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RESEARCH

Lab on a chip for detecting Clara cell protein 16 (CC16) for potential screening of the workers exposed to respirable silica aerosol

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Abstract

Early detection of pulmonary responses to silica aerosol exposure, such as lung inflammation as well as early identification of silicosis initiation, is of great importance in disease prevention of workers. In this study, to early screen the health condition of the workers who are exposed to respirable silica dusts, an immunoassay lab on a chip (LOC) was designed, developed and fully characterized for analyzing Clara cell protein 16 (CC16) in serum which has been considered as one of the potential biomarkers of lung inflammation or lung damage due to the respirable silica dusts. Sandwich immunoassay of CC16 was performed on the LOC developed with a custom-designed portable analyzer using artificial serums spiked with CC16 protein first and then human serums obtained from the coal mine workers exposed to the respirable silica-containing dusts. The dynamic range of CC16 assay performed on the LOC was in a range of 0.625–20 ng/mL, and the achieved limit of detection (LOD) was around 0.35 ng/mL. The assay results of CC16 achieved from both the developed LOC and the conventional 96 well plate showed a reasonable corelation. The correlation between the conventional reader and the developed portable analyzer was found to be reasonable, resulting in $R^2 \sim 0.93$. This study shows that the LOC developed for the early detection of CC16 can be potentially applied for the development of a field-deployable point-of-care testing (POCT) for the early monitoring of the field workers who are exposed to silica aerosol.

Keywords Immunoassay · Lab on a chip (LOC) · Point-of-care testing (POCT) · Early screening of respirable silica exposure · Detection of Clara cell protein (CC16)

1 Introduction

There has been a growing interest in point-of-care testing (POCT) for early monitoring of the workers who are potentially exposed to toxic aerosols such as nanomaterials and crystalline silica dusts for epidemiological research (Gulumian et al. 2006; Sato et al. 2006; Prakova et al. 2005; Altindag et al. 2003; Pingle et al. 2008; Trout and Schulte 2010; Howard and Murashov 2009). The National Institute for Occupational Safety and Health (NIOSH) published a Hazard Review report on the health effects of occupational exposure to respirable crystalline silica (RCS) (NIOSH 2002). Preventing or reducing silica-related diseases and development of methods for earlier detection or more definitive, non-invasive evaluation of silica-related pulmonary disease have been identified as critical research needs. Chest radiography (CR) using X-ray films and lung function abnormalities are currently used as clinical detection methods for diagnosis of silicosis in workers, but these methods

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have limitations for early monitoring of the workers who are potentially exposed to crystalline silica dusts for epidemiological research, since it can diagnose only the late severity of the silica induced disease (Gulumian et al. 2006).

On the other hand, toxicological studies on silica exposure in animals and humans, using blood or urine specimens, have indentified a couple of potential biomarkers for silicosis (Deniz et al. 2012; Morfeld et al. 2001; Bernard et al. 1994, 1998; Andersson et al. 2007; Schins et al. 1996; Schins 1996; Arcangeli et al. 2001). A comprehensive review study (Gulumian et al. 2006) reported several potential biomarkers for silica-induced silicosis, such as Clara cell protein-16 (CC16), tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), IL-6, and reactive oxygen species (ROS). Among them, CC16 has been considered as one of the most potentially suitable biomarkers for the early screening of silicosis (Sarkar et al. 2021; Naha et al. 2020; Thongtip et al. 2020; Nandi et al. 2021). Other studies showed that some biomarkers were increased with the progression of silicosis (Pingle et al. 2008; Altindag et al. 2003). Thus, the development of a new easy-to-use screening tool toward a POCT system, which is capable of rapid and direct monitoring of the biomarkers indicating lung inflammation or fibrosis, is very desirable for the assessment of worker exposure to silica aerosol at the early stage.

Recently, a rapid diagnostic test (RDT) was developed for screening of occupational silica dust exposed workers to early detect silicosis/silico-tuberculosis using a lateral-flow strip assay for semi-quantitative estimation of serum CC16 level (Nandi et al. 2021). However, since there is a large demand for the high-sensitive as well as quantitative analysis of the target biomarkers for screening of the workers exposed to silica aerosol in occupational setting, the new development of immuoassay-based POCT with a minimal user intervention is very desirable and allows us to detect the pulmonary response of the exposed workers (Foudeh et al. 2012) by overcoming the disadvantages of the existing assay methods.

Thus, a sample-to-answer LOC for the application of POCT was newly proposed and designed for performing the chemilunescence-based sandwich immunoassay in this work, as shown in Fig. 1. The LOC has reserviors to contain the reagents in liquid which were required for the immunoassay to be perforemed on the LOC. Pressure-driven reagents in liquid on the LOC could flow sequentially through the microchannels, which autonomously performed the immunoassay of CC16. To smartly monitor the flow sequece, a new flow monitoring method using infrared was developed and applied. A custom designed optical analyzer was also develelped for reading the LOC.

