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In situ lysis droplet supply to efficiently extract ATP from dust particles for near-real-time bioaerosol monitoring

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GRAPHICAL ABSTRACT

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ABSTRACT

Simultaneous improvement in detection speed and reliability is critical for bioaerosol monitoring. Recent rapid detection strategies exhibit difficulties with misinterpretation due to signal interference from co-existing non-biological particles, whereas biomolecular and bioluminescent approaches require long process times (> several tens of minutes) to generate readable values despite their better detection reliability. To overcome these shortcomings, we designed a system to achieve rapid reliable field detection of bioaerosols ($> 10^4$ relative luminescence units [RLU] per cubic meter of air) in < 3 min processing time (equivalent to 24 L sampling air volume) by employing a lysis droplet supply for efficient extraction of adenosine triphosphate (ATP) from particulate matter (PM) and a photomultiplier tube detector for signal amplification of ATP bioluminescence. We also suggested the use of the ratio of RLU (m^{-3}) to total PM ($\mu g\ m^{-3}$), or specific bioluminescence (RLU μg^{-1}), as a measure of the biofraction of PM (i.e., potential biohazards). A correlation between RLU and colony forming unit was also obtained from simultaneous aerosol sampling using an agar-inserted sampler.

1. Introduction

Biological particulate matter (PM) suspended in the air (bioaerosols such as airborne viruses, bacteria, fungi, and pollen) has an important impact on human health and the environment because of their induction of the toxins causing toxic effects and environmental disturbance [1,2]. In particular, the health threats from inhalation of and direct exposure to bioaerosols are closely associated with infectious and

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allergic diseases [3], since bioaerosol particles between 1 and 5 μm may remain suspended in the air for a long time. Bioaerosols are a major part of indoor air pollutants (typically 5% to 34%) alongside volatile organic compounds and nonbiological PM [4,5]. In order to protect humans and the environment from biological threats, much effort has focused on bioaerosol capture, inactivation and monitoring technologies. Even though rapid assessment of bioaerosols is the first task when attempting to efficiently manage and maintain air quality via capture and inactivation, culture-based colony counting (taking 3–5 days) is still the most widely employed method of estimating bioaerosol concentration [6]. However, the colony counting method is unsuitable both for continuous bioaerosol monitoring and for the estimation of total bioaerosols; microbes that cannot be cultured are not counted resulting in the underestimation of bioaerosols [4].

Against the shortcomings of the conventional culture-based method, rapid detection of bioaerosols via continuous signal acquisition has recently been proposed using light-induced fluorescence (LIF) [7–10], field-effect transistor [11], or quartz crystal microbalance (QCM; using antibody-functionalized surface to recognize biological signals in dust particles) devices [12]. Among these, LIF (e.g., wideband integrated bioaerosol sensor) is most frequently utilized as it enables real-time bioaerosol monitoring. Upon exposure to UV light, microbes emit fluorescent light as a result of the excitation of organic substances, such as riboflavin, amino acids, nicotinamide adenine dinucleotide, etc. borne on their surfaces [13–15]. However, the LIF approach is still only suitable for pure or well-isolated bioaerosols, and for reliable detection at high concentration at the laser irradiation region is required for the acquisition of readable bioaerosol signals. Interference from co-existing nonbiological (fluorescent or not PM causes under- or over-estimation of bioaerosol concentration [1,2,13]. Interference is also an issue with QCM-based bioaerosol monitoring [12], for which additional data or instrument calibration is required to achieve reliable (i.e., distinguishing between biological and nonbiological PM) bioaerosol detection [16]. A miniature biomolecular analysis device based on amplification of a particular region of DNA (real-time polymerase chain reaction [PCR]) that provides a result within 90 min has thus been developed to overcome the problem of misreading of bioaerosol concentration [17], while indirect predictions based on the PM monitoring data have also been considered as an inexpensive, simple and rapid solution [18,19]. However, for rapid bioaerosol detection the PCR method is still too slow, and the prediction approach may result in significant uncertainties without supporting biological assays. Moreover, these two strategies, as well as LIF analyses, require well-trained operators to perform the detection or data processing [7,8]. Therefore, a realizable, easy-to-use detection platform to reliably measure both culturable and nonculturable bioaerosols rapidly while avoiding the under- or over-estimation is eagerly awaited.

