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Mechanical desorption of immobilized proteins using carbon dioxide aerosols for reusable biosensors

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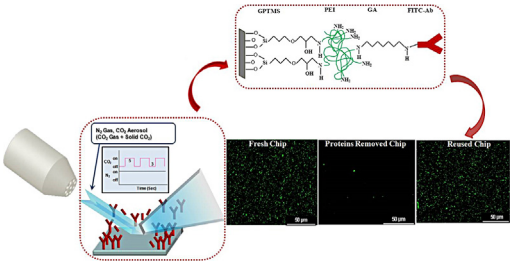
HIGHLIGHTS

- Immobilized proteins were removed using carbon dioxide aerosols.
- We observed high removal efficiencies due to the aerosol treatment.
- We confirmed the removal with FTIR and X-ray photoelectron spectroscopy.
- This CO_2 aerosol treatment did not undermine re-functionalization.
- This technique is a fast and damage-free method to reuse a sensor surface.

GRAPHICAL ABSTRACT



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ABSTRACT

Reusability of a biosensor has recently received considerable attention, and it is closely related with the effective desorption of probe molecules. We present a novel mechanical desorption technique to reuse biosensors by using periodic jets of carbon dioxide (CO_2) aerosols (a mixture of solid and gaseous CO_2), and demonstrate its feasibility by removing physically adsorbed and covalently bonded fluorescent proteins i.e., *Escherichia coli* fluorescein isothiocyanate antibody and bovine serum albumin (*E. coli* FITC–Ab and FITC–BSA) from silicon chips. The proteins on the chip surfaces were measured by fluorescent images before and after applying the aerosols. The removal efficiency of the aerosol treatment was measured for various concentrations (1–20 $\mu g\,mL^{-1}$) of *E. coli* FITC–Ab and FITC–BSA with two different removal cycles (5 and 11 cycles; each cycle: 8 s). We observed high removal efficiencies (>93.5% for physically adsorbed Ab and >84.6% for covalently bonded Ab) at 11 cycle aerosol treatment. This CO_2 aerosol treatment did not undermine re-functionalization, which was confirmed by the fluorescent images of FITC–Abs for fresh and reused chips. Desorption of the immobilized layers was validated by Fourier transform infrared and X-ray photoelectron spectroscopic analyses. We also conducted an experiment on the regeneration of *E. coli* sensing chips using this aerosol treatment, and the chips were re-used 5 times successfully. This mechanical desorption technique is a highly effective and novel strategy for reusable biosensors.

1. Introduction

Development of a reusable biosensor is currently a strong priority and has recently gained significant interest. Reusability or

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regeneration of a biosensor is closely related with the effective desorption of probe molecules such as antibody (Ab), bovine serum albumin (BSA), and deoxyribonucleic acids (DNAs). Many works have been conducted on the reusability or regeneration of sensing elements functionalized with several biomolecules [1–4].

The current reusability techniques can be categorized into two main methods: electrochemical desorption [5,6] and chemical solutions [7–10]. In the electrochemical desorption technique, negative or positive electric potential is applied to self-assembled monolayers (SAMs) on a gold electrode to detach these layers from the electrode. This method usually requires a gold electrode, and it can have limitations such as electrode peeling-off and electrolysis due to high electric potential [11,12].

Another technique is to use chemical solutions such as strong acid and base [7], detergents [13,14], and buffers [15] to remove immobilized proteins from a surface. This technique is relatively convenient and inexpensive, but the chemical solutions can damage sensing sites and restrict the fabrication process of a biosensor. Moreover, the chemical solution technique usually depends on sensing elements, immobilized proteins, and target analytes. For example, the proteins adsorbed on the surface of a quartz resonator in the quartz crystal microbalance were removed by soaking it in the oxidant piranha-solution [7]. Acidic or alkaline solutions with high salt concentration are often times used as a cleaning fluid for a quartz resonator, but such solutions can damage other parts of the sensing platform.

Here, we propose carbon dioxide (CO_2) aerosols (a mixture of solid and gaseous CO_2) to remove immobilized proteins towards reusable biosensor applications. In this technique, a great number of tiny solid CO_2 particles are generated via supersonic expansion of a high-pressure CO_2 gas as it passes through a nozzle, and they are projected at high velocity towards the target material or particles on a substrate for removal [16]. The mechanical impact of the high-speed solid CO_2 particles onto the target particles is well known as a primary removal mechanism [16]. Also, transient liquid CO_2 is generated when the solid CO_2 particles melt on an impact, facilitating the removal. These two are most commonly believed mechanisms for the CO_2 aerosol technique [16]. The CO_2 aerosol technique has several advantages, including simplicity of the equipment, residue-free action, and damage-free cleaning [16]. This technique has generally been used to remove dust particles from silicon wafers [16,17], and recently it was applied to remove bacterial biofilms from several surfaces [18,19].