In this work, a new field-deployable POCT with a sample-to-answer polymer LOC and portable analyzer was successfully developed, characterized, and applied for the detection of CC16 biomarker for the early screening of the field workers who were exposed to silica aerosol.

2 Design and microfabrication of LOC

2.1 Design of polymer lab on a chip (LOC)

The design of LOC was based on the design from our previous work (Thiyagarajan Upaassana et al. 2019) with some modifications made to address the several issues that emerged from this study. As shown in Fig. 1, the LOC contains three reservoirs for preloading the

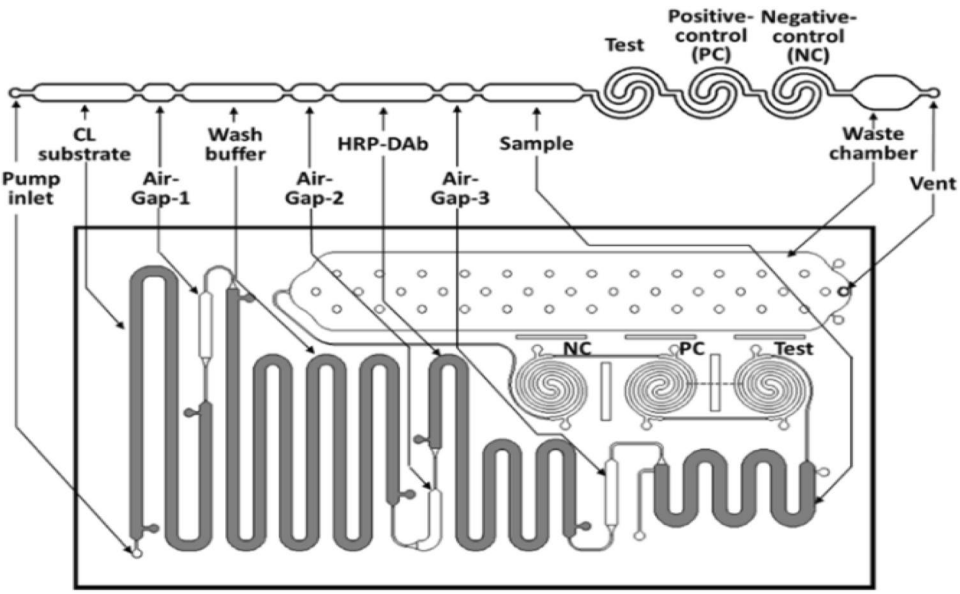

Fig. 1 Flow system (top) and LOC (bottom) design with reservoirs for on-chip reagents, air gap to prevent on-chip reagents from mixing, and spirals channels as reaction zones

chemiluminescence-based immunoassay reagents such as chemiluminesence substrate, washing buffer, and detection antibody conjugated with hydroperoxide enzyme (HRP-DAb). Air gaps in hydrophobic surfaces are placed between the reservoirs to keep the reagents be separated during storage. One main modification was that the volume of air gap between each reservoir was reduced to 2.0 µL to minimize the effect of airplane cabin vacuum pressure on the stability of the reagents' position during shipment via airplanes. The modified LOC with air gap reduced to 2 µL having hydrophobic surface coating and a pinching valve could minimize the moving of the reagents during the shipment, because the reduced air gap volume is less susceptible to vacuum pressure than larger volume air gap, preventing contamination of two reagents by mixing. This modification improved the reliability and reproducibility of the LOC data.

For performing the assay, sample is loaded first and then let the reagents stored in reservors sequentially flow through the spiral channels in sequence by pumping air to the pump inlet. The sample and reagents flow through the test sprial (TEST) coated with a target capture antobody (CsAb) first, then the positive control spiral (PC) coated with a control capture antibiody (PcAb) coated for control, and lastly the negative control spiral (NC) coated with blocking buffer to measure the background noise generated from any non-specific binding during the assay. The optical signal from the NC channel is subtracted from the LOC test channel (TEST) output to improve signal-to-noise ratio. In addition, new optical blocks, which are made of a simple slit, are added between the spiral channels to prevent the optical crosstalk through the polymer chip between the test and control spiral channels, since the optical signals from the two spiral channels are almost concurrently produced during the chemiluminescence assay. The spiral reaction chambers within the LOC device were designed to hold sufficient quantities of reagents, such as the Horseradish Peroxidase (HRP) conjugated detection antibody, wash buffer, and CL substrate. These chambers were precisely tailored to bolster the execution of the CL-based immunoassay. To offer a safe and user-friendly means for disposing of the LOC device, an extra chamber (i.e., waste chamber) was integrated. This particular chamber was intentionally designed to accommodate all utilized reagents and any potentially biohazardous samples. For a comprehensive insight into the dimensions and capacities of the chambers in the LOC, refer to Table 1.