A possible alternative could be to exploit adenosine triphosphate (ATP) bioluminescence. Since most viable microbes (i.e., metabolically active bacteria and fungi) contain the ATP molecule as an essential energy source, it could form the basis of a rapid and affordable procedure to efficiently quantify the presence of microbes. ATP bioluminescence can be generated according to the following reaction [20]:

$$\text{ATP} + \text{luciferin–luciferase} + O_2 \rightarrow \text{oxyluciferin–luciferase} + \text{adenosine monophosphate} + \text{pyrophosphate} + CO_2 + \text{luminescence}.$$

Our previous studies [21,22] and others [23–25] have validated the feasibility of rapid bioaerosol detection based on ATP bioluminescence, but this required a two-step preprocess (air sampling and analyte preparation) prior to bioluminescence detection by a luminometer. More recently, a method involving continuous monitoring of bioaerosol ATP signals has been introduced [26]. However, an aerosol-to-hydrosol preprocess and subsequent microfluidic control are required because delivering bioaerosol into a microfluidic channel as a droplet and maintaining the channel as clean to avoid detection interference are challenging issues [27], precluding it as an easy and inexpensive bioaerosol detection method. In addition, one relative luminescence unit (RLU) as measured using this approach is equivalent to approximately two colony forming units (CFU), which is of marginal use when attempting to classify bioaerosol levels at detection sites [28].

In this study, we addressed the two major issues of the ATP bioluminescence assay (achieving a convenient sampling procedure and intense bioluminescence signal) for rapid, reliable bioaerosol quantification by system optimization. Specifically, this study not only demonstrates an integrated bioaerosol detection platform that can be used to generate intense bioluminescence signals ($> 10^4$ RLU per cubic meter air) rapidly (3 min, corresponding to 24 L of sampling air) but also provides an index to evaluate bioaerosol fractions (RLU per unit mass of PM; RLU μg^{-1}) of PM suspended in the air. First we designed and fabricated a microfiber swab (darcon polyester) that can be inserted into the air sampler, the aim being to confirm the feasibility of direct PM deposition onto the swab surface; the swab is subsequently immersed into luminescence reagent to produce bioluminescence signals (Fig. 1A). PM-laden air passes through the swab, and the PM is directly deposited on its surface via mechanical filtration (Fig. 1B and Table S1). To improve contact of the deposited PM and therefore ATP extraction efficiency, droplet of lysis solution are automatically injected onto the swab surface for the last 30 s of air sampling (under the regulation of an Arduino–Bluetooth smartphone controller). Subsequently, bioluminescence from the swab is detected using a photomultiplier tube (PMT) luminometer. During sampling, an optical particle counter simultaneously acquires PM data. When sampling is complete, the optical particle counter results are combined with the bioluminescence data via a user interface program to provide a specific bioluminescence reading (RLU μg^{-1}) that represents the bioaerosol fraction the PM. Furthermore, corresponding counting of CFUs was simultaneously conducted using an agar-inserted sampler to examine reliability (correlation between RLU m^{-3} and CFU m^{-3}) of the developed method in this study.

2. Experimental

The test bacteria (*S. aureus*, KCTC 1621) were dispersed in deionized water at the chosen concentration. The dispersion was aerosolized using an atomizer (9302, TSI, USA) with clean air (as operating fluid, high efficiency particulate air [HEPA] filtered), as shown in Fig. S1. The bacteria-laden aerosol flow was then passed through a diffusion dryer and aerosol neutralizer (4530, HCT, Korea) for desiccation and surface charge neutralization, respectively. The laminar flow meter and mass flow meter were used to measure flow rates of the aerosol and clean air, respectively. The aerosol concentration was measured using an aerodynamic particle sizer (APS; 3321, TSI, USA) at a sampling flow rate of 5 L min^{-1}. The aerosol flow was injected into the sampler (10 L min^{-1}); a swab (PD-20/PD-30, Kikkoman, Japan) inserted configuration for ATP bioluminescence measurement) or an agar (tryptic soy)-inserted single-stage (650 nm cutoff diameter) impactor (28.3 L min^{-1}; for colony counting after incubation; TE-10-880, Tisch Environmental, USA) for 1 min after the aerosol flow (2.0 L min^{-1}) was diluted with clean air (26.3 L min^{-1}). After aerosol sampling, the swab was inserted into a PD-type luminometer (Lumitestor, Kikkoman, Japan) to measure RLU immediately after the reaction with the luminescence reagent (luciferin − luciferase with lysis buffer), while the agar plate was placed in an incubator for 24 h at 37 °C, and the number of colonies was counted to estimate CFU. For conventional counting of CFUs, aerosol was simultaneously sampled using a Spin Air (IUL S.A., Spain) sampler according to the protocol provided by manufacturer.

