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Detection of C-reactive protein in a portable microfluidic immunosensor from whole human blood

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ABSTRACT
C - reactive protein (CRP) plays a key role in determining the risks associated with cardiovascular diseases and can be detected via ELISA using a microfluidic chip inserted into a portable reader. Detecting CRP is a challenge because it accounts for less than 1% of all known blood proteins. This is due to interference from the cellular components of the blood and the high abundance plasma proteins (HAPs). Polydimethyl Siloxane (PDMS) chips were fabricated to remove blood cells and platelets from whole blood using filter membranes and remove plasma proteins, including albumin and human IgG using protein A surface functionalization. An incubation protocol was developed for the removal of HAPs and CRP was detected via a sandwich ELISA using fluorescence antibodies and fiber optics via a portable reader. A dose response curve was generated that correlated CRP concentration with the mean gray value of the detected signal. The limit of detection of CRP in blood was found to be 0.39 µg/mL and 0.61 µg/mL depending on the method of calculation, which would be comparable to clinical measurements.
Keywords: *microfluidics, C – reactive protein, CRP, blood, ELISA, protein A, portable device.*

1. INTRODUCTION
While Lab-on-a-Chip (LOC) devices have their origins in small scale analysis of air and liquid processes [1-3], the most common application for LOC has been in the field of medical diagnostics. One application is the diagnosis of cardiovascular diseases using C - reactive protein (CRP), a known inflammatory marker [4,5]. While normal CRP concentration levels in serum are between 5 to 10 µg/mL, higher levels can be found in older patients and in mild inflammations that are between 10 to 40 µg/mL and severe cases can occur resulting in concentrations greater than 40 µg/mL [4,6]. To determine the risk of heart disease, the American Heart Association developed a reference guide that states the a CRP level under 1.0 µg/mL in blood (1.85 µg/mL in serum) would be a low risk, 1.0 – 3.0 µg/mL in blood (1.85 – 5.56 µg/mL in serum) would be an average risk and over 3.0 µg/mL in blood (5.5 µg/mL in serum) would be a high risk for cardiovascular diseases [6]. The most difficult aspect in measuring CRP is its low concentration relative to the other constituents in whole blood. Blood cells and platelets account for 45% of the total volume of blood [7,8] and the remainder (plasma) consists of high abundance proteins (HAPs) that comprise 8% of its total volume [9,10]. These proteins include albumin (50 – 60% HAP), human immunoglobulin G (IgG) antibodies (20 – 30% HAP) and fibrinogen (4 – 7% HAP) [7-9,11]. The large sizes of the blood cells (6 – 8 µm diameter) and proteins (200 – 400 µm diameter) [7,12] act as barriers and obstruct accurate detection of CRP. Because of this, it is necessary to remove them before CRP detection can commence inside of the microchip.

Known methods for CRP detection include the use of a blood analyzer (nephelometer), which has the ability to either perform a normal or an hs-CRP (high sensitivity CRP) test [6], in terms of macroexperiments [13,14] and the use of specific antibodies to capture CRP, either with nanoparticles [15,16] or by engineering immunoglobulin Y (IgY) [17-19] in terms of microexperiments. However, since nanoparticles can be expensive to buy and avian antibodies have to be manipulated in order to be useful in mammalian assays, these methods are not useful for CRP detection. However, one method that is known for CRP detection is the use of an enzyme-linked immunoassay (ELISA) [20, 21]. This method does not require the use of special nanoparticles or engineered antibodies, only mammalian antibodies and a small amount of reagents to make use of a small detection area.

Some methods that are designed to remove blood cells and HAPs include precipitations (for proteins) [22] and centrifugation / ultracentrifugation (for both) [23], macroexperiments and dielectrphoresis [24] in microexperiments. Due to the use of outside equipment and the amount of fluid used in protein extraction, these methods are not suitable for LOC experiments. However, membranes for blood separation can be integrated on a microfluidic chip [25,26]. And the use of surface functionalization using protein A to remove IgG and antibodies that bind to albumin [27-30], can be integrated on a chip. In this work, these methods were used to detect CRP in whole human blood in a microfluidic chip in combination with a reader.

2. MATERIALS AND METHODS
2.1. Chemicals and Materials. For chip mold fabrication, two methods were utilized: soft lithography with silicon wafers [28] and hard lithography with aluminum molds [29]. For the soft lithography molds, silicon wafers were purchased from SI-Tech,