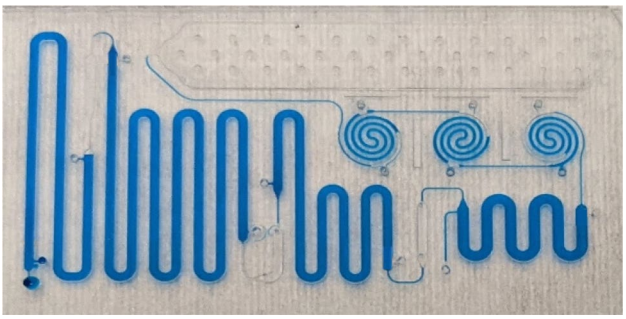

Fig. 2 Picture of the fabricated LOC filled with dyed DI water. The dyes filled in reservoirs proved the functionality of using air gaps to separate assay reagents and storing them for long-term stability

2.2 Microfabrication and surface control of LOC

The LOC was microfabriated with cyclic olefin copolymer(COC) using injection-molding technology as reported (Thiyagarajan Upaassana et al. 2019). Inlet and outlet holes were drilled on the injection-molded COC chip using a microdrilling machine. Thermal bonding of a blank COC chip over the injection-molded COC chip was made to form the microchannels and reservoirs on the LOC. Then, the surfaces of reserviors were coated with a hydrophilic coating solution of P100d (Joninn, Denmark) to prevent the surface from absorbing the reagents and target protein during storage and assay (Sohn et al. 2004).

During the storage of liquid reagents on the LOC at 4 °C, we noticed that the HRP-dAb solution moved out of the reservoir and contaminated the sample loading chamber, which needed to be prevented. To resolve these issues, we coated the Air-Gap-2 and Air-Gap-3 with Fluoropel 800 solution (Cytonix, MD, USA) which has a contact angle of 110°. Thus, the high hydrophobicity achieved on the air gaps prevents movement of the HRP-dAb solution during storage (Zhang et al. 2008). Picture of the fabricated LOC filled with dyed DI water is shown in Fig. 2.

Table 1 Sizes and capacities of various chambers within the designed LOC dedicated for both reagent storage and sample loading

Chambers on LOC	Width (mm)	Height (mm)	Length (mm)	Volume (µl)
Sample loading chamber	1.2	0.35	44	18.5
Spiral reaction chamber (for each TEST, PC, & NC)	0.35	0.25	45.7	4
Air gap between chambers	1	0.35	5.8	2
Detection antibody chamber	1	0.35	72	25
Wash buffer chamber	1	0.35	143	50
Chemiluminescence substrate chamber	1	0.35	72	25

2.3 Preparation of ready-to-test LOC

The ready-to-test LOC was prepared with several required processes which include the surface modification and the immobilization of capture antiboides through the sprial channels. First, capture antibody (CsAb; rat anti-human uteroglobin capture antibody) in a concentration of 64.0 µg/mL and positive control capture antibody (PcAb; goat anti-rat IgG) in a concentration of 160.0 µg/mL were applied to the test spiral channel (TEST) and the positive control spiral channel (PC), respectively, and then incubated both for 10 min at room temperature. Then, blocking buffer was applied and incubated in all three spirals for 10 min at room temperature. Finally the LOC was vacuum-dried at 95 kPa for 15 min at room temperature to make a dry format of antibodies on the surface of spiral channels. After completing the vacuum-drying process, all required assay reagents in liquid were loaded onto the chip and the chip was immediately sealed, packaged, and then stored at 4 °C as a sealed package. All the on-chip assay reagents from the LOC were prepared, as specified in Table 2.

After loading liquid reagents in the LOC, a sheet of ThermalSeal RTS™ Sealing Film (Excel Scientific, CA, USA) was covered on the LOC surface to seal all the ports on LOC. The seal was first pressed onto the chip with a plastic paddle, then an even pressure of 25 psi was applied evenly onto the LOC top surface by a hydraulic press (Wabash Press P3H-15-CLX) to achieve a firm seal. Then, the LOC was placed inside a heat-seal aluminum pouch and sealed for packaging. All the packaged LOCs were then stored at 4 °C.

2.4 Operation and test protocol

The LOCs were stored at 4 °C for up to 1 month before testing with human samples. Before test the packaged LOC was brought up to room temperature for 15 min. Afterwards, the sample loading port and the sample loading vent were unsealed, then the sample or standard was pipetted into the sample reservoir before being sealed again. The waste chamber vent and pump inlet port were opened, and the LOC was placed into a gasket-seal frame built for pump operation.