In this study, we demonstrate that this CO_2 aerosol technique can be utilized to regenerate or reuse a biosensor by removing immobilized proteins on a sensing surface as well as bacterial cells captured on these protein layers. The proteins were immobilized via physical adsorption using polyethylenimine (PEI) as well as covalent binding using (3-glycidyloxypropyl) trimethoxysilane (GPTMS). PEI is a hydrophilic polycationic polymer that can be directly adsorbed to silica surfaces via electrostatic interaction between the positive charges of PEI and the negative charges of the silica surface. It is shown to be practically irreversible and exhibits excellent properties for the immobilization of biomolecules [20]. A SAM of functionalized silanes such as GPTMS has also attracted much attention for the immobilization of biomolecules i.e., proteins, enzymes and DNA since they are easy to form, and silanization provides a uniform layer on a desired surface and medium to tailor the surface properties [21,22]. We measured the desorption effectiveness of the CO_2 aerosol treatment as the concentration of proteins was varied for these physical adsorption and covalent binding cases. We also validated the desorption of the immobilized proteins with Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS) analysis, and demonstrated that this CO_2 aerosols technique can be used several times for bacteria detection sensors by regenerating the sensing surface.

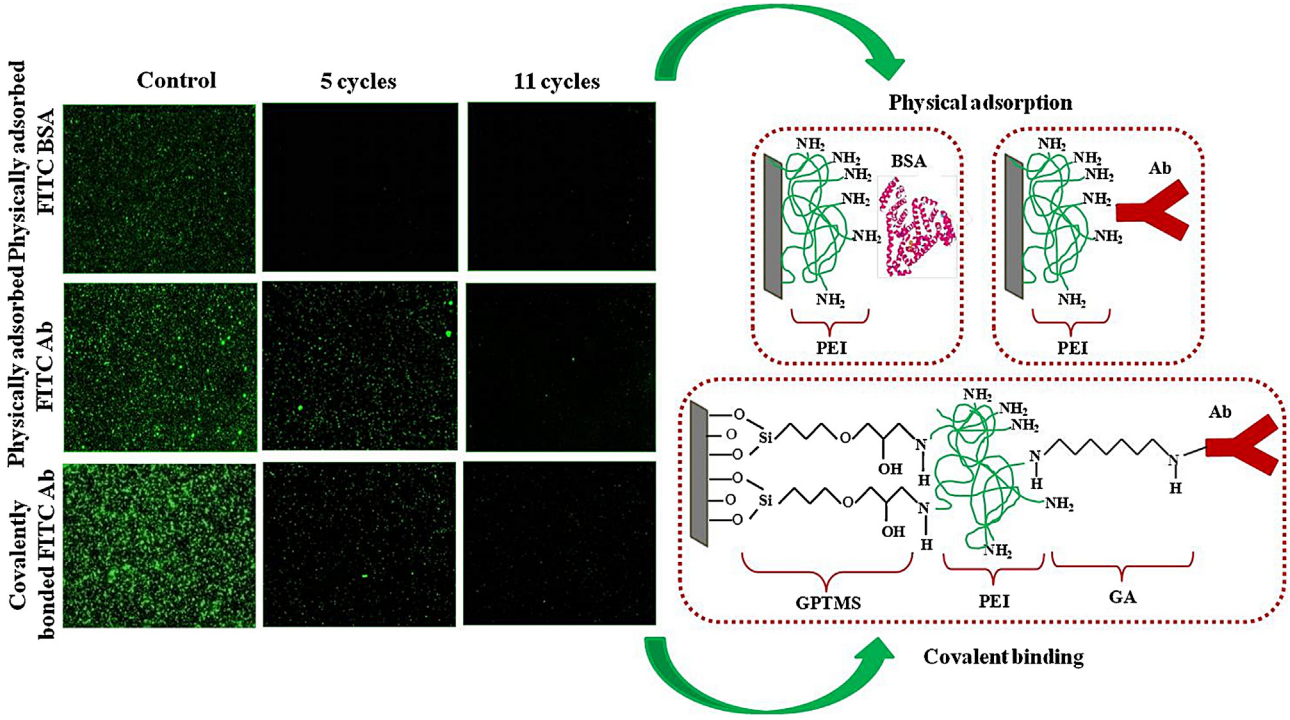

Fig. 1. Fluorescent images of physically adsorbed FITC–BSA, *E. coli* FITC–Ab, and covalently bonded *E. coli* FITC–Ab for control (20 μg mL^{-1}) and two different removal times (5 and 11 cycles) and the schematic of used physical adsorption and covalent binding methods.

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2. Materials and methods

2.1. Chemicals and reagents

Fluorescein isothiocyanate (FITC)–BSA, GPTMS, glutaraldehyde (GA), and PEI were procured from Sigma–Aldrich, USA. Toluene (99%) was obtained from Junsei Chemical Co., Tokyo, Japan. *Escherichia coli* FITC–Ab was obtained from thermo scientific, USA. *P*-type silicon (Si) wafers were obtained from Shin-Etsu, Japan. All other chemicals were of analytical grade. Deionized water (dH_2O) (resistance: ~18.2 MΩ) from the Millipore water purification system was utilized for the preparation of the desired aqueous solutions (molecular biology grade). All the solutions and glassware were autoclaved prior to being used.

2.2. Protein immobilization on Si chips

Diced Si chips (10 mm × 10 mm) were cleaned using piranha solution (hydrogen peroxide (30%): sulfuric acid (96%) = 1:1 v/v) for 20 min, rinsed with dH_2O, and dried with pure nitrogen gas. The contact angles of a distilled dH_2O droplet on the chips and surface roughness of the chips were 17.0 ± 1.5° and 22.6 ± 0.8 nm, respectively [18].

