ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

Strategy To Enhance the Wettability of Bioacive Paper-Based Sensors

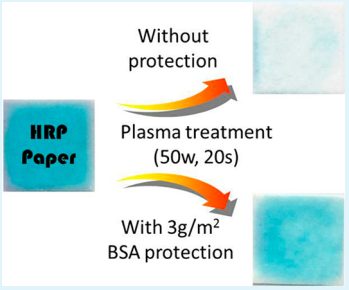
Junfei Tian,[†] Purim Jarujamrus,[‡] Lizi Li,[†] Miaosi Li,[†] and Wei Shen*[,†]

[†]Department of Chemical Engineering, Monash University, Wellington Road, Clayton, Vic. 3800, Australia

[‡]Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400

Supporting Information

ABSTRACT: This paper reports a potential method that can restore the wettability of bioactive paper-based sensors while maintaining their bioactivity. This study is driven by the need to increase the wettability of the antibody-loaded blood typing paper devices in order to increase the blood typing assaying speed using such paper devices. Plasma treatment is used to improve the wettability of bioactive paper; the protective effect of bovine serum albumin (BSA) to biomolecules against plasma deactivation is investigated. In the first stage, horseradish peroxidase (HRP) was used as a model biomolecule, because of the convenience of its quantifiable colorimetric reaction with a substrate. By using this protection approach, the inactivation of biomolecules on paper during the plasma treatment is significantly slowed down. This approach enables plasma treatment to be used for fabricating paper-based bioactive sensors to achieve strong wettability for rapid penetration of liquid samples or reagents. Finally, we demonstrate the use of plasma treatment to increase the wettability of antibody treated blood typing paper. After the treatment, the blood typing paper becomes highly wettable; it allows much faster penetration of blood samples into the plasma treated testing paper. Antibodies on the paper are still sufficiently active for blood typing and can report patients' blood type accurately.



KEYWORDS: *paper-based, bioactivity, wettability, low-cost diagnostics, blood typing, plasma treatment*

INTRODUCTION

The recent rapid advancement in bioactive paper-based diagnostics has shown enormous promise of this new platform technology in improving human health in the developing world.[1−5] Some novel proof of concept studies focusing on specific diagnostic assays and methods of assay result transmissions have demonstrated that paper-based diagnostics have superior application potentials to many currently available technologies in obtaining rapid diagnoses under the unsupported field conditions.[2,4,6] Major research and development groups in this field have estimated that real applications of bioactive paper diagnostics are getting closer to becoming reality, although there are still hurdles to overcome.[7−9]

Among the engineering considerations of an up-scaled production of bioactive paper devices, a top priority is to retain all the required properties of the substrate and biomolecular materials in a fabrication process so that the performances of the fabricated devices are at their optimum. For most bioactive paper-based devices, paper wettability and paper bioactivity are two of the most important properties, which determine the performance of the devices. In the fabrication of some devices, it was found that the introduction of bioactive reagents can significantly reduce the wettability of bioactive paper, seriously compromising the performance of the device in a diagnostic test.

An example from our recent experience is the bioactive paper device for blood typing.[6,10,11] The working principle of a paper-based blood typing device relies on the introduction of blood typing antibodies into the paper first. Penetration of antibody solution into the pores of the paper results in deposition of antibody molecules and an additive, such as BSA, on the fiber surface. The so-formed bioactive blood typing paper is expected to function when a blood sample is added onto the paper. First, the blood will redissolve the deposited antibody from the fiber surface into the blood serum. The dissolved antibody will be able to interact with the antigens on the surface of the red blood cells (RBCs). The antibody-RBC interaction may either cause agglutination of the red cells or have no effect on them. With the aid of saline washing, the agglutination or non-agglutination of RBCs will be revealed.[6,10,11]