The frame was hooked to the peristaltic pump to drive the flow. The assay operation was performed with sequential steps of loading the reagents and incubation of the reagents in the spiral channels. For the on-chip chmemiluminescence assay described in Figs. 1 and 3, the sample in a form of serum or human plasma was loaded into the spiral channels. CsAb in TEST channel captures the target antigen in the sample. After 10 min of incubation, the air in Air-Gap-3 pushes the sample into the waste chamber and the HRP-detection antibody (HRP-DAb) conjugate reagent was loaded into the spiral channels and incubated for 10 min. The HRP-DAb bound to the captured target antigen in TEST channel, while a PcAb with epitopes for DAb captured the HRP-DAb in the PC channel to indicate the functionality of DAb and identify false-negative result. Finally, washing buffer (i.e., Phosphate Buffered Saline [PBS]) was flown through the spiral channels to remove any signal altering residues in the spiral channels before chemiluminescent (CL) substrate was loaded into the spiral channel for reading. Since the three spiral channels were positioned similar to the locations of wells in 96-well microplate, the LOC was able to be read using a conventional 96-well plate reader. TECAN reader (Infinite M200 Pro, TECAN, Austria) and

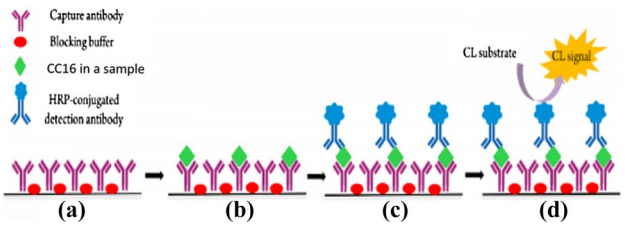

Fig. 3 Immunoassay protocol based on chemiluminescence (Thiyagarajan Upaassana et al. 2019). **a** Capture antibodies; **b** immobilization of CC16; **c** detection antibodies; and **d** chemiluminescence substrate

Table 2 Assay reagents in LOC

	Diluting buffer	CC-16	
		Reagent	Concentration
Test CsAb	PBS	Rat anti-human uteroglobin capture antibody	64 µg/mL
Positive-control capture antibody PcAb	PBS	Goat anti-rat IgG	160 µg/mL
HRP-dAb	Reagent diluent, 1% BSA in PBS, 0.2 um filtered	HRP–stretavidin-biotinylated rat anti-human uteroglobin detection antibody	16 µg/mL
Washing buffer	n/a	PBS	
Chemiluminescent substrate	n/a	UniGlow™—one-component chemiluminescent substrate (Rockland, PA, USA)	
Blocking	n/a	Liquid plate sealer® stabilizer coating (Candor, Germany)	

Biotek reader (Synergy H1, BioTek, USA) were used in facilities in South Korea and USA, respectively.

2.5 LOC immunoassay optimization for CC16

The chemiluminescence-based immunoassay protocol is summarized in Fig. 3 (Thiyagarajan Upaassana et al. 2019; Jung et al. 2013). For the optimization of chemiluminescence-based sandwich assay on the developed LOC, Opti-96™ microfluidic microplate (Mico Biomed Inc. USA) was used, since the LOC was designed and developed to have the same spiral microchannels of Opti-96™ microfluidic microplate for performing and comparing both immunoassays. Thus, antibody concentrations were optimized first with the chemifluorescent (CF)-based assay using Opti-96™ microfluidic microplate. To obtain the optimal antibody concentration, CF-based CC16 immuoassay was performed in Opti-96™ microfluidic microplates using all the assay reagents at a fixed concentration except for the antibody to be optimized (Jung et al. 2013; Kai et al. 2012). The concentrations of antibody were varied over a wide range from the results of the assay, thus the antibody concentration exhibiting the highest CF signal was to be determined as the optimal concentration. For all optimizations, 1.0 ng/mL of human CC16 antigen concentration was used as a reference.

Using the same optimization protocol used for the Opti-96 microfluidic microplate, optimization assays for the capture antibody (CsAb) as well as the detection antibody (dAb) in terms of Human CC16 were performed only over the spiral channel of the LOC platform. Figure 4a shows the obtained assay results which were matched well with those obtained from the Opti-96™ microfluidic microplates. From Fig. 4a, the optimized capture antibody (CsAb) concentration was found around at 64.0 µg/mL. For optimizing the detection antibody (dAb), CsAb concentration was fixed at the optimized value, 64.0 µg/mL, and the dAb concentration was varied from 4.0 µg/mL to 64 µg/mL. As shown in Fig. 4b, the optimal dAb concentration was found around 16.0 µg/mL.

Similarly, the optimization assay of positive control PcAb concentration was performed by varying capture antibody concentration from 20 µg/mL to 1280 µg/mL with a fixed optimized dAb concentration of 16.0 µg/mL. Figure 5 shows the optimal PcAb concentration was found at 160.0 µg/mL (Goat anti-rat IgG) for Human CC16.

With the optimal antibody concentrations, the assay conditions for CC16 on the LOC were set to achieve a optimized assay performance.