The attenuation of ATP bioluminescence signals due to interference by non-viable particles was confirmed via co-deposition of S. aureus and PSL (as model PM; 5074 A, Thermo Scientific, USA) particles on a swab at 10 L min^{-1} for 2 min. The RLU values with different PSL ratios were recorded using a luminometer. For field tests, both sampling

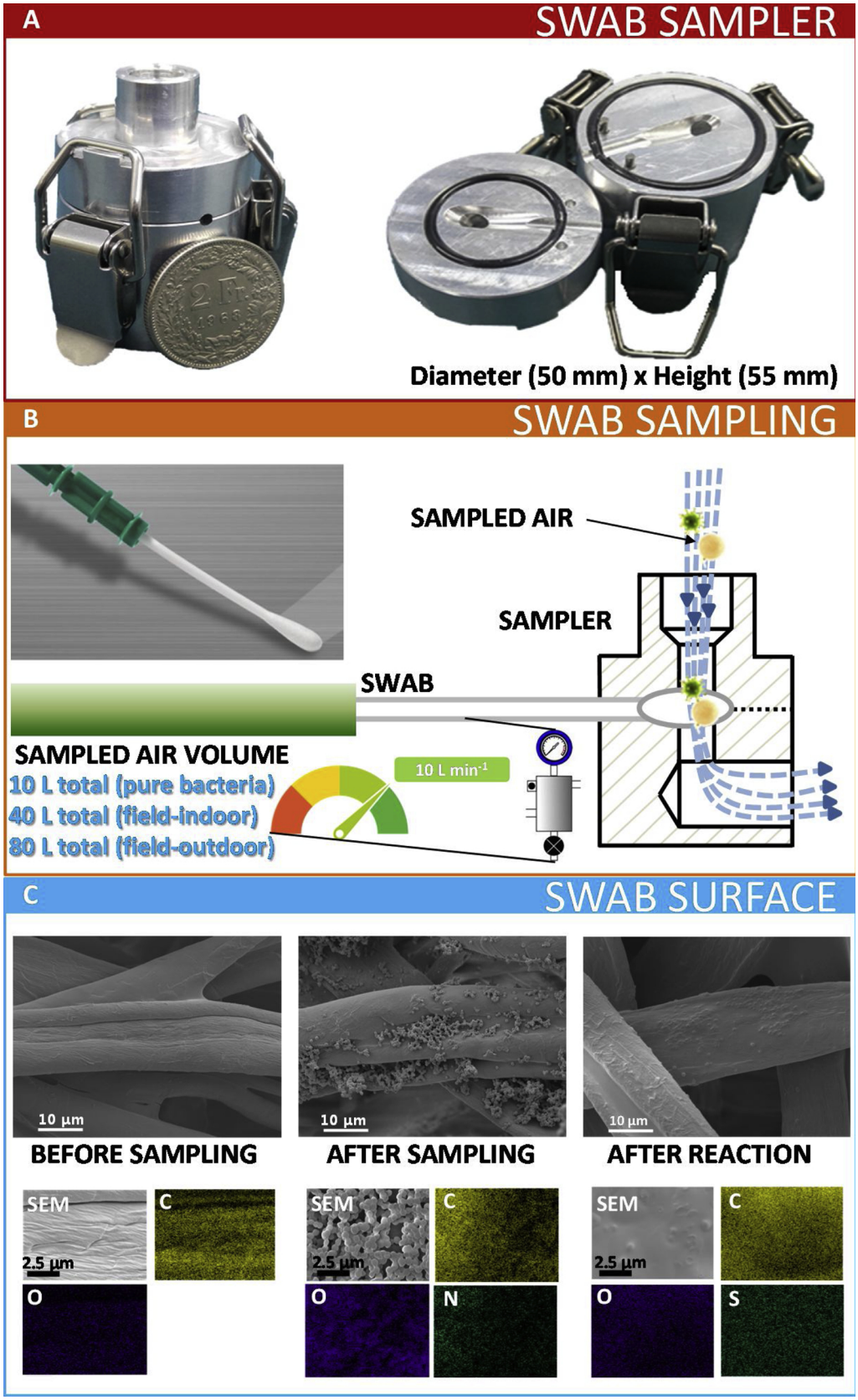

Fig. 1. Sampling of aerosolized *S. aureus* on surfaces of a swab for ATP bioluminescence detection. (A) Digital images of the sampler developed in this study. A swab is placed inside the sampler before sampling begins, and air containing the particles to be sampled is passed through the swab. (B) Schematic of direct *S. aureus* deposition through mechanical filtration. The microfibers of the swab act as filter fibers to collect aerosolized *S. aureus* in clean air. The bacterial aerosol flow rate was 10 L min^{-1}, and the sampling time varied (1–8 min) depending on particles to be sampled (*S. aureus* aerosol, indoor air, and outdoor air). (C) SEM images and EDS maps of swab surfaces showing pristine swabs (before sampling), swabs bearing deposited *S. aureus* (after sampling), and swabs after reaction with the luminescence reagent (after reaction) to demonstrate the feasibility of direct sampling for ATP bioluminescence generation. (C, carbon; O, oxygen; N, nitrogen; S, sulfur.).

