC, LM, IG, SC, RG, NemesioP and NickP have no competing interests.

Jonathan Grigg reports financial support was provided by Barts Charity. Jonathan Grigg reports financial support was provided by The Medical College of Saint Bartholomew's Hospital Trust. Professor Grigg received personal fees from AstraZeneca, GSK, Novartis, Vifor Pharma, OM Pharma, and Omron outside the submitted work.

## Data availability

Data available upon reasonable request to the corresponding author.

## Acknowledgements

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