3 Analysis of CC16 on the developed LOC using human samples

3.1 Collection of human sample for CC16

A group of 20–30 retired male coal mine workers who had been exposed to the coal mine dust containing silica, were

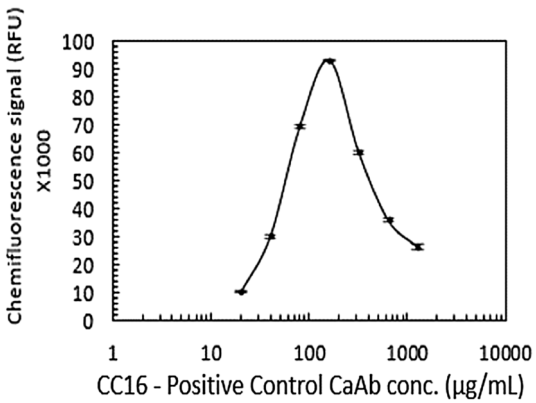

Fig. 5 Fluorescence assay results obtained from Opti-96™ microchannel microplate for positive control capture antibody (PcAb) optimization for CC16 positive control

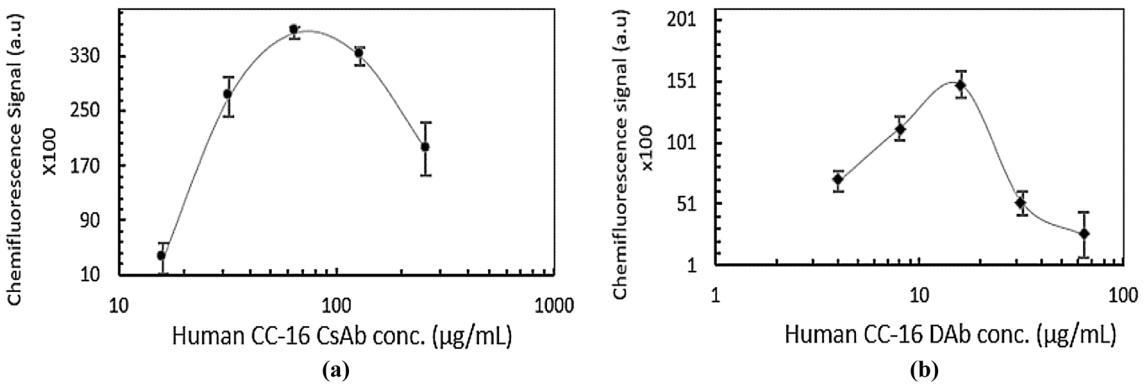

Fig. 4 Optimization assay results performed only over the spiral channel of the LOC platform for the capture antibody (CsAb) (**a**) and the detection antibody (dAb) (**b**) in terms of Human CC16

examined for pneumoconiosis (potentially silicosis) at an affiliated hospital of South Korea Workers' Compensation & Welfare Service (COMWEL) for this study. Among them, 10 non-pneumoconiosis miners and 10 miners with pneumoconiosis were chosen for testing the developed LOCs and evaluation of LOC performance. Personal information including age, body weight, height, job history, and smoking status was obtained using a structured questionnaire. All study subjects gave written informed consent on the use of their biosamples for research purposes. The biosample (i.e., serum) and personal information of each study subject were obtained by the Institute of Occupation and Environment (IOE), South Korea and approved by the Institute of Review Board (IRB) of IOE and NIOSH.

Pulmonary function test (PFT) was conducted in accordance with guidelines recommended by the American Thoracic Society (ATS)/European Respiratory Society (ERS) Task Force (Brusasco et al. 2005) using spirometry (Vmax22, SensorMedics, San Diego, CA). The measured parameters were forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) predicted, and $\%FEV_1$/FVC (Morris et al. 1971). Chest radiographs for pneumoconiosis were obtained by the digital chest X-ray (Digital Diagnostics, Philips, Netherlands). Diagnosis of pneumoconiosis was in accordance with the guidelines of the pneumoconiosis review committee of COMWEL in South Korea and were categorized by the International Labor Organization (ILO) classification scheme (ILO 2002). The coal miners who did not have pneumoconiosis (ILO classification of 0/0) were chosen as the control group and the miners with ILO classifications of 1/1 to 2/3 used as the exposed group. The PFT and information about workers for control and exposed groups are summarized in Table 3. The concentration of CC16 in silica dust-exposed workers (exposed group) was found to decrease due to the damage of epithelial cells, in other word, lung injury, compared to that of CC16 for healthy workers (control group), as reported in the literature (Gulumian et al. 2006).