2.2.1. Protein immobilization via physical adsorption method using PEI

These pre-cleaned Si chips were incubated in PEI polycation solution (PEI:dH_2O at a ratio of 1:100) for 15 min at 25 °C, and the positively charged PEI were electrostatically attached to the Si surface. The chips were rinsed with autoclaved dH_2O followed by mild air drying. Either *E. coli* FITC–Ab or FITC–BSA was then incubated onto the Si chips for 2 h at 37 °C followed by rinsing with autoclaved dH_2O and N_2 drying. The PEI can also attract the negatively charged proteins leading to uniform immobilization. The antibodies and BSA were immobilized non-covalently onto a physically adsorbed PEI layer (Fig. 1).

2.2.2. Protein immobilization via covalent binding method using GPTMS

A GPTMS SAM was prepared on the pre-cleaned Si chips as reported by Kumar et al. [23]. The pre-cleaned Si chips were immersed in a 1% (v/v) solution of GPTMS in toluene for overnight (~18 h) at 25 °C to form an epoxysilane monolayer having many active tail epoxy groups for reacting readily with protein amino groups. The GPTMS modified chips were subsequently rinsed with toluene and water to remove any physically absorbed epoxysilane from the surface followed by N_2 drying. These chips were then incubated in PEI (PEI:dH_2O at a ratio of 1:100) for 15 min at 25 °C, and they were rinsed with autoclaved dH_2O followed by mild air drying. GA was used as a cross linking agent onto the PEI–GPTMS–Si chips for 30 min at 25 °C followed by rinsing with autoclaved dH_2O. *E. coli* FITC–Ab was then immobilized onto the GA–PEI–GPTMS–Si chips for 2 h at 37 °C followed by rinsing with autoclaved dH_2O and N_2 drying. Here, the antibodies were covalently attached to PEI covalently bonded to the silanized Si surfaces (Fig. 1).

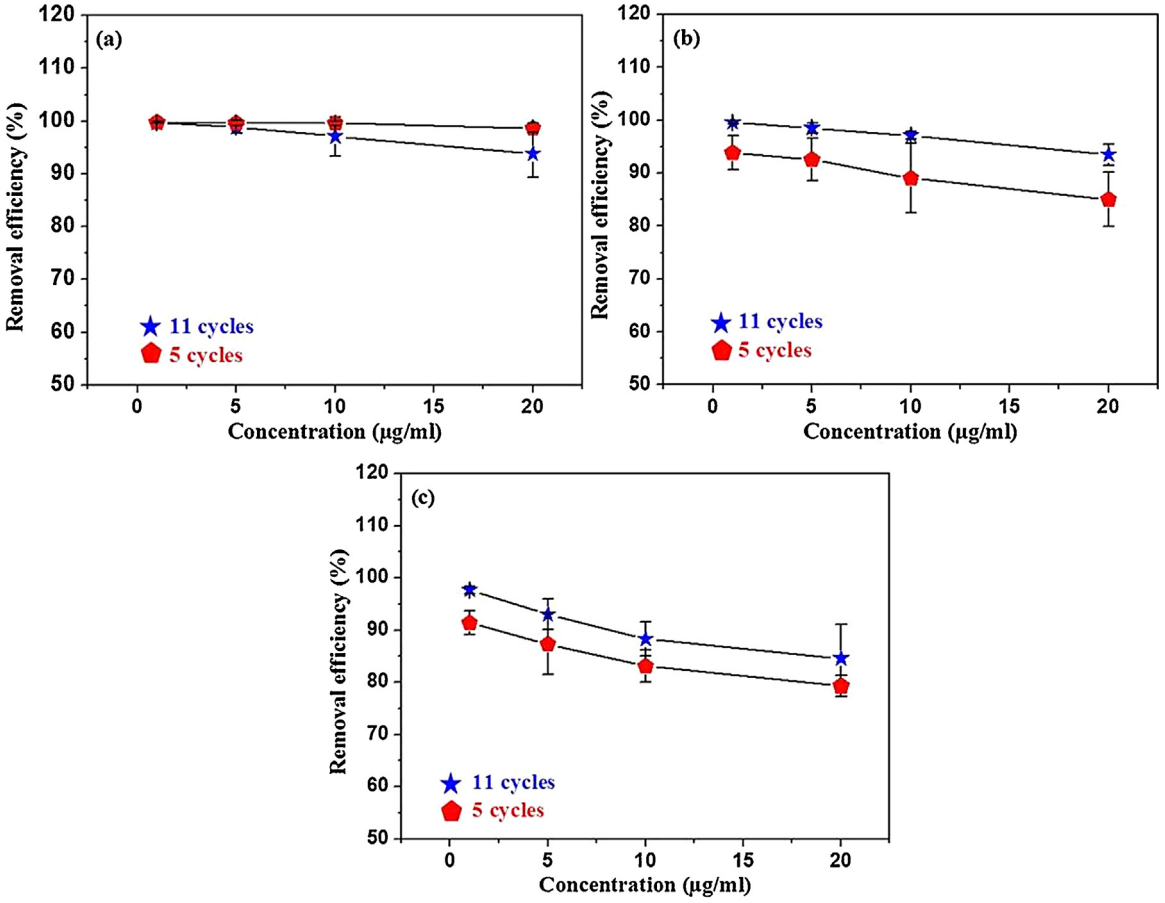

Fig. 2. Removal efficiencies of (a) physically adsorbed FITC–BSA, (b) physically adsorbed *E. coli* FITC–Ab, and (c) covalently bonded *E. coli* FITC–Ab by the CO_2 aerosol treatment for various concentrations (20, 10, 5, and 1 μg mL^{-1}) and two different aerosol treatment times (5 and 11 cycles). The error bars indicate one standard deviation.

