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

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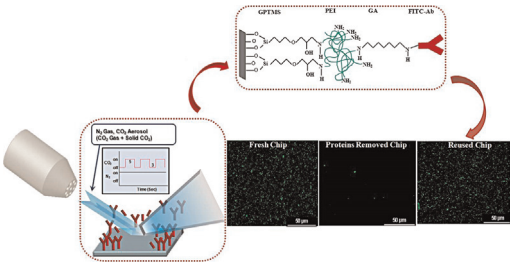
HIGHLIGHTS

- 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.

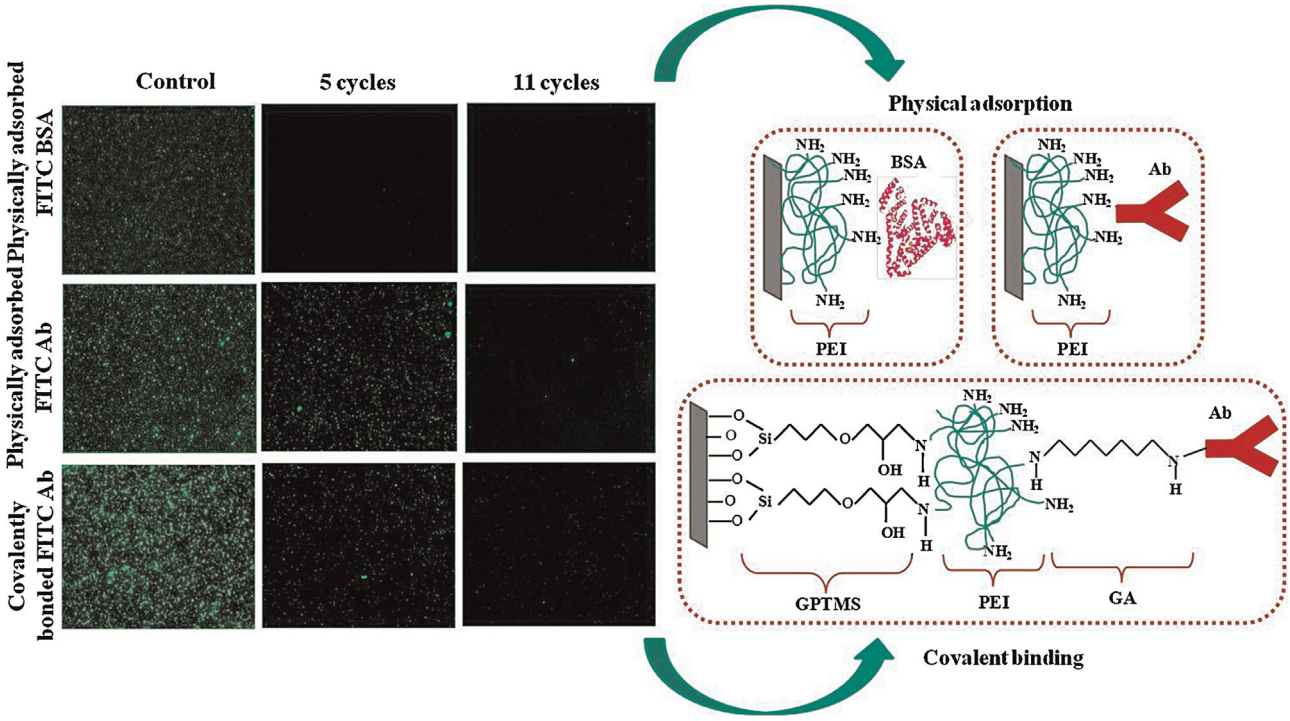

Fig. 1. Fluorescent images of physically adsorbed FITC–BSA, *E. coli* FITC–Ab, and covalently bonded *E. coli* FITC–Ab for control (20 μg mL^{-1}) and two different removal times (5 and 11 cycles) and the schematic of used physical adsorption and covalent binding methods.