This simple design has confronted an unexpected problem of reduced wettability of the antibody loaded paper by the blood sample when antibodies from commercial sources are used.[10] Figure 1 shows the degree of poor wetting of the antibody-loaded paper compared with paper without antibody. The poor wettability of the paper significantly reduces the speed of liquid penetration. An easy and upscalable solution for restoring the wettability of the paper could be by plasma treatment. In a plasma environment, energetic electrons, ions, and radical species impinge on the surface, leading to physical and chemical changes of the surface in three main ways: 1) Etching. Plasma treatment is used to remove materials from solid surfaces. 2)

Received: August 18, 2012
Accepted: November 14, 2012
Published: November 14, 2012

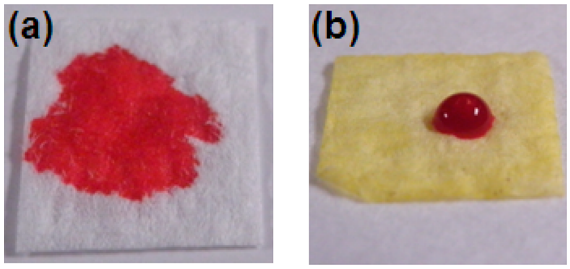


Figure 1. (a) 4 μL of blood penetrates into untreated paper and (b) 4 μL of blood stands on an antibody treated paper.

Activation. It is used to chemically and/or physically modify the surfaces so that the modified surfaces carry active species. 3) Coating. It is used to deposit a thin film of material on solid surfaces.[12] Martinez et al. and Abo et al. respectively demonstrated the use of plasma treatment as a step of μPADs fabrication to increase channel wettability.[1,13] Li et al. and Tian et al. also used plasma treatment to increase the wettability of the thread-based microfluidic device and V-groove microfluidic device on polymer film by removing the contaminant deposits on the surface.[14,15] In another study by Li et al., plasma treatment has been utilized as a patterning method to selectively etch hydrophilic patterns onto a piece of hydrophobic filter paper to create the μPADs.[3] After plasma treatment, the sample delivery channels and detection areas of these devices become highly wettable. Aqueous solution of biomolecules and detection reagents can be easily loaded into the paper by absorption, to form a complete sensor.

While plasma treatment is an easy and matured technology for surface treatment to increase materials' wettability, it can significantly reduce the activity of biomolecules.[16−18] Plasma treatment has been widely used as a method for biodecontamination.[17,19,20] Von Keudell showed that plasma treatment can efficiently inactivate a range of biocontaminants including micro-organisms, bacteria and proteins, etc.[16] Plasma treatment allows energetic particles to destroy or modify surface chemical bonding of materials, causing a variety of proteins to denature.

In our bioactive paper studies, however, we found that plasma treatment imposes a much weaker destructive effect on blood typing antibodies than expected. Blood typing antibodies do not lose their functions in blood typing assays. This surprising observation was found to be reproducible. One possible reason for this observation is the presence of certain protection addictives in the antibody reagent. Based on the supplier's specification, the antibodies contain 1−5% BSA as a protection additive. There are reported bioactivity protection methods in the literature, but most of these methods focus on the protection of the activity and stability of proteins and enzymes from the inhibition induced by temperature, soluble additives, and even radiation.[21−24] In those methods, BSA is a widely used stabilizer that prevents the thermally induced inactivation of biomolecules. Moreover, BSA has also been reported to have a protection effect on enzyme activity from the inhibition caused by a toxicant. Our hypothesis is that BSA may contribute to the protection for antibody during the plasma treatment. If the destructive effect of plasma to biomolecules can be reduced through a simple method, then plasma treatment can be used to improve the wettability of bioactive papers; it will greatly improve the fabrication of low-cost sensors.