devices were also employed (with flow rates as above) to measure RLU and CFU (except sampling time was extended to 4 min for the indoor environment and 8 min for the outdoor environment) to secure readable RLU and CFU values.

3. Results and discussion

Direct PM deposition on a swab surface was qualitatively confirmed using scanning electron microscopy (SEM; 7800 F, JEOL, Japan) and

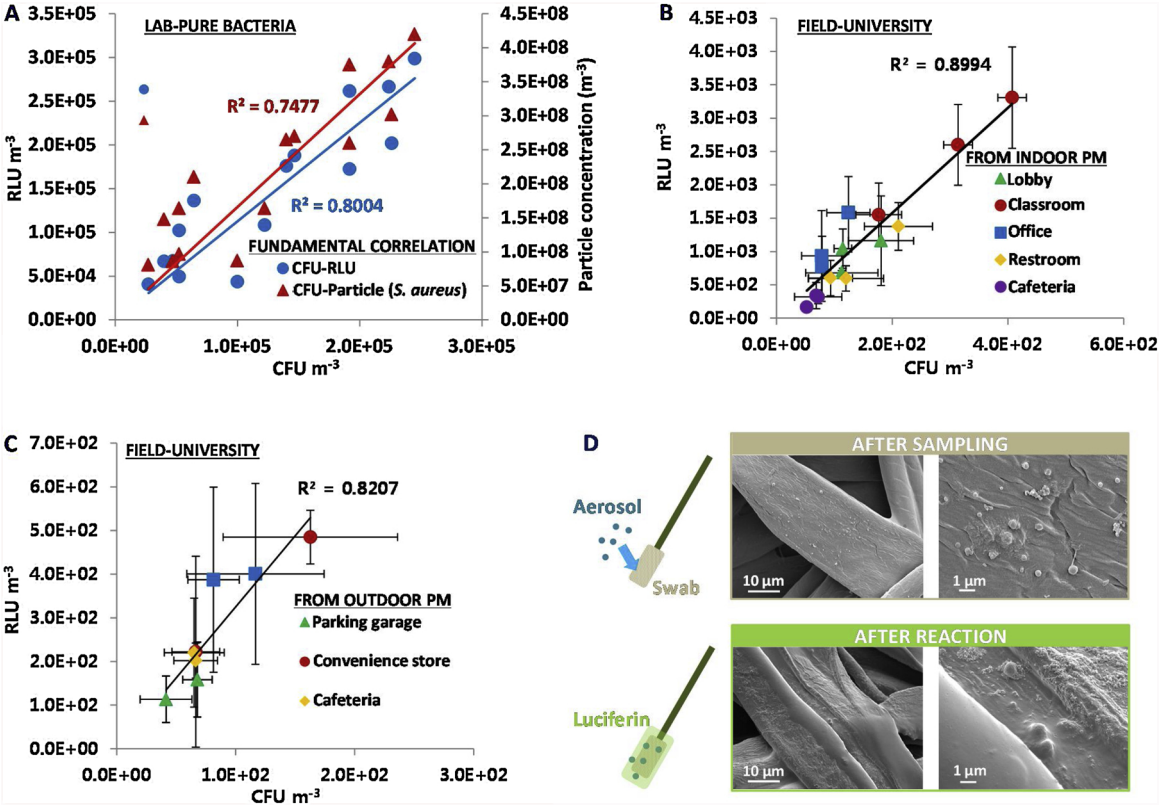

Fig. 2. Efficacy of ATP bioluminescence detection to quantify bioaerosols. Correlation between CFU m^{-3} and RLU m^{-3} (or particle concentration, m^{-3}) for pure *S. aureus* aerosol (A), indoor PM (B), and outdoor PM (C). In the case of the *S. aureus* aerosol, particle concentration as measured using an APS was compared with CFU to examine any correlation (R^2). (D) Representative low- and high-magnification SEM images of swab surfaces after indoor air sampling and subsequent ATP bioluminescence generation.