Inc. (Topsfield, MA) with SU-8 2050 photoresist and developer purchased from MicroChem Corp. (Newton, MA) and AP300 adhesion promoter from Silicon Resources, Inc. (Chandler, AZ). Piranha solution was created on site using 50% hydrogen peroxide (H_2O_2) (Ricca Chemical Company, Arlington, TX) and 50% sulfuric acid (H_2SO_4) (Acros Oragnics, Geel, Belgium). Photomasks were custom made by CAD/Art Services, Inc. (Bandon, OR) and isopropyl alcohol (C_3H_7OH) was purchased from Fisher Scientific (Fair Lawn, NJ). For hard lithography molds, aluminum was given to use from the University Of Rhode Island Department Of Mechanical Engineering (Kingston, RI). Milling was performed using an EMCO Controlled Milling 55 miller (Hallein, Austria) with milling bits purchased from McMaster-Carr (Elmhurst, IL). For both methods, polydimethyl siloxane (PDMS) and curing agent (Slygard 184) was purchased from Dow Corning (Midland, MI). Compressed oxygen and nitrogen gases were purchased from AirGas (Warwick, RI). Other materials used in chip manufacturing included a spin coater (Laurell Technologies, Corp., North Wales, PA), hot plates (HS40A, Torrey Pines Scientific, Inc., Carlsbad, CA), a UV-light oven (i-line, Karl Suss MJB-3, Suss Microtech, Garching, Germany) and plasma asher (Femto, Diener Electronic, Reading, PA). All safety regulations were followed, including the proper use of lab coats, gloves and safety glasses, especially with the handling of PDMS, piranha solution and SU-8 photoresist ensuring bodily safety.

For the immunoassay, bovine serum albumin (BSA), glutaraldehyde, phosphate buffer saline (PBS), PBS with 0.05% Tween (PBST) and protein A were purchased from Sigma-Aldrich (St. Louis, MO).

Mouse monoclonal anti-human CRP IgG antibody and Goat polyclonal anti-human CRP IgG antibody labeled with FIT-C were purchased from Abcam (Cambridge, UK) with protein A/IgG binding buffer purchased from Thermo Scientific (Rockford, IL). Anti-albumin IgG antibodies were also purchased from Thermo Scientific. CRP pentomers and CRP-free serum was purchased by Fitzgerald Industries (Acton, MA). Blood was withdrawn safely from samples at the University of Rhode Island Health Services (Kingston, RI). Gloves, lab coats and safety glasses were worn with all experiments, especially ones that dealt with the handling of blood.

Detection was performed via the use of a spectrometer with fiber optic cables (Ocean Optics, USB4000, Dunedin, FL) inside of a portable detection device previously created from earlier projects (shown in figure 1 [29]). An LED (light emitting diode) (Quadica Developments, Brantford, ON, Canada) was used in order to excite the FIT-C on the detection antibodies. The light traveled through a series of bandpass filters that that emitted a wavelength of 470 nm onto the detection system (Edmund Optics, Barrington, NJ). The filters have a bandwidth of 10 nm, a minimum transmission of 45% and an optical density (OD) of 3 in order to block excess light and eliminate background noise in the system. Detection and readings were performed using the Ocean Optics program Overture. The reading wavelength was adjusted to 200 nm and read at 493 Hz. A spike was generated at that specific frequency. The higher the spike, the more "full" the detection area would be. The intensity level was recorded and plotted using Microsoft Excel against the concentration of the CRP [28-30].

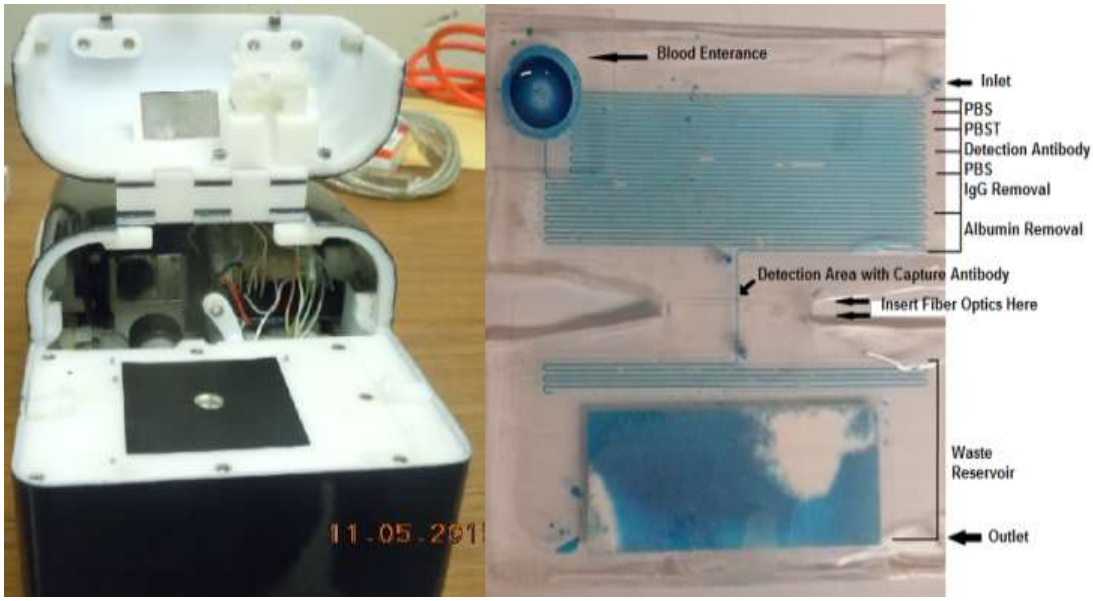

Figure 1. Portable box used for CRP detection (left) and microfluidic chip used to detect CRP with specified inlets, outlets and removal areas (right).