In this paper, we used horseradish peroxidase (HRP) as a model biomolecule to demonstrate the influence of plasma treatment to the activity of biomolecules on paper. The choice of HRP is based on the fact that its activity can be visually shown by using a suitable liquid substrate; HRP therefore provides an easy way to assess the activity loss of the biomolecule. We compared the activity of HRP paper loaded with a range of concentrations of HRP solutions before and after plasma treatment. The effect caused by plasma with increasing treatment time on the activity of HRP paper loaded with a given concentration of HRP solution was also studied. An effective strategy that can protect the biomolecules on paper from being deactivated by plasma treatment was investigated. This strategy offers a significant flexibility to the fabrication of bioactive paper based sensors; this flexibility allows the enhancement of the device's wettability and the addition of bioactivity to the paper to be performed in any desired sequence, effectively reducing practical fabrication constraints in device production.

EXPERIMENTAL SECTION

Materials and Equipment. Horseradish peroxidase (HRP) (Type I, lyophilized powder, 100 KU) was obtained from Sigma-Aldrich. HRP powder was dissolved in phosphate-buffered saline (PBS, pH 7.4) to make stock solution (1 mg/mL). A liquid substrate system of HRP, 3,3′,5,5′-tetramethylbenzidine (TMB), was obtained from Sigma-Aldrich and used for HRP activity analysis. The activity of enzymatic paper containing HRP molecules can be calorimetrically assessed by using TMB substrate. After applying the liquid substrate to an HRP loaded enzymatic paper, the enzyme-catalyzed reaction will occur. The intensity of the blue color developed through this reaction reflects the activity of HRP in paper. The blue color of the reacted substrate was then scanned into a computer, and the activity of HRP was quantitatively analyzed using computer software.

Commercial antibody solutions of red blood cell antigens A, B, and D (Epiclone Anti-A, Anti-B, and Anti-D monoclonal grouping reagents) were obtained from CSL, Australia. Anti-A and Anti-B are color-coded blue and yellow solutions, respectively, whereas Anti-D is a clear solution. These antibodies are made of immunoglobin M (IgM). Normal saline (0.85 g NaCl in 100 mL of deionized water (18.2 MΩ cm^{-1})) was used as diluent and washing solution in this study. Blood samples were collected from 3 adult volunteers; the samples were kept in standard plastic vials containing lithium heparin anticoagulant. Antibody solutions and blood samples were stored at 4 °C, and blood samples were used within 5 days.

Filter papers (Whatman grade 4) were cut into 1 cm × 1 cm squares for fabricating HRP and HRP-BSA paper. A Kleenex paper towel was cut into 5 mm × 5 mm squares which were used as the base substrate for making the blood typing device, while standard blotting papers (drink coaster blotting, 280 g/m^2) were used to remove excess liquids during device preparation.

K1050X plasma asher (Quorum Emitech, U.K.) was used for plasma treatment. The vacuum level for the treatment was 6×10^{-1} mbar. The paper samples were always placed in the center of the chamber during the treatment for a consistent effect of treatment.

Test of Activity of HRP Paper without Plasma Treatment. HRP stock solution was diluted with to get serially diluted HRP standard solutions with the concentrations of 0, 1, 2, 3, and 4 μg/mL. About 10 mL of HRP solution in a plastic Petri dish (I.D. = 8.5 cm) was used for soaking paper. Five filter paper squares were respectively soaked in each diluted HRP solution for 2 s. The wet paper squares were not blotted. They were put onto a piece of plastic film and dried in a fume hood for 20 min. After drying, each paper square received an addition of 15 μL of TMB liquid substrate solution. These paper squares carrying TMB were then placed into a dark box for 2 min to develop color changes of the substrate on paper. Color intensity of scans of the filter paper squares was measured using Adobe Photoshop