3.2 Examination and analysis of human sample for CC16

Serum was centrifuged from whole blood samples of study subjects and stored at -80 ℃ until assay. Serum levels of CC16 were measured by enzyme-linked immunosorbent assay (ELISA) kits (CC16: Human Uteroglobin Quantikine ELISA Kit, DUGB00, R&D Systems, Inc.) according to the manufacturer's protocol (see Fig. S1 in the supplementary information for standard curve for CC16 in serum obtained from the ELISA kit). The DuoSet ELISA kits used in the LOCs were purchased from R&D Systems (Minneapolis, MN USA) and included capture antibody, detection antibody, standard, and streptavidin–HRP for CC16. Chemiluminescent substrate (UniGlow™) was purchased from Rockland (PA, USA). The correlation between the LOC with portable analyzer and conventional reader was examined by Origin software (OriginLab Corporation, Northampton, MA), as shown in Fig. S2.

3.3 Assay results of CC16 on the developed LOCs

The assay standard curves for CC16 using the developed LOCs were obtained, as shown in Fig. 6. A range of concentration from 0.625 ng/mL to 20 ng/mL for CC16 was spiked in artificial serum Serasub™ (CST technologies, USA) as the standard samples. In Fig. 6, the assay results

Table 3 Age, BMI, working duration, pulmonary lung function and CC16 concentrations in serum for control group (i.e., workers without pneumoconiosis) and exposed group (i.e., workers with pneumoconiosis)

Parameters	Workers without pneumoconiosis[d] ($n=10$)	Workers with pneumoconiosis[d] ($n=10$)
Age (year), mean ± SD (range)	60.9 ± 9.2 (46–73)	65.8 ± 6.3 (57–79)
[a]BMI (kg/m^2), mean ± SD (range)	24.3 ± 2.5 (19.6–27.0)	23.8 ± 3.2 (17.8–29.0)
Working duration (year), mean ± SD (range)	19.3 ± 5.8 (8–30)	19.2 ± 8.0 (4–30)
Number of current smokers with COPD[b]	2	2
Number of current smokers without COPD	3	2
ILO category	0/0	1/1, 1/2, 2/2, 2/3
%FVC, mean ± SD (Range)	95.0 ± 7.6 (80.3–105.9)	88.4 ± 17.7 (120.6–55.9)
$\%FEV_1$, mean ± SD (Range)	95.5 ± 17.2 (50.9–118.1)	85.8 ± 23.1 (125.2–44.3)
$\%FEV_1$/FVC, mean ± SD (range)	72.2 ± 13.2 (37.7–83.5)	67.4 ± 9.1 (54.7–81.4)
CC16 (ng/mL), mean ± SD[c] (range)	21.37 ± 10.14 (7.88–40.90)	15.51 ± 8.03 (6.58–33.60)

[a]BMI stands for body mass index, which is a measure of body fat based on height and weight of a worker, and defined as a weight in kilograms divided by the square of height in meters

[b]COPD means chronic obstructive pulmonary disease and was determined based on the value of FEV_1/FVC during the pulmonary function test, which was less than 70%

[c]CC16 concentrations were determined using the 96-well plate ELISA. All samples were run in duplicate

[d]All workers are male

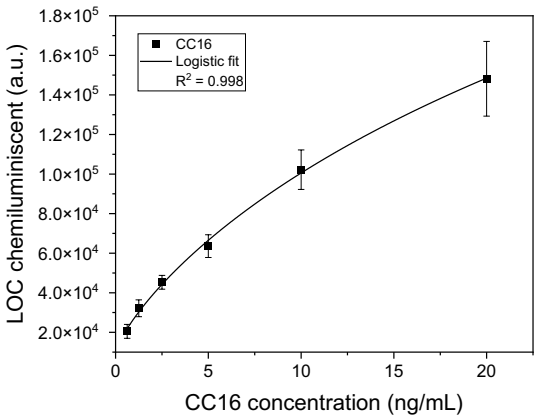

Fig. 6 CC16 LOC standard curve. Each data point represents a replication of 3

were calculated by subtracting the relative signal of the negative control spiral channel (NC) from the signal of the test spiral channel (TEST). The limit of detection (LOD) was calculated based on limit of blank (LOB) and test replicates of an analyte for the LOC: LOB = mean blank + 1.645(SD for blank), LOD = LOB + 1.645(SD for lowest concentration sample), where SD is the standard deviation. Limit of quantification (LOQ) was calculated as LOQ = mean blank + 10 * SD (for blank). All sample concentrations were analyzed in triplicate. It was found that the LOD was about 0.35 ng/mL and lower LOQ was about 0.61 ng/mL. The overall assay time was about 30 min per sample, which was much faster than the 96 well plate ELISA.