2.2. Microfluidic Mold and Chip Fabrication

2.2.1. Soft Lithography Method.

To prepare the soft lithography molds for the microfluidic chips, silicon wafers are cleaned with piranha solution for 15 minutes which was followed by a rinse with distilled water. A wafer is placed on the spin coater. 2 – 3 mL of AP300 adhesion promoter is added to the wafer and spun for 25 sec at 300 rpm and then for 30 sec at 3000 rpm. SU-8 2050 photo resist is then added to the center of the wafer and spun at 300 rpm for 45 sec and then at 600 rpm for 50 sec. The wafer is removed and placed on a

leveling tray and baked on a hot plate. The baking consists of a three part sequence: a constant 2°C/min rise from 50°C to 95°C, a constant 95°C, bake for 3 hours and a constant 1°C/min cooldown from 95°C to 22°C. Photomasks are designed using Adobe Illustrator which consists of single- and multi-channel geometries (straight lines, curved lines) and holders for the membranes. Once completed, the photomasks are sent to CAD/Art Services, Inc. where they are printed on transparent plastic. To etch the pattern on the wafer, the photo mask is placed over the resist and into a UV-light oven, where UV light at a wavelength of 365 nm hardens the exposed photo resist for 6 minutes. The wafer is then placed on a hotplate at 45°C for a three step bake: a constant 2°C/min rise from 45°C to 85°C, a constant 85°C bake for 45 minutes and a constant 1°C/min cooldown from. 85°C to 22°C. The molds are then developed using a series of two SU-8 developers for approx. 10-20 minutes followed by a wash in isopropyl alcohol for 1 minute and then blown dry. The last step would be to bake the chip at 150°C for 30 minutes, with a 1°C/min heat up and cool down before and after baking [28]. Using this method, simple one-channel molds can be created for initial CRP detection and HAP removal.

2.2.2. Hard Lithography Method. Some issues with creating molds via soft lithography include the amount of hazardous chemicals used, the limitation of the UV oven etching the channels and the limited repeated use of the molds. In its place was a method of milling molds using aluminum. This is performed by first designing the mold in SolidWorks and then translating this model into code that would etch the mold using a computer numerical control (CNC) miller. Molds are etched out of aluminum due to its lightweight, high heat tolerance and capital cost. The molds are etched with mill bits that created raised structures for the microfluidic channels that had a minimum channel height of 300µm. Using this method, more complicated networks of channels can be incorporated onto a single chip, allowing for multiple reagents and detection to occur on the same chip.

2.2.3. Microfluidic Chip Fabrication. The microfluidic chips are made by using a mixture of liquid PDMS and curing agent in an 8:1 – 10:1 PDMS:Curing Agent ratio. Depending on the size of the mold and the thickness of the final chip (between 0.4 and 0.6 mm thickness), the mass of the PDMS mixture varies between 30 and 50 grams. The liquid mixture is poured onto the mold and a blank petri dish (bottom) in equal proportions. The molds are then placed in a vacuum chamber to remove all air bubbles from the molds. The mold is then placed on a hot plate at 95°C for a bake time of 2 hours. The chips are removed from their respective molds, trimmed of excess PDMS and holes are punched at the channel openings. The chip halves are then placed in a plasma asher for approximately 7 minutes. This includes 3 minute vacuum, 1 minute of introducing oxygen gas and 3 minutes of activating the asher. The chips are then taken out and are bonded together and baked on a hot plate at 95°C to bake for 1 hour in order to facilitate binding. The ashing process is then repeated with the membrane holder slab in order to attach the filter to the chip [29]. A top view of the chip showing its various channels and their function and the various inlets, outlets and waste collection area was well as the reader box are shown in figure 1 [29].

2.3. Surface Functionalization. The channels that are used to remove the high abundant proteins (HAPs) and where the detection area is are functionalized by the following method. First BSA in PBS (1.5 mg/mL) is loaded and incubated for 4 hours. This solution is washed with PBS buffer and replaced with a mixture of glutaraldehyde and water (0.4% v/v, to serve as a linker between the BSA and protein A). This is allowed to incubate for 1 hour and is then washed away. Afterwards, protein A in PBS (50 µg/mL) is loaded and allowed to incubate for 1 hour. This is also washed away and replaced with a storage buffer (10 mM Tris Buffer Saline (TBS) in distilled water, 0.05% BSA (w/v), 0.05% Proclin 300 (v/v), 5% glycerol (v/v)) while the chips are in storage. Figure 2a shows the state of functionalization to this point [28-30].