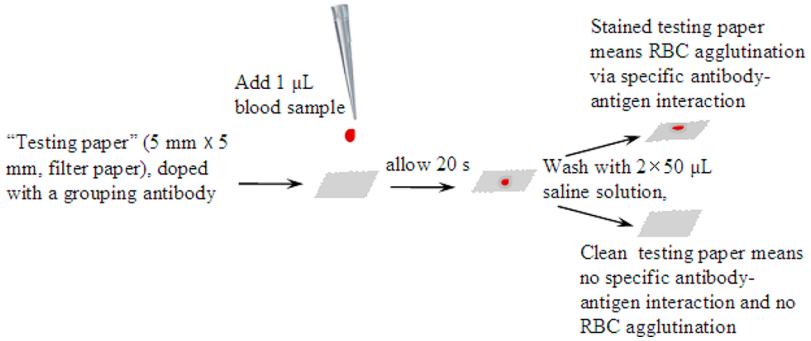


Figure 2. A schematic diagram showing blood typing test protocol using paper and result interpretation.

CS software; the calibration curve was then obtained. Error bars (relative standard deviation) were obtained from five repeats of the whole experiment.

Test of Activity of HRP-BSA Paper without Plasma Treatment. Seven filter paper squares (1 cm × 1 cm) were soaked in diluted HRP solution (4 μg/mL) and dried in a fume hood for 20 min. Then, 15 μL of BSA solution with the concentrations of 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, and 4% (w/v) were deposited onto each square, respectively. After drying in the fume hood for 20 min, 15 μL of TMB liquid substrate solution was added onto each of the filter paper squares. These filter paper squares were then incubated for 2 min to allow the color development. Finally, the filter paper squares were scanned to get clear images. Error bars (relative standard deviation) were obtained from five repeats of the whole experiment.

Test of Activity of HRP Paper after Plasma Treatment. *Paper Loaded with Different Concentrations of HRP Solution Treated with Plasma for a Fixed Treatment Time.* HRP stock solution was diluted to standard solutions with the concentrations of 0, 1, 2, 3, and 4 μg/mL. Five filter paper squares (1 cm × 1 cm) were soaked in each diluted HRP solution and dried in a fume hood for 20 min. Then, the paper squares were plasma treated (50 W) for 60 s. Plasma treatment was activated in vacuum, with a background air pressure of 6×10^{-1} mbar. TMB liquid substrate was added to the paper squares following the same procedure used in the previous section.

Paper Loaded with a Known Fixed Concentration of HRP Solution Treated with Plasma for Different Times. Six filter paper squares (1 cm × 1 cm) were soaked in diluted HRP solution (4 μg/mL) and dried in a fume hood for 20 min. Then, the paper squares were plasma treated (50 W) for 0, 5, 10, 20, 40, and 60 s, respectively. TMB liquid substrate was added to the paper squares following the same procedure used in the previous section.

Test of Activity of HRP-BSA Paper with Plasma Treatment. *Paper Loaded with a Fixed Concentration of HRP Solution and BSA Solution Treated with Plasma for a Fixed Treatment Time.* Ten filter paper squares (1 cm × 1 cm) were soaked in diluted HRP solution (4 μg/mL) and dried in a fume hood for 20 min. Then, 15 μL of BSA solution with the concentration of 2% (w/v) was deposited onto these squares. After drying in the fume hood for 20 min, the paper squares were plasma treated (50 W) for 0, 5, 10, 20, 40, 60, 80, 100, 200, and 300 s, respectively. TMB liquid substrate was added to the paper squares following the same procedure used in the previous section.

Paper Loaded with a Fixed Concentration of HRP Solution and Different Concentrations of BSA Solution Treated with Plasma for a Fixed Treatment Time. Seven filter paper squares (1 cm × 1 cm) were soaked in diluted HRP solution (4 μg/mL) and dried in a fume hood for 20 min. Then, 15 μL of BSA solution with the concentration of 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, and 4% (w/v) were deposited onto each paper square, respectively. After drying in the fume hood for 20 min, the paper squares were plasma treated (50 W) for 20 s. TMB liquid substrate was added to the paper squares following the same procedure used in the previous section.