energy dispersive X-ray spectroscopy (EDS; JED-2300, JEOL, Japan). *Staphylococcus aureus* (*S. aureus*; KCTC 1621) bacteria were first aerosolized with clean air and the pure bacterial air flow was passed through a swab for 1 min. Fig. 1C shows SEM-EDS images comparing fibers of a pristine swab, a swab after sampling, and a swab after reaction with the luminescence reagent. The pristine swab shows clean surfaces on fibers approximately 25 μm diameter, the fiber consisting mainly of carbon (C) and oxygen (O). After bacterial sampling, the swab surface is covered with groups of spheres, and unlike the pristine swab, nitrogen (N) content is significant; this is a major component of *S. aureus*, and implies direct deposition of the injected bacterial aerosols. After application of the luminescence reagent used for bioluminescence generation the swab exhibits a smooth surface resulting from luciferin (pigment) conjugation corresponding to the number of sulfur (S) atoms on the swab originating from elemental S in the luciferin molecules. These results confirmed the feasibility of direct swab sampling for ATP bioluminescence assay of bioaerosols.

The feasibility of the proposed approach was validated by observing the correlation between RLU and CFU for pure *S. aureus*-laden air (Fig. 2A; 10 L of air), as well as for indoor (Fig. 2B; 40 L of air) and outdoor (Fig. 2C; 80 L of air) air at various university sites (gross area: ~850,000 m^2; number of colleges: ~10; number of students and staff: ~30,000). The data were acquired using a photodiode (PD) luminometer (PD-30, Kikkoman, Japan) and an agar-inserted single-stage impactor (TE-10-880, Tisch Environmental, USA), respectively (Fig. S1). The R^2 values for the sites were greater than 0.8, comparable with previous reports [21,22]. This suggests that direct PM deposition on a swab may be feasible for achieving bioluminescence signals for reliable bioaerosol quantification, as well as for simplifying the preprocess for ATP assay. The linear correlation between *S.aureus* abundance (measured using an aerodynamic particle sizer [3321, TSI, USA]) and CFU was also confirmed (Fig. 2A), proving the feasibility of using direct swab deposition. The physical changes (Fig. 2D) in surface morphology of a swab after field air sampling and subsequent bioluminescence generation corresponded to the results from pure bacteria (Fig. 1C), although the density and shape of deposits on the swabs are different. The bacterial species suspended in the indoor air are listed in Table S2, identified using matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-8020, Shimadzu, Japan). Of these, it was mostly the Gram positive bacteria that contributed to producing bioluminescence signals in the indoor field tests, and these bacteria appeared to be dominant in the deposits on the swab surface after the air sampling (Fig. 2D). The closeness of R^2 values for luminescent reaction between the laboratory and field bioaerosol tests further demonstrates the potential and the robustness of this sampling method for wider application in bioaerosol monitoring. Nevertheless, the acquired RLU values from the field tests were under 100 in most cases, which is comparable with the lower limit of quantification for commercial ATP devices (below which ATP bioluminescence can be measured but ATP levels cannot be reliably quantified or classified) [29]. A similar issue was demonstrated for an extended use of the sampling method for detecting aerosol viruses (Table S3). Even though the method could detect different aerosol viruses using a commercial influenza antibody test kit (JW influenza A&B Test, JW Bioscience, Korea), the detection ability was limited by the need to sample a large volume of air (~50 L) with a high virus concentration ($> 10^6\ m^{-3}$).

Low levels of RLU are generally related to the sensitivity of a luminometer, but they may also be because of co-existence of non-biological PM causing interference to bioluminescence signals. To examine the effect of interference (Fig. 3A), a sampler was employed to deposit *S. aureus* and polystyrene latex (PSL; as a model for non-biological PM; 5074 A, Thermo Scientific, USA) aerosols simultaneously