3.4 Correlation between the LOCs and 96-well plate ELISA measurements

Finally, the CC16 assays on the develoepd LOC were performed with the human sera from the workers in South Korea as described in the previous section. Then, the obatained assay results from the LOCs were compared with those from 96-well plate. Given the limited number of human samples and the insufficient numbers of available LOCs due to the transport limitation to South Korea (e.g., some leakage of the liquid reagents stored in the LOCs), we plotted all data points we acquired from each LOC against the CC16 biomarker concentration from standard 96 well plate ELISA as a cluster plot (Fig. 7). Figure 7 shows all the human subject sera CC16 levels range between 10.0 and 41.0 ng/mL. The results showed that the CC16 chemiluminescence signal from the LOC devices correlated with the conventional assay results in Fig. 7a. The correlation curve between the two measurements in the CC16 range of 10–41 ng/mL was obtained using a four parameter logistic (4PL) fit in Fig. 7a. The curve fit resulted in $R^2 = 0.85$, which is simliar to that of the linear fit ($R^2 = 0.82$, Fig. S2 in the supplementary information). Figure 7b shows LOC response as a function of CC16 concentration compared to the 96 well plate data, giving a correlation of $R^2 = 0.80$, indicating that our LOC data have a reasonable correlation with the 96-well assay measurement. Thus, based on this reasonably good corelation and validation of the CC16 assay results obtained from the developed LOCs, we were able to move to perfom the CC16 assay on the developed LOCs with the custom-designed portable analyzer as a POCT application (see Figs. S3, S4, S5 and S6 for the flow monitoring sensor and custom-designed portable analyzer, as decribed in supplimentary information and to be published in other journal).

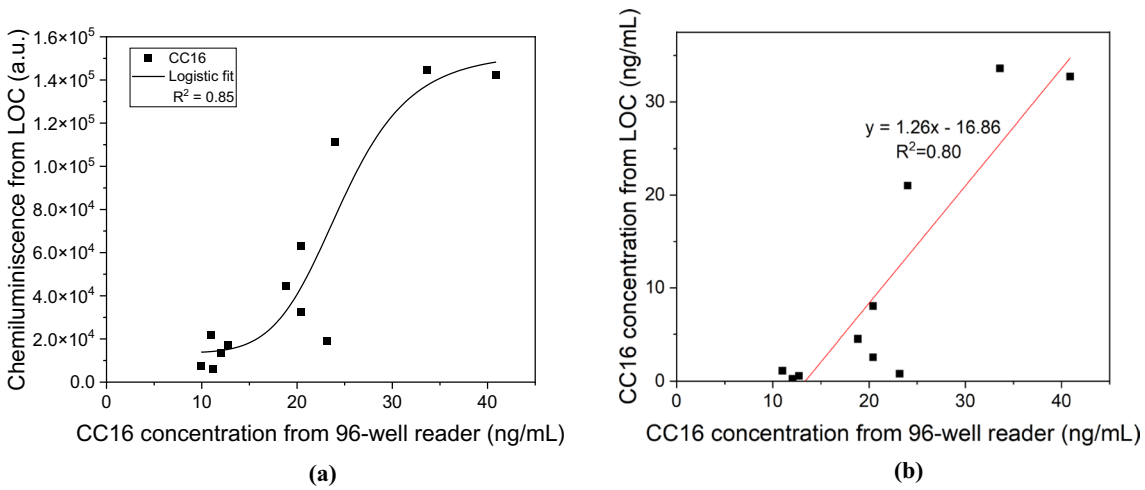

Fig. 7 **a** Chemiluminescence signal of CC16 in workers' serums from LOC compared to CC16 concentration obtained using 96-well reader. Four parameter logistic fit gives regression $R^2 = 0.85$. **b** CC16 concentration levels from LOC compared to that from 96-well reader

3.5 Assay results with the portable analyzer as a POCT

Human sera CC16 assay was performed on the develelped LOC using the portable analyzer. The assay reagents on the LOC were driven using a piezoelectric pump and the entire assay was automatically performed with the help of a fluid flow monitoring system (see Figs. S3 and S4 in the supplementary information). The new microfluidic flow monitoring method has been developed using infrared sensors, and then fully implemented and successfully characterized using an immunoassay lab-on-a-chip which needs precise monitoring and control for sequential flow of reagents in the portable analyzer.