Quantification of the Activity of Bioactive Paper from Scanned Images. After each test, the colored paper squares were imaged with a desktop scanner (Epson Perfection 2450, color photo setting), then imported into Adobe Photoshop software, and converted into CMYK mode. The mean cyan intensity was obtained using the histogram function. This is because the cyan channel can provide a larger dynamic range than other channels and further increases the accuracy of the analysis of the developed color on the paper squares. The ultimate mean intensity value was generated by subtracting the measured average intensity of the colored paper squares from the mean intensity of the blank control.

Blood Typing by Using Plasma Treated Testing Paper. A 5 mm × 5 mm Kleenex paper square was cut and treated by doping it with 3.5 μL of undiluted (1×) commercial grouping antibody agent and allowed to dry in a fume hood for 10 min. This Kleenex paper square was then treated in a vacuum plasma reactor for 1 min, at an intensity of 50 W. The vacuum level for the treatment was 6×10^{-1} mbar. A further 3.5 μL of undiluted (1×) commercial grouping antibody agent was then introduced to the paper square (double dope). The plasma treatment was then repeated once again. The antibody-treated papers (with 2 × plasma treatments) are referred to as the "testing papers" and are shown as the white squares in Figure 2. The testing papers treated with Anti-A, Anti-B, and Anti-D are specifically referred to as "paper A", "paper B", and "paper D", respectively.

A one-microliter blood sample was introduced onto the antibody treated testing paper (Figure 2). Most of the sample was absorbed by the testing paper. The nonabsorbed blood sample passed through the testing paper and was absorbed by blotting paper. Twenty seconds was given to allow interactions of RBCs with the antibody in paper. After that, the testing paper was placed onto another blotting paper. Two aliquots of 50 μL saline solution aliquot saline solution were gradually introduced by a micropipet onto the blood sample-loaded testing paper; the saline solution penetrated through the testing paper and was absorbed by the blotting paper underneath the testing paper. The testing paper was then separated from the blotting paper for visual inspection. Visible blood stain on the testing paper indicates that RBC agglutination occurred and the test is positive. On the other hand, if no blood stain is observable on the testing paper, RBC agglutination did not occur, and the test is negative. Following this principle an ideal testing-result-matrix is presented in Figure S1.

RESULTS AND DISCUSSION

Effect Plasma Treatment of HRP Activity. Proteins, including enzymes, can be denatured when they are exposed to extreme conditions such as high temperature, mechanical forces, radiation, plasma treatment, chemicals, and many transition metal ions. Figures 3 (a) and 3 (b) clearly show that plasma treatment has the effect of deactivating the HRP paper. Since the TMB solution was added onto HRP paper, the blue color was developed resulting from the enzyme−substrate reaction (Figure 3 (a)). The HRP activity is indicated by the intensity of the blue color. Figure 3 (c) shows the activity of HRP paper treated with a series of concentrations of HRP solution. However, after 60 s plasma treatment (50 W), the blue color intensity was greatly reduced indicating a significant