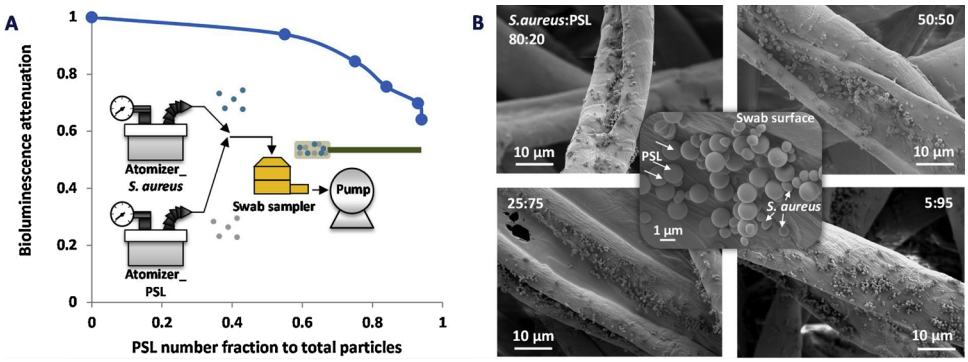

Fig. 3. Evaluation of bioluminescence signal attenuation upon PSL particle co-deposition on a swab. (A) Bioluminescence signal attenuation characteristics for different proportions of PSL particles. The *S. aureus*:PSL particle ratio was modulated by supplying aerosols from two separate atomizers, as shown in the inset, and bioluminescence signals were measured at different ratios. (B) Representative SEM images upon co-deposition of *S. aureus* and PSL aerosol particles on swabs at ratios (*S. aureus*:PSL) of 80:20, 50:50, 25:75 and 5:95. A representative high-magnification SEM image (center) exhibits co-existence of *S.aureus* and PSL on a swab surface.

on a swab using two identical atomizers (inset of Fig. 3A; 9302, TSI, USA). As the proportion of PSL increased the bioluminescence signals were attenuated, matching the different densities of PSL on the swab surface (Fig. 3B). The high-magnification SEM image shows the co-existence of *S. aureus* and PSL on the swabs. The PSL particles limits access of the lysis solution to the surface of the *S. aureus*, reducing ATP extraction and leading to poorer bioluminescence generation. This implies that an effective lysis strategy is particularly important for field monitoring of bioaerosols. In addition, achieving high values for RLU ($> 10^4$) is critical for correlation with CFU for the detection of bacterial pathogens [30,31]; therefore, further improvements in detection sensitivity and ATP extraction are needed for the reliable detection of bioaerosols in the field.

With the aim of resolving both issues, we modified the sampler by adding a vibrating nozzle to supply lysis solution droplets onto the swab surfaces during the last 30 s of air sampling in the field (Fig. 4A). Air sampling and lysis droplet supply were controlled using an Aduino–Bluetooth smartphone controller, with a sampling time of 3 min (equivalent to an air volume of 24 L). An inertial impact mechanism was employed for efficient deposition of the PM with lysis solution droplets on swab surfaces [32], and the collection efficiency of PM was found to be 92.1% (1 μm cutoff diameter; 96.8% of overal collection efficiency [< 20 μm]). In addition, the cell viability of airborne bacteria collected on swabs was 90.4% when distilled water droplets were supplied instead of lysis droplet, suggesting that most bioaerosols might be viable in the absence of lysis solution. A PMT detector (NovaLum II, Charm Sciences, USA) was employed to detect bioluminescence instead of a PD detector. Fig. 4B shows the signal (RLU m^{-3}) enhancement with and without lysis droplet supply duringt field testing. Supplying lysis droplets intensified the bioluminescence signals (> 10-fold, and $> 10^4$ RLU m^{-3} in most sites). These results demonstrated that bioluminescence generation could be enhanced by the use of a PMT detector combined with a modified ATP extraction method. The corresponding CFU values were assessed using a Spin Air agar-inserted sampler in 284 field tests ($R^2 = 0.9027$). When the CFU and RLU results were correlated the following relationship could be derived:

$$\text{CFU } (m^{-3}) = 3.2 \times \text{RLU}^{0.34} \ (m^{-3}).$$

When this formula was used to calculate and compare CFU values with RLU values obtained using both the PMT data and data from a PD detector (Fig. 4C), values for PMT were always greater than those for PD. These results suggest that the modified platform is less likely to underestimate bioaerosol levels; furthermore, the calculated CFU values lie within the range of concentrations for airborne microbes (< 100–1000 CFU m^{-3}) given in guidelines suggested by the World Health Organization [8].

Even though the modified platform met the requirements (i.e., immediacy coupled with intense signal) for rapid reliable bioaerosol detection, individual RLU values do not provide definitive classification of bioaerosol levels, concentrations or relevant biological risks. Hence, we proceeded to consider the use of specific bioluminescence (given by the ratio of RLU to PM) as a measure of the biological fraction of PM:

Specific bioluminescence (RLU μg^{-1}) = RLU (measured using a luminometer) / PM (measured using a PM monitor).