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2.3. Protein removal via CO_2 aerosol treatment

The general experimental procedure of the CO_2 aerosol treatment was described by Cha et al. (Fig. S1) [18]. The Si chips functionalized with either *E. coli* FITC–Ab or FITC–BSA via the two methods were placed horizontally and positioned 2 cm from the nozzle in an aerosol flow. The nozzle axis was maintained at 40° angle relative to the chip surface. The chips were enclosed by a large acrylic cage. A dual gas unit (K6-10DG; Applied Surface Technologies, NJ, USA) was used for aerosol generation. The CO_2 gas pressure was 5.5 (±0.2) MPa and the N_2 gas pressure was 0.7 MPa. The measured solid CO_2 diameter ranged from 0.4 to 9.6 μm with the peak of 0.7 μm and the measurement procedure is shown in the Supporting information (Fig. S2). The CO_2 aerosols were off for 3 s during each 8-s removal cycle, and the total number of removal cycles was 5 and 11 (Fig. S1). The average room temperature was 24.8 (±1.1) °C, and the average relative humidity was 25.8 (±3.4)% during all aerosol treatments.

2.4. Regeneration of E. coli sensing chips

We also conducted an experiment on the regeneration of bacterial sensing chips using this CO_2 aerosol technique. The analyte for this test was *E. coli* XL1-blue. This *E. coli* strain (rod-shaped, 2 μm long and 0.5 μm in diameter) was transformed with pAmCyan plasmid (Clontech) which provides ampicillin resistance and encodes for the cyan fluorescent protein. 10 mL of Luria–Bertani (LB) broth with ampicillin (100 μg mL^{-1}) was inoculated in a sterile 50 mL conical tube with a single colony of the bacteria, and it was incubated with shaking at 160 rpm and 37 °C. The *E. coli* suspension was then serially diluted with LB broth until the OD_{600} reached 0.8, whose corresponding bacterial density was 8×10^8 cells mL^{-1}.

Firstly, *E. coli* FITC–Ab (10 μg mL^{-1}) were immobilized on the pre-cleaned Si chips via the covalent binding method using GPTMS as described earlier. The Si chips were incubated in 600 μL of *E. coli* suspension (OD_{600}: 0.8) at 25 °C for 15 min followed by gentle rinsing in 0.05% Tween-20 in PBS and dH_2O, and then N_2 drying. The 11 cycle aerosol treatment was then applied to these chips for regeneration of the sensor surface. This experiment was repeated 5 times by sequential immobilization and desorption.

2.5. Fluorescence and scanning electron microscopic analysis

The control and CO_2 aerosol-treated chips that had been functionalized with *E. coli* FITC–Ab and FITC–BSA were characterized by scanning electron microscopy (SEM) and fluorescence microscopy. For SEM analysis, the functionalized chips were coated with a thin layer of platinum for better resolution, and imaged with a scanning electron microscope (s-4800, Hitachi). All fluorescence microscope images were taken with 40 × lens (Nikon, NA: 0.75) and an epifluorescent microscope (Eclipse 80i; Nikon) connected to a CCD camera (Cool SNAP HQ2 Monochrome; Photometrics). The fluorescent dots of *E. coli* FITC–Ab and FITC–BSA were quite uniform in the images with few protein agglomerates (Fig. S3) and the number of fluorescent dots *N* was measured using ImageJ. The desorption effectiveness of the CO_2 aerosol treatment can be