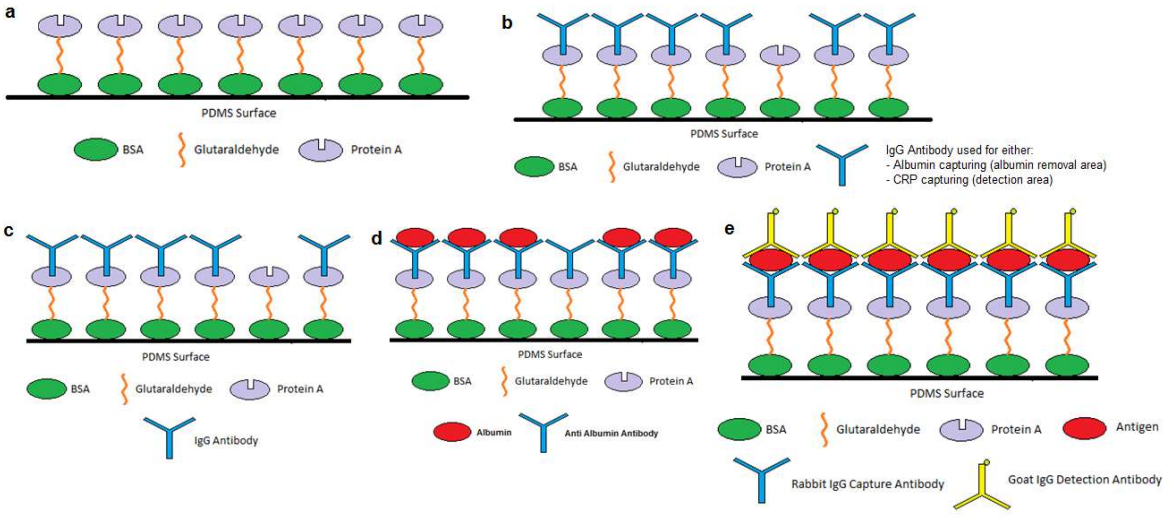

Figure 2. Diagrams of initial protein A surface functionalization (**a**), functionalization of anti-albumin IgG and anti-CRP capture IgG antibodies (**b**), removal of human IgG (**c**), removal of albumin (**d**) and detection of CRP in sandwich assay (**e**).

To further prepare the channels for the removal of IgG, albumin and for the detection of CRP, 200 µg/mL mouse monoclonal anti-human CRP IgG antibodies in Protein A/IgG binding buffer are loaded into the detection area channel which has its own inlet and outlet ports. In the meantime, anti-albumin antibodies are loaded into the channels where albumin is to be removed. Both of these antibodies are captured by the protein A and are thus immobilized. The solutions in both channels are allowed to incubate for 7 minutes. Afterwards, the channels are loaded with PBS not only for washing, but also to keep the functionalization intact. Figure 2b shows schematically the functionalization of the channels where albumin will be removed and the detection area functionalization up to this point.

3. EXPERIMENTAL SECTION

3.1. Immunoassay.

To conduct the ELISA, serum spiked with a pre-determined amount of CRP pentomers (between 0.7 and 100 µg/mL CRP in serum) is loaded into the chip's main inlet. The serum is first held in the functionalized area to specifically remove the IgG antibodies for a period of time discussed in the following section. The IgG antibodies are removed by being captured by protein A. The serum is then pushed into the next area which is functionalized to remove albumin by the anti-albumin antibodies. Figures 2c and 2d show the conjugates formed in the IgG and albumin removal areas respectfully. After a certain incubation period, the serum is pushed into the detection area and remains there for 5 minutes. All channels are then washed and a solution of detection antibodies in PBS (50 µg/mL, Goat polyclonal anti-human CRP IgG antibody labeled with FIT-C) are pumped from the main inlet into the detection area and is incubated for 5 minutes. A final wash and incubation with PBST for 5 minutes is required before inserting PBS into the detection area in order to record measurements. The final sandwich assay formed in the detection area is shown in figure 2e [28-30].

3.2. HAP Removal Experiments.

Multiple experiments demonstrating the ability of protein A to bind to IgG are implemented. A solution of IgG and PBS is inserted into the chip and experiments are based on the concentration of IgG in solution and incubation time. The first experiments involve IgG labeled with FIT-C and are detected using fiber optics. Based on these experiments, a buffer solution with IgG and CRP is included for CRP detection. The buffer flows into the chip, incubates for IgG removal and then for CRP detection. Data is recorded based on the ratio between IgG and CRP, as well as incubation time. Based on these results, serum with a set amount of CRP is integrated into the system. Experiments are performed based on the same procedure as before and conclusions can be made about IgG removal. Afterward, these experiments are repeated, but with the removal of albumin with the use of anti-albumin IgG. Once a suitable method of CRP detection is found, a titration curve is drawn that relates CRP concentration to intensity. Once that curve is found, experiments with blood can commence.

3.3. Limit of Detection.

Two methods are used to calculate limit of detection (LoD): signal-to-noise calculation [31] and limit of blank (LoB) [32]. In order to calculate the LoD using signal-to-noise, a plot between the concentrations and signals for the lower end of the curve is constructed where the slope (SL) is obtained. Using the standard deviations (SD) for each signal, the following equation is used to calculate LoD [31]:

$$LoD = \frac{3.3(SD)}{10(SL)} \quad (1)$$

Using the LoB method, separate measurements of a blank sample have to be measured and the mean and standard deviation for it is calculated. LoB is then found using the following equation [32]:

$$LoB = mean_{blank} + 1.645(SD_{blank}) \quad (2)$$

A sample of a low amount of CRP is measured and the standard deviation is recorded. LoD is found using the following equation:

$$LoD = LoB + 1.645(SD_{low\ conc.\ sample}) \quad (3)$$

4. RESULTS SECTION

4.1. Capturing of IgG with Protein A.

4.1.1. IgG – Protein A Affinity.

Figure 3 displays the results of the ability of protein A to remove IgG. Based on this result, a considerable amount of IgG can be captured using only protein A only after 2 minutes of incubation. However, this ability decreases over time, which is why a constant signal is displayed after 2 minutes. At longer incubation times (> 5 minutes), the ability of protein A to capture IgG is poor due to the saturation limit of the protein. This is shown by the decrease in signal after 5 minutes. Based on this result, an optimal time of 2 to 5 minutes of incubation is required in order to extract an appropriate amount of IgG.