An optical particle counter (OPC-N2, Alphasense, UK; 63.5 × 60 × 75 mm) was integrated into the detection platform, and the apparatus was connected with a user interface program (built with Microsoft Excel macros and Python) to generate specific bioluminescence data automatically (Fig. S2). Fig. 5A and B show measured RLU and PM data, respectively, for the estimation of RLU μg^{-1} at a university (gross area: ~2,700,000 m^2; number of colleges: ~20; number of students and staff: ~40,000) and a university hospital (number of hospital beds: ~1000; number of operating rooms: ~20; number of staff: ~2000). Fig. 5C illustrates the estimated RLU μg^{-1} values (derived from the data in Fig. 5A and B), which clearly show site-dependent bioaerosol distributions that in some cases differ markedly from the PM values. In particular, the dental clinic site exhibits a greater RLU μg^{-1} value than those from most other sites in spite of its low PM (RLU m^{-3}) value (Fig. 5A). This shows that the suspended PM at the dental clinic contained a significant biological fraction, probably originating from the dental cleaning procedures that can aerosolize oral bacteria [33]. In addition, the high level in the student cafeteria might be caused by various activities, such as eating, cooking, talking, and dishwashing, which is consistent with a previous report [34]. The site-dependent characteristics were also valid for the specific CFU (CFU per unit mass [μg] of PM) plots based on calculations of CFU m^{-3} via the formula (Fig. S3). Considering the weight (in the order of femtograms) [35,36] and number (1 − 1000 bacterial cells per CFU) [37] of individual bioaerosols as components of the PM, the resulting CFU values from the calculations were plausible, where different biological fractions in a microgram of PM at the detection sites may derive different CFU μg^{-1} values due to cellular proliferation. Interestingly, the plots from the university hospital mostly exhibited greater specific CFU values than those from the university, which might suggest that PM at medical facilities has greater potential for producing biologically adverse effects from bioaerosols. This type of information may be helpful to efficiently respond to biological risks for maintenance of air quality with quantitative decision criteria.

4. Conclusions

An integrated platform for rapid, reliable detection of bioaerosols was developed by combining a lysis droplet supply with a PM sampling swab. Resulting RLU (m^{-3}) data were merged with PM data (μg m^{-3}) to produce specific bioluminescence (RLU μg^{-1}, a measure of relative potential biohazard of PM suspended in the air. In particular, the specific bioluminescence gave a measure of site-dependent bioaerosol distribution, which could facilitate site-customized (i.e., bioaerosol dominant vs. nonbiological-PM dominant) management of air quality. The detection process required a sampling time (or sampling air volume) of < 3 min (or 24 L) to achieve intense bioluminescence (> 10^4