dx.doi.org/10.1021/am301699m | ACS Appl. Mater. Interfaces 2012, 4, 6573−6578

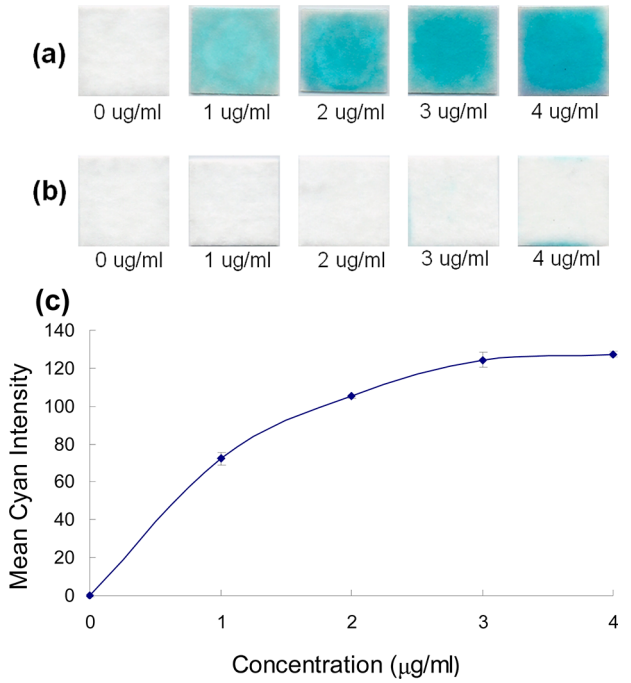


Figure 3. The comparison of the activities of a series of HRP paper squares (a) without plasma treatment and (b) with plasma treatment (50 W, 60 s); (c) measured mean cyan intensity of catalytic product on HRP paper. (a) First, paper squares were treated with different concentrations of HRP solution and dried in a fume hood. Then, 15 μL TMB was added on the squares. Image was obtained after 2 min incubation. (b) First, paper squares were treated with different concentrations of HRP solution and dried in a fume hood. Then, these paper squares were treated with plasma (50 W) for 60 s. After that, 15 μL TMB was added on the squares. Image was obtained after 2 min incubation.

loss of enzymatic activity (Figure 3 (b)). This observation clearly shows the devastating loss of HRP activity in paper by plasma treatment.

To quantify the effect of plasma treatment on the activity loss of HRP, the dried HRP paper, which was prepared by soaking the paper in 4 μg/mL HRP solution, was chosen for further investigation; the paper squares were treated with plasma (50 W) for different times, and the HRP activity was measured in the same way. Figure 4 shows the rapid loss of HRP activity on paper with the increase of plasma treatment time. It can be seen from Figure 4 that there is a sharp decline of mean cyan intensity with the increasing treatment time from 0 to 10 s,

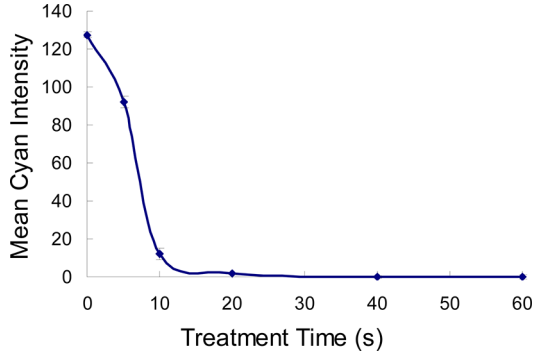


Figure 4. The effect of plasma treatment on the activity of HRP on paper.

showing that deactivation of HRP occurred in a very short time under plasma treatment. After 20 s of plasma treatment, HRP was almost completely deactivated. This study indicates the activity of HRP is extremely susceptible to plasma treatment.

Protection of HRP from Total Inactivation Caused by Plasma Treatment. The above results are in agreement with many studies reported in the literature that plasma treatment has devastating effects on activities of biointerfaces. In fabrication of low-cost diagnostic devices such as bioactive paper devices, however, it is ideal that bioactivities can be protected from total inactivation by plasma treatment. At least two scenarios can be perceived where such protection will be necessary. First, if bioactivities can be protected from plasma inactivation, then the fabrication process can be carried out with more flexibility; plasma treatment of the device does not have to be performed before the addition of bioactive reagents into the device. Second, the biochemical method used for protecting the plasma inactivation could be used as a method of patterning of bioactivity. In this study, however, we focus on proving the concept of using BSA to protect the bioactivity from total inactivation by plasma treatment.