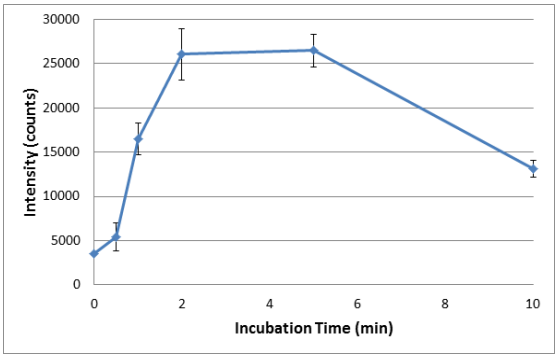

Figure 3. Direct IgG capturing with varying incubation times.

4.1.2. Relation with CRP Detection.

At this point, it is necessary to see how well CRP can be detected if IgG was the only other plasma protein present in

solution. To perform this, two variables are considered: incubation time and IgG concentration. For incubation time, a 1:1 IgG:CRP ratio was considered with incubation times that ranged between 0 and 5 minutes. For the concentration difference, IgG:CRP concentration ratios varied between 0:1 and 4:1, while the incubation time remained at a constant 5 minutes.

Figure 4 displays the differences between the incubation times. Based on this, it can be stated that as the incubation time increases, the CRP detection signal also increases. However, as shown in figure 5 (CRP detection based on varying IgG concentrations), the signal decreases as IgG concentration increases. This is due to the increased interference with the IgG due to the amount of IgG still in solution. This is problematic because once blood is inserted into the system; there is a higher ratio of IgG to CRP and only a limited amount of protein A present on the channel surface.

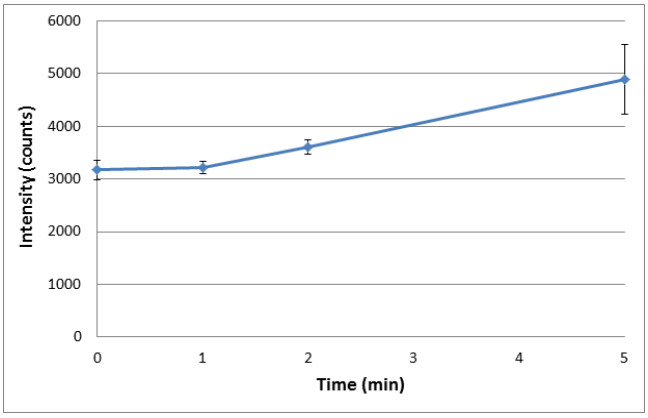

Figure 4. CRP detection with IgG present with dependence on incubation time.

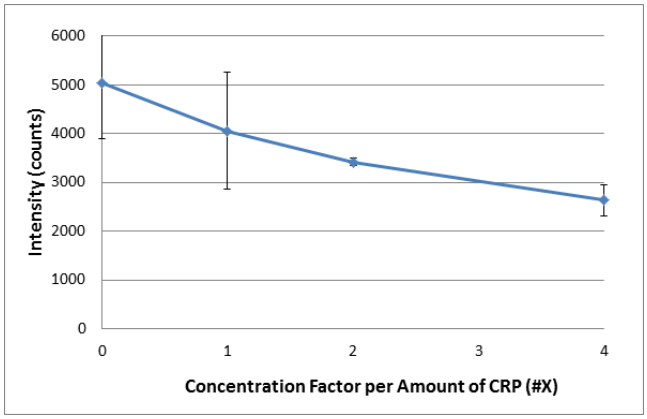

Figure 5. CRP detection with IgG present with dependence on IgG concentration.

4.2. Multiple Removal Areas.

4.2.1. IgG Removal in PBS Buffer.

Since it is assumed that the channel surfaces are fully concentrated with protein A, modification is not applicable unless the chip is completely redesigned. However, it is worth noting that due to the amount of plasma that would be used (less than 5 µL), underutilized channel space in the chip is readily available. Because of this, multiple removal areas can be utilized in order to capture more IgG. Figures 6 and 7 display the results of CRP detection with 0 to 3 IgG removal areas in the chip. As the number of removal areas increased, the intensity detected also increased, within error after 2 removal areas. This was found to be true for both solutions containing double and quadruple the concentrations of IgG to CRP. As a result, this method could be utilized to detect CRP in a buffer with IgG present.

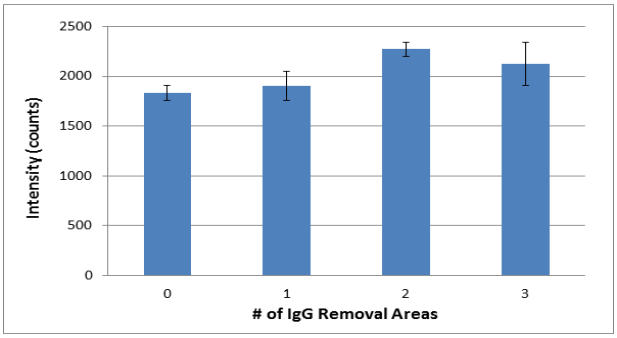

Figure 6. 10 µg/mL CRP in PBS with 2X the concentration of IgG with multiple removal areas.