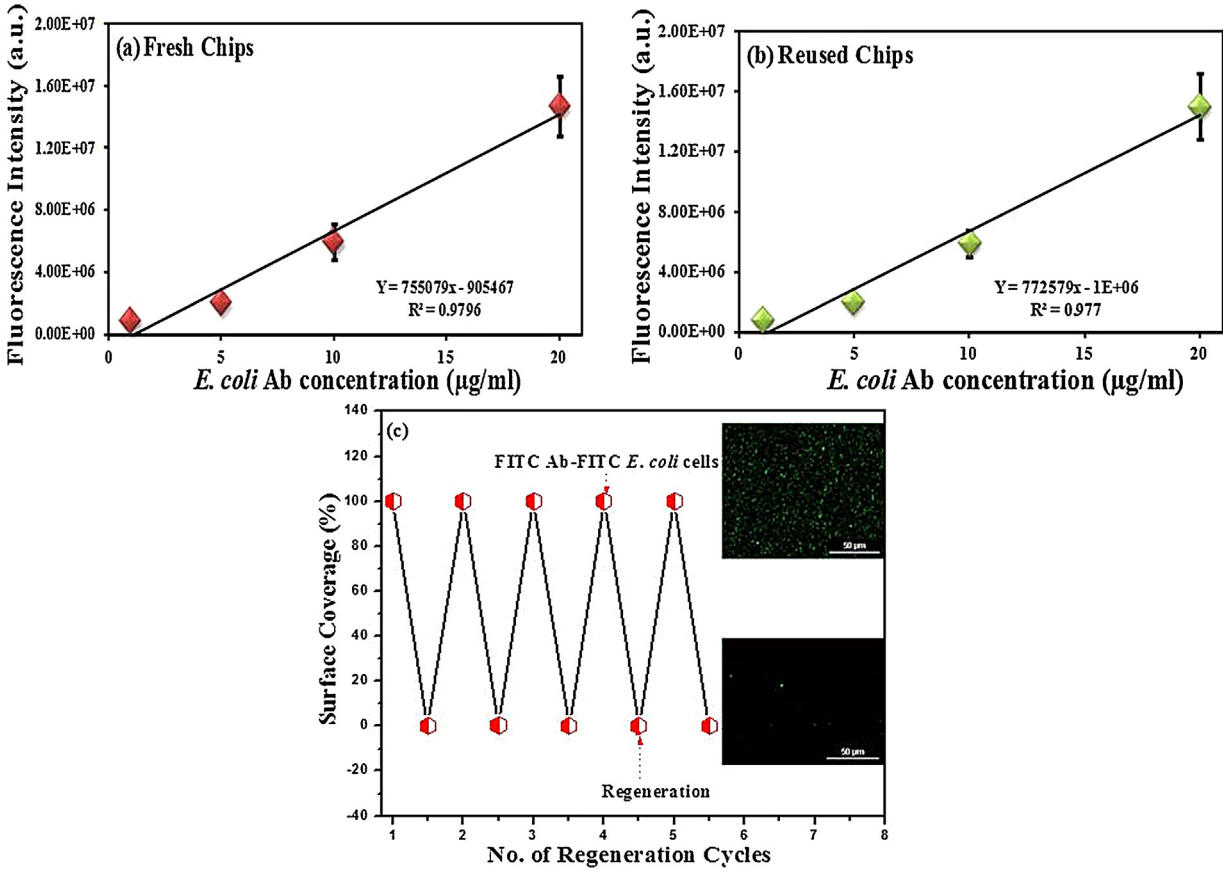

Fig. 3. Fluorescent intensity vs. FITC–Ab concentrations (20, 10, 5, and 1 μg mL^{-1}) for (a) fresh chips and (b) reused chips. (c) Surface coverage of the fluorescent dots as a function of the number of regeneration cycles, which consisted of *E. coli* cell capture by FITC–Ab (10 μg mL^{-1}) specific to *E. coli* immobilized onto GPTMS modified Si chips and subsequent removal using the aerosol treatment. The surface coverage was based on the number of fluorescent dots. The error bars indicate one standard deviation.

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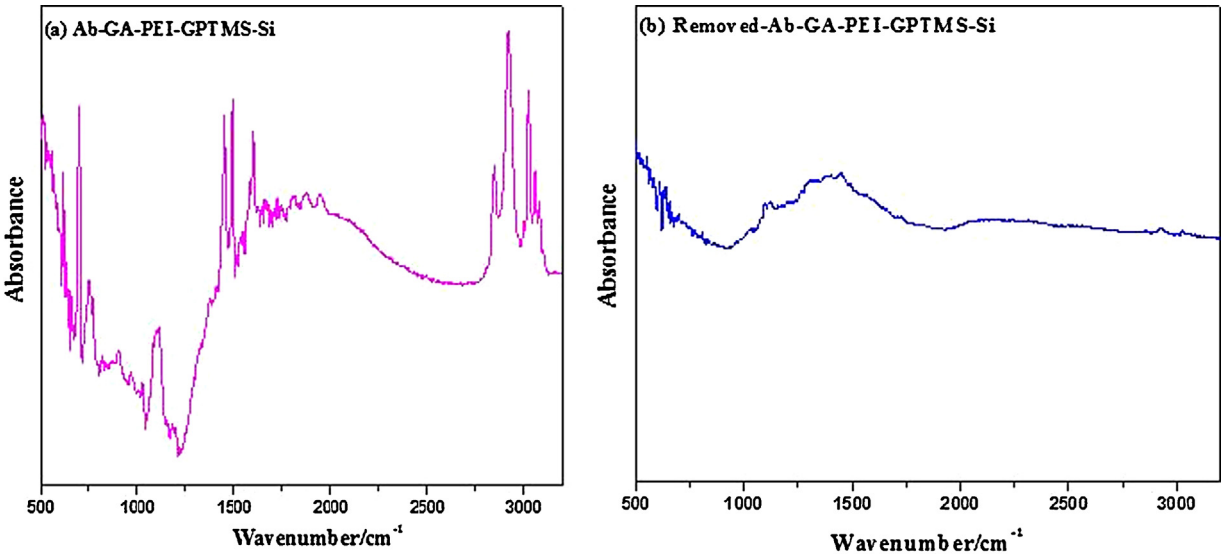

Fig. 4. FTIR spectra of (a) Ab–GA–PEI–GPTMS–Si, and (b) removed-Ab–GA–PEI–GPTMS–Si.

expressed in terms of the removal efficiency, which was computed according to the following formula:

$$100 \times \frac{N\,\text{of untreated chips} - N\,\text{of treated chips}}{N\,\text{of untreated chips}}.$$

2.6. FTIR spectroscopic and X-ray photoelectron spectroscopic analysis

A FTIR spectroscopic Varian 4100 (Agilent, USA) instrument was used for the structural characterization of the immobilized layers. XPS measurements were also performed using a K-Alpha X-ray Photoelectron Spectrometer (Thermo Fisher, UK).