In order to quantitatively evaluate the protective action of BSA against the inactivation of HRP paper caused by plasma treatment, a range of concentrations of BSA solution was prepared to create a protective layer over the HRP paper. In order to understand the addition of BSA to the activity of HRP on the paper, HRP activities on paper with and without BSA were compared. It can be seen from Figure 5 (a) that the

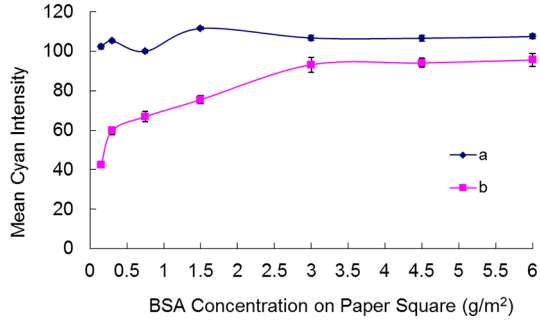


Figure 5. The protective action of BSA on HRP paper: (a) untreated HRP-BSA paper and (b) plasma (50 W, 20 s) treated HRP-BSA paper. Filter paper squares were soaked in 4 μg/mL HRP solution. After drying, these squares were dosed with 15 mL of different concentrations of BSA solution. After drying in a fume hood for 20 min, HRP-BSA paper was fabricated. The calibration curve to convert color intensity to equivalent concentration of HRP solution (ug/mL) can be seen in Figure S3.

addition of a BSA layer over the HRP paper slightly reduced the activity of HRP, nevertheless the HRP still remain active. A possible reason for this observation may be that the protective layer of BSA slows down the penetration of the TMB solution, which restricted the accessibility of TMB to the HRP molecules that are covered by the BSA layer. Therefore, the shorter reaction time led to a slightly weaker development of color.

The most interesting result can be seen in Figure 5(b), which clearly shows that HRP molecules on the HRP-BSA paper retain a substantial level of activity after plasma treatment (50 W, 20 s). Compared with Figure 4, the inactivation of HRP on paper is significantly slowed down. This result suggests that the protection layer of BSA offers protection to HRP in paper against damage by plasma treatment. The effect of protection

dx.doi.org/10.1021/am301699m | ACS Appl. Mater. Interfaces 2012, 4, 6573−6578

could be improved with the increase of the concentration of BSA up to approximately 2 wt %. Fifteen microliters of 2 wt % BSA solution can provide a BSA layer with the concentration of 3 g/m^2 on paper square. Further increase of BSA concentration solution beyond 2 wt % offers little improvement of the protection of HRP in paper. The results show that a stable protection layer which was made of 2 wt % BSA solution offers a significant level of protection to HRP against plasma deactivation of HRP although such protection is not 100%. The HRP activity that survived the plasma treatment shows that BSA offers a practical means to protect the bioactivity in paper, and the level of protection is sufficient to maintain its bioactive function. The possible mechanism of the protection of HRP by BSA may be due to the physical barrier effect of BSA which shields the HRP molecules from the direct exposure to the energetic particles in the plasma environment.

Kylián et al. studied the plasma etching of a deposited BSA layer on a silica surface. They found that the deposited BSA layer can be etched away by plasma treatment.[25] However, the reduction of BSA layer under the plasma treatment was not instantaneous; instead, it was shown to be a gradual process with the increase of the amount of plasma treatment (i.e., intensity × time).

Figure 6 compares the stability between HRP paper and HRP-BSA paper under plasma treatment. With increasing

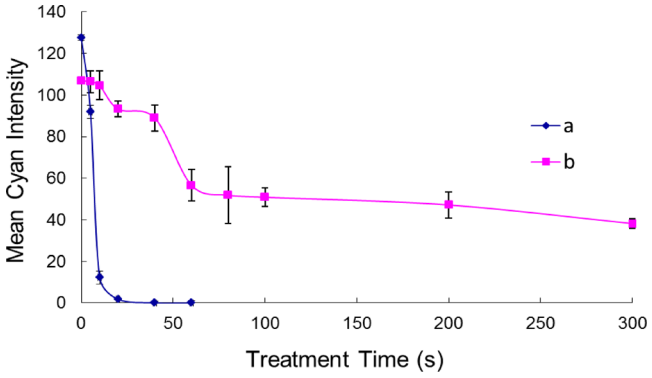


Figure 6. The stability of HRP paper (a) and HRP-BSA paper (b) under plasma treatment (50 W). Filter paper squares were soaked in 4 μg/mL HRP solution. After drying, these squares were treated with 15 mL of BSA solution (2 wt %). After drying in fume hood for 20 min, the preparation of an HRP-BSA paper sample was completed. The calibration curve to convert color intensity to equivalent concentration of HRP solution (μg/mL) can be seen in Figure S4.