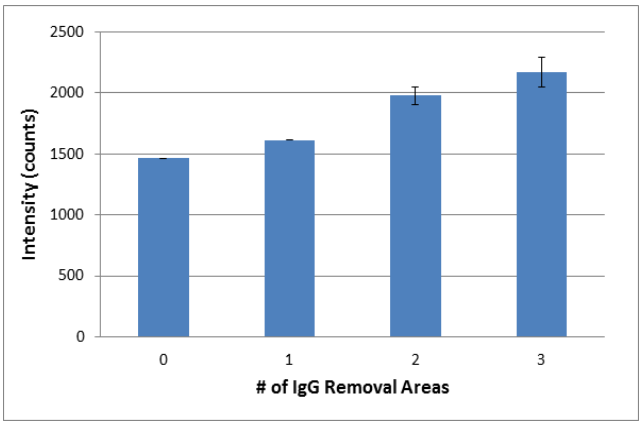

Figure 7. 10 µg/mL CRP in PBS with 4X the concentration of IgG with multiple removal areas.

4.2.2. IgG Removal in Serum.

The next issue for this would to see of this system worked for serum. To perform this, CRP free serum inoculated with different CRP concentrations was added: 5, 10 and 100 µg/mL CRP in serum. As shown in figure 8 (only 10 µg/mL CRP), as the amount of removal areas increases, a higher intensity signal can be found. This was also true for the CRP concentrations of 5 and 100 µg/mL. Based on the design of the chip and this result, it was determined that two IgG removal areas were sufficient to remove enough IgG to detect CRP. Figure 9 displays the result of the protocol with those 3 CRP concentrations as compared to no HAP removal (bottom line) and CRP in PBS buffer only (top line). While there is an increase in signal compared to no HAP removal, it is still considerably lower than that of CRP in PBS. This is still due to the amount of albumin in the serum. To solve this, the same protocol is applied in order to remove albumin from serum.

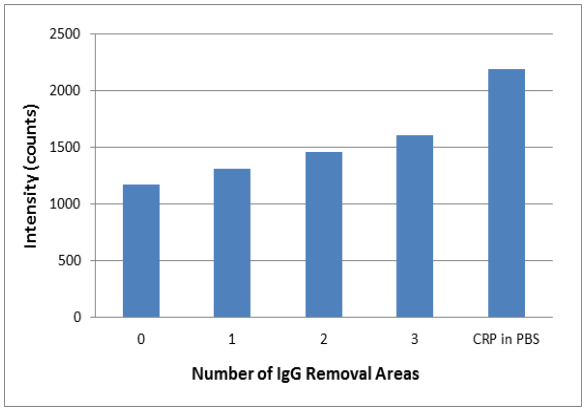

Figure 8. Implementation of multiple IgG removal sites in CRP detection chip with serum.

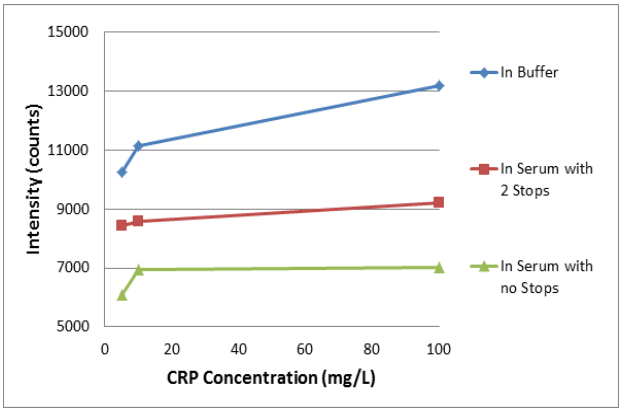

Figure 9. CRP detection in serum compared to the CRP in buffer and CRP in serum with no stops.

4.3. Introduction of Albumin Removal.

In figure 10, on the far left, an increase in signal can be obtained with multiple albumin removal areas with only one IgG removal area. However, there was no noticeable difference in distinguishing between the two CRP concentrations. This was due to the interference between the IgG still remaining in the serum. A second IgG removal area is added to the protocol and it shows that not only a high signal can be obtained, but a different intensity value can be distinguished between the two CRP concentrations. Based on this result, it was determined that the proper protocol would be two IgG removal areas followed by three albumin areas, with each incubation lasting only 5 minutes.

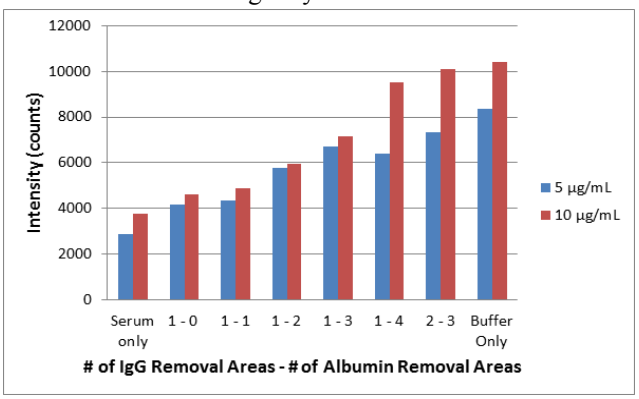

Figure 10. Addition of albumin removal areas for 1 and 2 IgG removal areas for CRP in serum.