The Infrared sensor unit (ISU) consists of an infrared (IR) LED emitting 1550 nm wavelength (LED1550L, ThorLabs) through a microchannel and an InGaAs IR-photodiode (FGA01, ThorLabs) detecting the IR intensity from the other side of the microchannel as shown in Fig. S3a. The output voltage with air inside the microchannel is considered as the baseline signal. Since water constitutes a large percentage of biological reagents, water molecules in the reagent absorb a considerable amount of IR signal emitted from the LED causing a significant reduction in the output voltage from the baseline, as shown in Fig. S4a. Hence, the output voltage of the photodiode can be used to indicate the presence of any fluid inside the microchannel. We tested the signal response of IR Sensor Unit (ISU) to different fluids in the sample chamber (Fig. S3b). When air is inside the microchannel, the air channel acts as an optical waveguide interfere light to transmit though the COC chip (knowing that COC has a refractive index of 1.53). When air is inside microchannel, it acts as an index-matching material with COC and allows more IR light to transmit to the photodiode, causing a higher output voltage. When water-based reagents present in the microchannel, it absorbs IR wavelength and causes the output signal to reduce, as shown in Fig. S4b. This new microfluidic monitoring method can envisage a functional microfluidic tracker for counting and characterizing oil droplets in a microfluidic droplet generator, cells in cell counter, as well as in microchannel flow immunoassays. The simple, low-cost ISU-based microfluidic tracker can be integrated as a key feature of an autonomous POCT system developed in this system. Once the sample was added to the LOC the incubation periods and the fluid flow was completely automated with no human intervention required. Optical signal output from the LOC at the end of the CL assay was directly read from the portable analyzer. The obtained optical outputs for the assay results of CC16 are summarized in Table 4. Figure 8 shows the assay results for CC16 obtained using the portable analyzer and the conventional BioTek reader, respectively. Both assay results have shown a similar linear trend within a dynamic range of 1.25 ng/mL to 20.0 ng/mL. The correlation between the conventional reader and portable analyzer was found to be reasonable, resulting in $R^2 \sim 0.93$ for a linear fit, while it was slightly higher using the logistic fit, giving $R^2 \sim 0.97$ (Fig. S7). The mean concentrations measured by the portable analyzer follow a trend to that measured by the conventional reader in the range of concentrations. Although the error for each data point of the analyzer is relatively high compared to the conventional reader, which needs to be improved further, once the sample was loaded on the LOC, the entire assay processes were performed automatically in the analyzer. The analyzer data obtained through a minimal human intervention in this study seem to be promising for the potential use of it as POCT in the occupational field. Recent studies showed successful biomarker detection using microfluidic platforms for various biomedical POCT applications (Wu et al. 2023; Lin et al. 2023; Chen et al. 2023; Yin et al. 2022; Singh et al. 2017),

Table 4 Mean and standard deviation of relative luminescence units measured from the conventional reader (BioTEK Synergy) and voltages measured from the portable analyzer

CC16 concentration (ng/mL)	Conventional reader		Portable analyzer	
	Mean (a.u.)	SD (a.u.)	Mean (Vol)[a]	SD (Vol)
1.25	2750.25	701.86	0.26918	0.01834
2.5	4169.25	1696.48	0.27628	0.03267
5	6951.5	765.71	0.27704	0.03499
10	11,194	1728.21	0.28570	0.01991
20	22,103.75	2884.64	0.29415	0.02106

[a] Vol stands for voltage

The mean and SD are of three to four replicate samples at each concentration

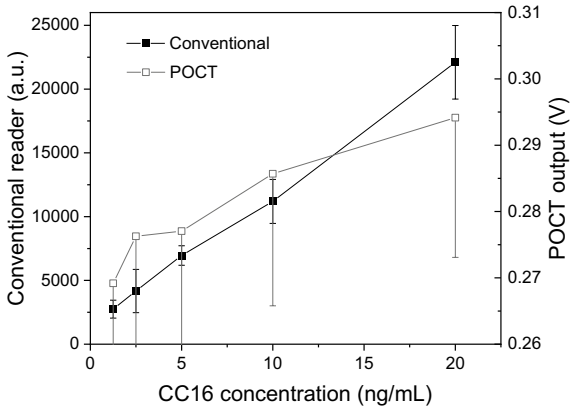

Fig. 8 Human CC16 standard curve results obtained from COC LOC and portable chemiluminescence analyzer and the corresponding table (Table 4) with the relative luminescence units measured from BioTEK Synergy and voltage measured from the developed portable analyzer, where the standard deviations (SD) were ranged within 0.0183–0.0350 (V)

indicating that the POCT based on the LOC developed in the study may be much more needed for rapid and field portable detection of the target biomarker.

As a final remark, it should be worth noting that the results from the small groups of workers in our study and the developed portable analyser should serve only to determine whether the developed LOCs and the analyzer would have the potential for reliable detection of the potential biomarker CC16, not to identify at-risk workers. More worker biosamples would be needed to rigorously test the developed LOC device for screening of health conditions for workers on site in the workplace.

4 Conclusions

In this work, a new sample-to-answer polymer LOC as an immuoassay platform for POCT applications was designed, microfabricated and fully characterized for detecting CC16 to early screen the health condition of the workers who are exposed to respirable silica dusts. Sandwich immunoasay of CC16 was performed on the LOC developed with a portable analyzer using artificial serum spiked with CC16 protien as well as human serum obtained from the coal mine workers exposed to the respirable silica-containing dusts. The achieved limit of detection (LOD) was around 0.35 ng/mL which could be applicable to the detection of CC16 for the early monitoring of the field workers who were exposed to silica aerosol. The chemiluminescence-assay LOC and platform developed in this work has a good potential to be used as a field-deployable POCT for early screening health condition of workers who are exposed to toxic aerosol exposures, such as respirable crystalline silica, even desiring further rigorous tests of the LOC and POCT using more worker's samples.

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Declarations

Conflict of interest There are no conflicts to declare.

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