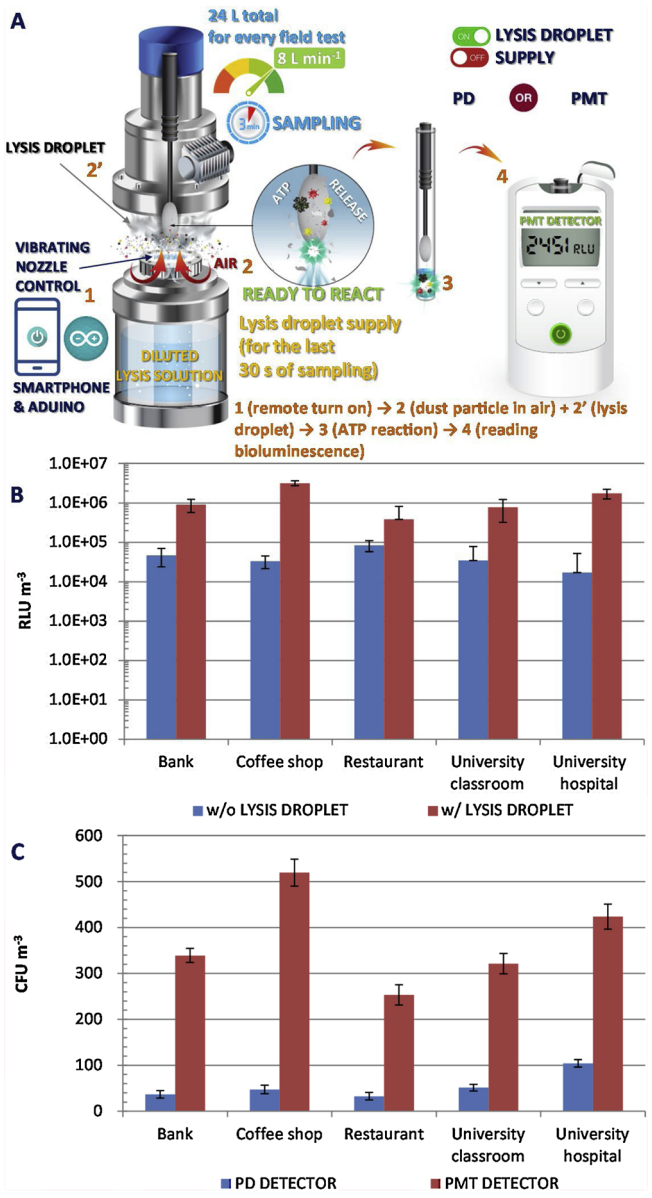

Fig. 4. Bioluminescence signal amplification during field tests in which lysis droplets were supplied during air sampling and a PMT detector was used instead of a PD detector. (A) Schematic of simultaneous lysis droplet supply (for the last 30 s of sampling) via an automatic vibrating nozzle under the control of an Aduino–Bluetooth smartphone system during air sampling onto a swab. The sampling flow rate for all field tests was 8 L min^{-1} (sampling time: 3 min, 24 L total). The droplet supply was applied to both PD and PMT detectors to validate bioluminescence amplification and CFU calculation. (B) Averaged RLU values (determined using a PMT detector) of PM in a bank, coffee shop, restaurant, university classroom and university hospital with and without lysis droplet supply. (C) CFU values in the same locations as calculated by the formula correlating RLU (PMT detector) and CFU.

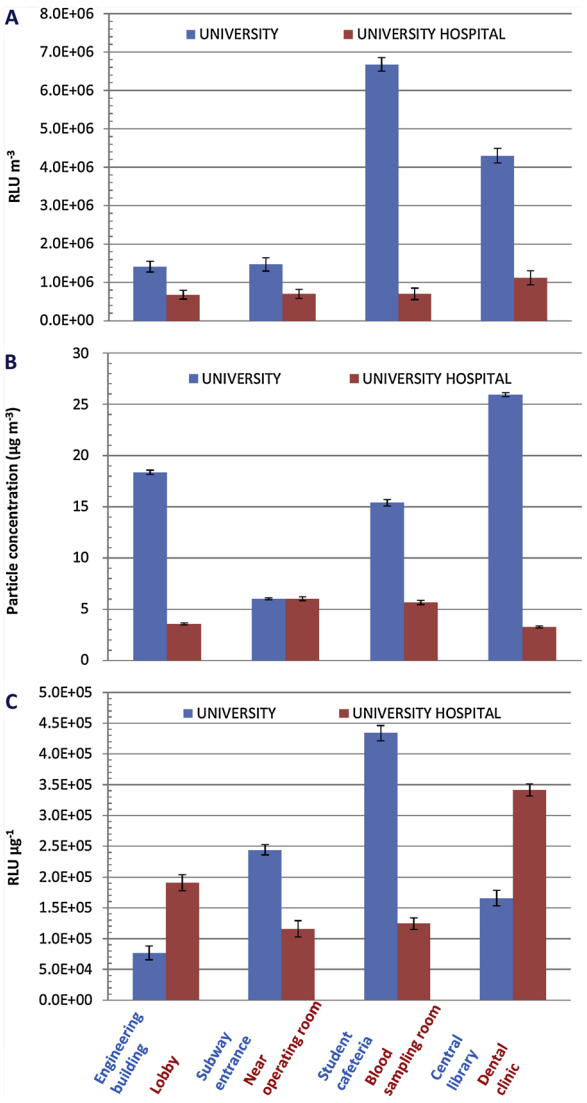

Fig. 5. Merge of averaged bioluminescence and PM data to derive specific bioluminescence for PM suspended in air. (A, B) RLU m^{-3} and μg m^{-3} values of the suspended PM at the locations at a university (engineering building, subway entrance, student cafeteria, and central library) and a university hospital (lobby, near operating room, blood sampling room, and dental clinic). (C) Specific bioluminescence at the same locations (ratio of bioluminescence to PM). These averaged values were automatically calculated by retrieving the bioluminescence and PM data within the user interface (Fig. S2).

RLU m^{-3}, also modulatable by controlling sampling flow rate [or time] and amount of lysis droplet) during field tests. The detection and data analysis were conveniently conducted by connection to an Aduino–Bluetooth smartphone controller and user interface program and via use of a lysis droplet swab sampler. Even though simultaneous counting of CFUs derived a workable correlation between RLU m^{-3} and CFU m^{-3}, more experimental and statistical studies with different monitoring conditions are needed to realize the developed system for practical uses. Nevertheless, these findings confirm the feasibility of developing a platform for rapid, reliable analysis of bioaerosol levels at target sites, offering a compact and digitizable platform for bioaerosol detection, and an easy-to-use methodological concept for ensuring near real-time response to bioaerosol levels (or fractions) for efficient air quality management.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

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