4.4. CRP Detection in Serum and Blood.

Figure 11 displays the results of the detection of multiple CRP concentrations in serum ranging between 0.7 and 100 µg/mL utilizing this protocol. The results show that the signal for this method was almost that of the signal for CRP in PBS. The reason that it was not a perfect match was due to the amount of other HAPs (e.g. fibrinogen) in serum. The one thing to point out is that at high CRP concentrations, the signal levelled off at around 14000. This was due to the saturation of the antibodies in the detection area. This "levelling off" occurs at a CRP concentration greater than 15 µg/mL. Because of this, this system would be ideal at measuring CRP at low serum concentrations (less than 15 µg/mL) but not at high CRP concentrations. It would be wise to look into redrawing the detection area for concentrations higher than 15 µg/mL.

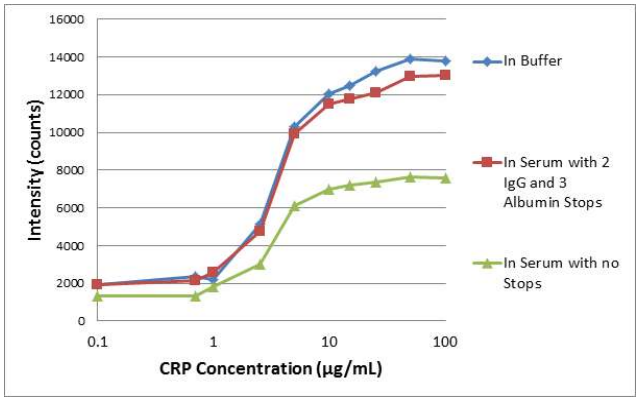

Figure 11. CRP detection in buffer (blue line), serum with high abundant protein removal (red line) and serum without high abundant protein removal (green line) with CRP concentration ranges from 0.7 to 100 µg/mL.

Due to the amount of CRP present already in in blood, it is more difficult to obtain a baseline (zero point) when performing experiments with whole blood. Any additional CRP would make it so that the system could not be readable. However, the CRP concentration in blood can be estimated using the same curve for that of serum since concentration is only a measurement of mass per liquid volume. (Since CRP mass is constant; there is indirectly proportional relationship between volume and concentration.) Because of this, the CRP concentration in blood can be estimated by multiplying the CRP concentration in serum by 0.55.

Figure 12 displays the curve for serum has been applied at two measurements in blood at a certain level. The top graph displays a cubic trend line, while the bottom graph displays a quartic trend line. The cubic line displays great accuracy at higher CRP concentrations, but not at lower CRP concentrations, since the trend line does not level off at a specific base point. For the quartic trend line, the line does level off at lower concentrations, but it is inaccurate at higher concentrations. To solve this, it was determined that for CRP concentrations less than 5 µg/mL (~9500 intensity) the quartic trend line is applicable, while the cubic line is for concentrations greater than 5 µg/mL (9500 intensity).

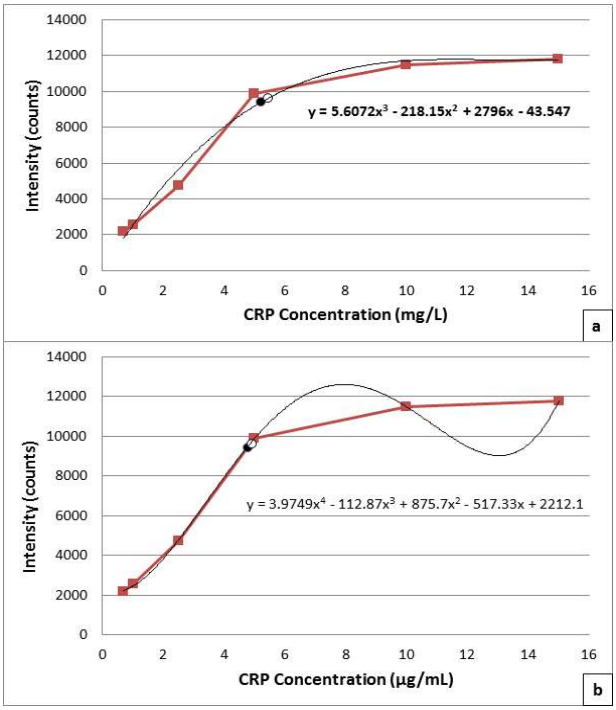

Figure 12. Trend line for CRP in Serum for CRP concentrations greater than 5 µg/mL (a) and less than 5 µg/mL (b).

Based on the equations generated in figure 12, two plots are created in figure 13. The top plot (serum) shows only the combination of the two trend lines at their specifications. The bottom plot is fitted for tests with blood with the volume change in effect the equations for both plots are on each graph. As far as the limit of detection, the values obtained using the signal-to-noise ratio method was determined to be 0.7 µg/mL CRP in serum and 0.39 µg/mL in blood, while the limit of blank method showed a result of 1.10 µg/mL for serum and 0.61 µg/mL for blood.