2.7. Data analysis

Each set of the experiments was performed in quadruplicate for error analysis. The Student *t*-test was used to determine whether two sample means were significantly different or not.

3. Results and discussion

3.1. CO_2 aerosol treatment confirmed by fluorescence microscopy and SEM

Fluorescence images of the functionalized Si chips were taken for various concentrations (20, 10, 5, and 1 $\mu g\,mL^{-1}$) before and after 5 and 11 cycle treatment (Fig. 1). The FITC–BSA was physically adsorbed, and *E. coli* FITC–Ab was physically adsorbed as well as covalently bonded. As the treatment time increased, the number of green dots (*E. coli* FITC–Ab and FITC–BSA) in the images decreased from the surfaces, meaning that these proteins were removed by the CO_2 aerosol treatment. The effectiveness of the CO_2 aerosol technique for removal of these proteins was also observed in the SEM analysis (Fig. S4).

3.2. Removal efficiency analysis

All the fluorescence images were used to compute the removal efficiency (Fig. 2, Table S1). The removal efficiencies for physically adsorbed BSA and *E. coli* FITC–Ab for 5 cycles were not significantly different from those for 11 cycles (*t*-test, $P > 0.0660$ and $P > 0.0529$, respectively). That is, the treatment for only 5 cycles (40 s) showed the equivalent removal of physically adsorbed *E. coli* FITC–Ab and BSA for the tested range of concentrations. The removal efficiencies for FITC–BSA were slightly larger than those of the Ab at each concentration, which implies that the adhesion strength of FITC–BSA to the Si surface was lower than that of the Ab.

The removal efficiencies of covalently bonded *E. coli* FITC–Ab for 5 cycles were significantly smaller than those for 11 cycles at 1 $\mu g\,mL^{-1}$ case (*t*-test, $P = 0.0141$). The removal efficiencies for covalently bonded FITC–Ab were lower than those for the physically adsorbed FITC–Ab at each concentration, which means that the covalent binding offered higher adhesion strength. This observation is consistent with the reason why covalent binding methods have been used frequently for the functionalization in many biosensing platforms.

As the Ab concentration increased, the removal efficiency decreased for both binding methods. The removal efficiencies for 20 $\mu g\,mL^{-1}$ case were significantly different from those for 1 $\mu g\,mL^{-1}$ case (*t*-test, $P = 0.013$ for physically adsorbed Ab, and $P = 0.0277$ for covalently bonded Ab) at 11 cycle treatment. It may be attributed to the fact that the total number of generated solid

Table 1
XPS analysis results for Si and Ab–GA–PEI–GPTMS–Si chip before and after aerosol treatment.

Chips	Element									
	C_{1s}		N_{1s}		O_{1s}		Si_2p		S_2p	
	At (%)	BE (ev)	At (%)	BE (ev)	At (%)	BE (ev)	At (%)	BE (ev)	At (%)	BE (ev)
Si	0	–	0.86	401.08	25.32	532.93	58.98	99.87	0	–
Ab–GA–PEI–GPTMS–Si	69.40	285.84	12.03	399.46	12.28	532.03	13.53	99.43	3.21	167.88
Removed-Ab–GA–PEI–GPTMS–Si	14.49	285.90	5.51	400.28	21.14	532.23	32.79	99.22	0	–