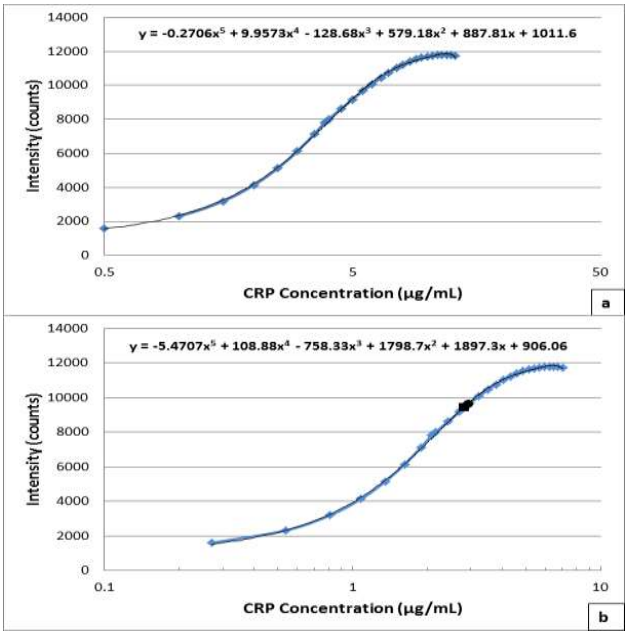

Figure 13. Modified plot for CRP detection in serum (a) and in whole blood with quadratic equation relating concentration (x) with intensity reading (y) (b).

4.4. Comparison with a Previous Method.

To further emphasize the point of this study, these results are compared to a previous work with a similar method. In this case, the same experiment is performed, however there are notable differences. These include the number of removal areas for both HAPs and a longer CRP incubation in the detection area. Three different experiments were compared against each other: CRP detection in buffer, serum and whole blood [30]. For both experiments involving buffer, CRP detection is similar based on the signal achieved for a specific concentration. However, compared to this study, the experiments shown with CRP in serum and in blood revealed that a wider range of concentrations can be detected. For example, in the previous study, at a CRP concentration of 3 µg/mL in buffer, serum and blood, the intensities were found to be at 3700, 630 and 510 respectfully (shown in figure 14). With the new method, the intensities recorded at the same CRP concentration (3 µg/mL) are found to be at 6000 for buffer (a bit higher, but within error), 5500 for serum (much higher and closer to the buffer measurement) and 9500 for blood (~5.5 µg/mL CRP in serum, as estimated by figure 13) (also shown in figure 14). This can be due to possible remnants of IgG and albumin present in the serum. This can also be explained by the protocol used in both methods. The previous method relied on one IgG and one albumin removal area, while this method utilizes two IgG and three albumin removal areas. This allows a higher rate of IgG and albumin removal, which results in a higher signal. This can be also said for the tests revolving whole blood. Because of this process, it has been shown that this newer method of multiple removal areas is beneficial for an accurate measurement of CRP in whole blood.

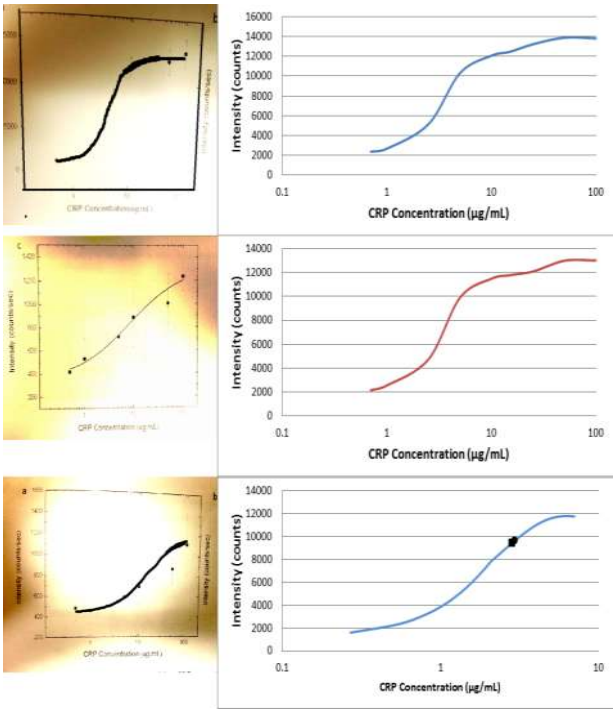

Figure 14. Comparison of experiments performed with a previous method (left column) [30] and in this study (right column) for CRP detection in buffer (top row), serum (middle row) and blood (bottom row).

5. CONCLUSIONS

It was shown that CRP in whole blood can be detected via ELISA in a microchip. This was due to the addition of multiple removal sites for the removal of both human IgG and albumin. Detection of CRP was high and intensities can be correlated with specific CRP concentrations, in order to create a curve that relates these two factors. This curve is shown to be much higher than that of serum without HAP removal and was within the range of pure CRP detection in PBS buffer. With whole blood, it was shown that a signal could be emitted and a concentration of CRP could be determined based on the aforementioned graph. Based on this and the explanation of the results, it can be concluded that low CRP concentrations can be detected in whole blood by use of a microfluidic chip.

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