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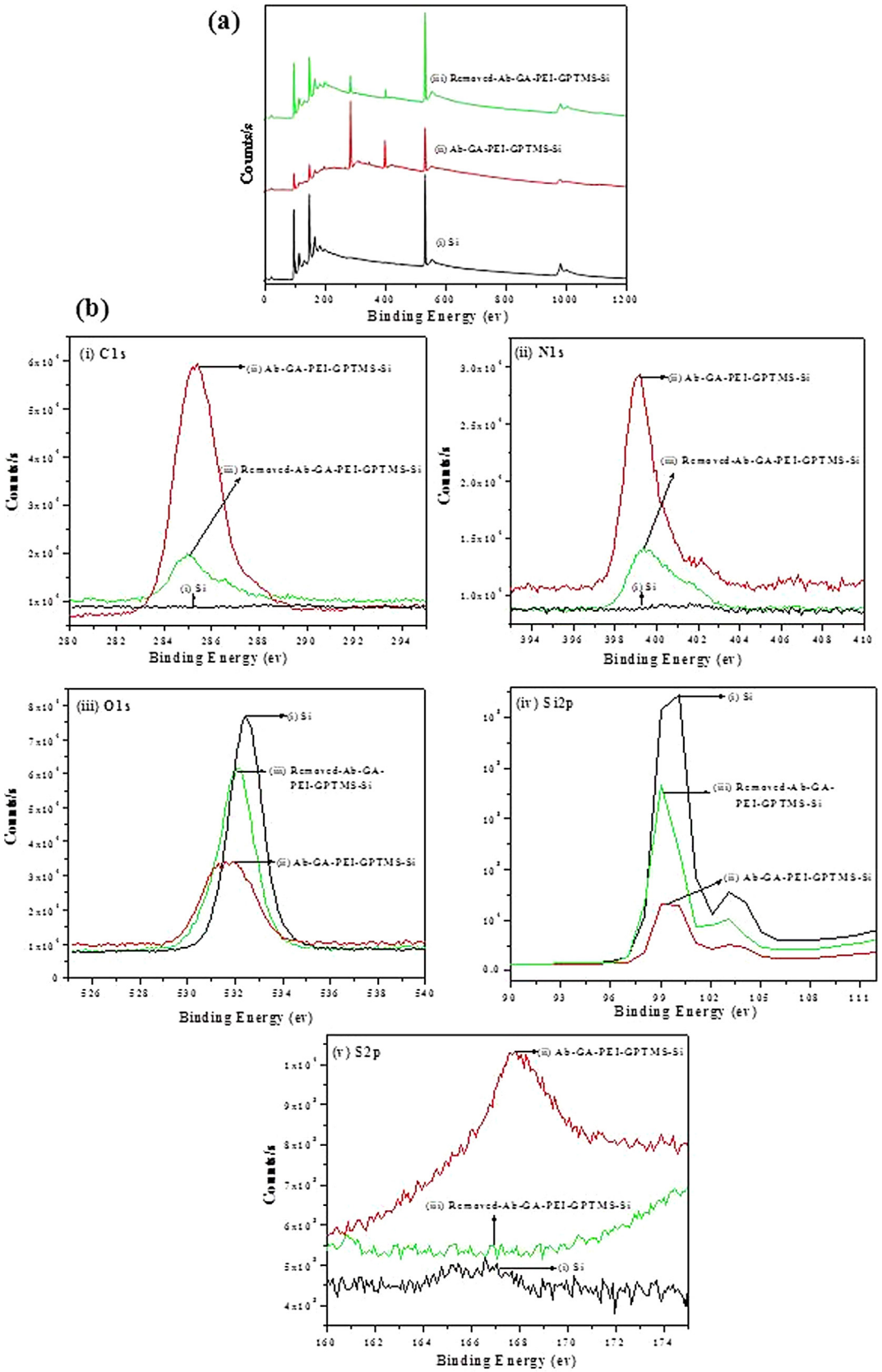

Fig. 5. Full XPS spectra of (a) (i) Si, (ii) Ab–GA–PEI–GPTMS–Si, (iii) Ab–GA-PEI–GPTMS–Si after aerosol treatment, and (b) (i) C_{1s} core level spectra (ii) N_{1s} core level spectra (iii) O_{1s} core level spectra (iv) Si_2p core level spectra (v) S_2p core level spectra.

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CO_2 particles, that is, the total removal power is almost constant, and the removal efficiency can depend on the area density of Ab immobilized on the surface. That is, if the density is small, the given number of CO_2 particles can be sufficiently large for removal, but if it is very large, the CO_2 particles may not be enough to remove the entire immobilized Abs. Moreover, the adhesion strength played an important role in the removal efficiency. For example, the removal efficiencies for covalently bonded Ab were not significantly different from those for physically adsorbed Ab at 5 cycles (t-test, $P > 0.1275$), but they were at 11 cycles (t-test, $P < 0.0412$). That is, the difference in the adhesion strength between the two binding methods became distinct at high removal power.

3.3. Calibration curves of fresh chips vs aerosol-treated Si chips

Fig. 3(a) and (b) illustrates the fluorescence intensity graphs of the (a) fresh and (b) reused chips via the aerosol-treatment as a function of the concentration (20, 10, 5, and 1 $\mu g\,mL^{-1}$) of the covalently bonded FITC–Abs. These experiments showed highly linear behaviors vs. Ab concentration, where the regression coefficients (R^2) were more than 97%. The relative difference in the fluorescence intensities of FITC–Abs between the fresh and reused chips was less than 3%, indicating that the aerosol-treated chips can be re-functionalized without any significant change in the Ab immobilization.

3.4. Regeneration of E. coli sensing chips

We also conducted an experiment on the regeneration of *E. coli* sensing chips by the CO_2 aerosol technique. This experiment of sequential immobilization and mechanical desorption using CO_2 aerosols was repeated five times (Fig. 3(c)). The number of fluorescent dots for a bare Si chip and the FITC–Ab/*E. coli* cells immobilized chips were set to be 0 and 100% respectively because both Abs and *E. coli* cells can be seen in the same image through an FITC filter. This test showed that the CO_2 aerosol technique was effective in removing *E. coli* bacterial cells as well as the covalently bonded protein layers from the Si chips, and that these chips can be re-functionalized for further use.

3.5. FTIR spectroscopic analysis

The FTIR spectra of Ab immobilized GA–PEI–GPTMS–Si chips before and after 11 cycle aerosol treatment are shown in Fig. 4 (spectra (a) and (b)). The FTIR spectrum of GPTMS showed the absorption bands around 840 cm^{-1} and 940 cm^{-1} (an epoxy absorption band) and it is due to Si—OH symmetric stretching vibration and asymmetric ring deformation. The Si—O—Si asymmetric stretching vibration appeared as a strong and broad band around 1034 cm^{-1}. The ether linkage in the glycidoxy group is known to be absorbed in the same region and it overlaps with the strong Si—O—Si absorption. The peak around 800 cm^{-1} was observed due to the symmetric Si—O—Si stretching vibration. The peaks seen at 571 cm^{-1} are attributed to the O—Si—O vibrations. The out-of-plane bending vibrations of the C—OH groups were seen as a weak peak at 664 cm^{-1} (Fig. 4(a) and Fig. S5 (a)) [23]. The absorbance bands for C—N stretching (1179 cm^{-1}) and C—H deformation (1437 cm^{-1}) occurred due to the presence of PEI (Fig. 4(a) and Fig. S5(b)). Shift of the C—OH stretching vibration from 1073 cm^{-1} to 1037 cm^{-1} shows the presence of glutaraldehyde (Fig. 4(a) and Fig. S5(c)). The amide bond formation between glutaraldehyde activated PEI–GPTMS–Si and Ab showed two new bands at 1552 cm^{-1} and 1642 cm^{-1}, which are mutually exclusive from the amide bands associated with the bound FITC–Ab (Fig. 4(a)). It should be noted that all the characteristic peaks of the immobilized layers vanished from the FTIR spectra of the Ab immobilized GA–PEI–GPTMS–Si chip after the CO_2 aerosol treatment (Fig. 4(b)). It can be concluded from this FTIR spectrum (b) that the CO_2 aerosol treatment yielded a bare Si surface that can be further re-used for another functionalization.

3.6. XPS analysis

The binding and removal of functional layers onto a Si chip was also proved by XPS, which is considered an effective tool to characterize the binding and removal of the functional layers. Fig. 5(a) shows the full XPS spectra of Si and Ab immobilized GA–PEI–GPTMS–Si chip before and after 11 cycle CO_2 aerosol treatment. Fig. 5(b) exhibits the core level spectra of (i) C_{1s}, (ii) N_{1s}, (iii) O_{1s}, (iv) Si_{2p}, and (v) S_{2p} elements. Table 1 shows the XPS analysis results for Si and Ab immobilized GA–PEI–GPTMS–Si chip before and after the aerosol treatment. The Si survey scan showed the presence of Si_{2p}, N_{1s}, and O_{1s} at 99.87, 401.08, and 532.93 eV binding energy (BE) with the atomic (At) concentration of 99.87, 0.86, and 25.32% (spectrum (i) of Fig. 5(a)). In a survey scan of Ab immobilized GA–PEI–GPTMS–Si chip, C_{1s}, N_{1s}, O_{1s}, and Si_{2p} signals at the BE of 285.84, 399.46, 532.03, and 99.43 eV, respectively, was observed at At concentration of 69.40, 12.03, 12.28, and 13.53%, respectively (spectrum (ii) of Fig. 5(a)). The presence of C_{1s}, increase in N_{1s} and decrease in the O_{1s} peaks in the Ab immobilized GA–PEI–GPTMS–Si chip confirmed the binding of the GPTMS, PEI, GA and Ab on the Si chip. Additionally, the S_2p signal at 167.88 eV with an At concentration of 3.21% appeared due to the presence of sulfur group in the Ab. After the aerosol treatment, the C_{1s} and N_{1s} signals decreased to 14.49 and 5.51% respectively (spectrum (iii) of Fig. 5(a)). On the contrary, the O_{1s} and Si_{2p} signals increased up to 21.14, and 32.79% respectively. Furthermore, the S_{2p} signal was not observed in the CO_2 aerosol treated chips, revealing the removal of Ab. From these results, it was confirmed that all the functional groups was almost removed from the chips by the aerosol treatment and the chips were ready to be re-functionalized.

3.7. Parameters affecting the removal efficiency

It was observed that the removal efficiency depended on a protein, protein concentration, and the binding method. There are several choices to increase the removal efficiency including longer treatment time. It should also be noticed that the cleaned area due to the CO_2 aerosol treatment ranged from several hundreds of micron to several millimeters, which may allow selective regeneration of sensing areas.

In comparison to other reusability techniques, this approach has several advantages. This does not depend much on probe molecules, substrate material, and target analytes because the immobilized proteins are removed mostly by the mechanical impact of CO_2 solid particles [16]. Moreover, it is readily reusable and can be switched to detect different analytes easily. In fact, it took a small amount of time (40–88 s) for regeneration of the sensing surface up to 99%. However, this technique also poses several drawbacks. The surface should not be immersed in liquids for effective desorption and the surface may suffer from high pressure due to the aerosol jets.

4. Conclusions

We presented a novel protein desorption technique using carbon dioxide (CO_2) aerosols to reuse a biosensor and demonstrated its feasibility by removing physically adsorbed FITC–BSA and *E. coli* FITC–Ab, and covalently bonded FITC–Ab from Si chips for two removal times (5 and 11 cycles). The removal efficiency due to 11 cycle treatment varied from 93.85% to 99.65% for physically

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adsorbed FITC–BSA, from 93.50% to 99.60% for physically adsorbed *E. coli* FITC–Ab, and from 84.6% to 97.69% for covalently bonded *E. coli* FITC–Ab. The removal of the fluorescent proteins using the aerosol treatment was shown by fluorescence microscopy and SEM. FTIR spectroscopy and XPS also revealed the confirmation of removal of the functional groups from the Si chips. The fluorescence intensity graphs of fresh and reused chips showed that the CO_2 aerosol desorption process did not influence the sensor sensitivity. We also conducted an experiment on the regeneration of *E. coli* sensing chips. These chips were re-used 5 times by sequential immobilization and desorption using CO_2 aerosols, and they can be re-used more times. This mechanical desorption technique can be a good alternative to the current reusability techniques for biosensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.11.006.

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