treatment time, the intensity of developed cyan color of the catalytic product on HRP-BSA paper was reduced. The trend shown by Figure 6 (b) indicates that the activity of the HRP-BSA paper underwent slower loss with the increase of plasma treatment time, compared with the HRP paper without BSA. This result shows that BSA has a positive protection effect on HRP activity against plasma treatment. Normally, 20−30 s of plasma treatment (50 W) can significantly improve the wettability of low-wettable paper. After 20−30 s of plasma treatment at 50 W, the measurement of HRP catalyzed color change shows that greater than 70% of color intensity can still be observed. This color intensity corresponds to near 40% of enzyme activity. Even after 300 s of plasma treatment at 50 W, measurement of HRP catalyzed color change shows that greater than 40% of color intensity can still be observed indicating that there is still an amount of enzyme molecules remaining active. This finding shows the stability of HRP-BSA paper is greatly improved over HRP paper, whose activity was almost completely lost after 20 s of plasma treatment at 50 W.

Plasma Treatment of Papers Carrying Blood Grouping Antibodies. We return to the practical application of improving blood wettability of blood typing paper that carries the blood typing antibodies. As was mentioned earlier, when blood typing antibodies from stated source are introduced on the paper, the blood wettability of the paper reduces significantly (Figures 7 (a) and 7 (b)). Such a reduction in

Figure 7. Profile of blood in/on (a) paper; (b) antibody paper (contact angle = 122.2°); and (c) plasma treated antibody paper. (a) Kleenex paper square without any treatment. (b) Kleenex paper square treated by adding antibody solution twice. (c) Kleenex paper square treated using the same treatment procedure as blood typing paper. Four microliters of blood was deposited onto each paper square respectively. After 3 s, images were taken to compare the status of blood sample on each paper. Blood penetrated into paper (a) and plasma treated antibody paper (c) in less than 3 s. However, water does not completely penetrate into antibody paper (b) within 30 s.

blood wettability negatively impacts on the performance of the blood typing paper, since slow penetration of sample will slow down the assaying speed. The most effective way to overcome this problem is to restore the wettability of the paper.

Blood typing antibodies from stated source contain 1−5% BSA as the stabilization reagent.[10] Because of the hydrophobic nature of BSA, its adsorption on cellulose fiber surfaces will reduce wettability. However, based on our study of the HRP-BSA system, the wettability of the paper carrying blood typing antibodies should be restored by plasma treatment and the blood typing antibody molecules should still be active. Followed by this hypothesis, paper carrying blood typing antibodies were plasma treated for 60 s at 50 W. After the treatment, the blood wetting of the paper was restored; a blood sample can completely penetrate into the treated paper within 3 s (Figure 7 (c)). This level of wettability fulfills the blood penetration requirement of blood typing paper.

Figure 8 shows the blood typing results using the plasma treated papers (50 W, 60 s). These results show that plasma treatment can be used as a method to improve the wettability of bioactive papers, provided that appropriate protection measures are taken to prevent the plasma deactivation of the biomolecules.

CONCLUSION

The concept of using plasma treatment to improve the wettability of bioactive paper for rapid spreading and penetration of liquid sample into paper was investigated. Plasma treatment is shown to rapidly deactivate HRP molecules on paper. In this study, we found that BSA can act as a molecular chaperone to protect HRP in paper against inactivation caused by plasma treatment. The successful protection of biomolecules by BSA enables plasma treatment to be used as a part of the fabrication process for the mass production of paper-based sensors in which strong wettability of bioactive areas such as detection areas on a device is

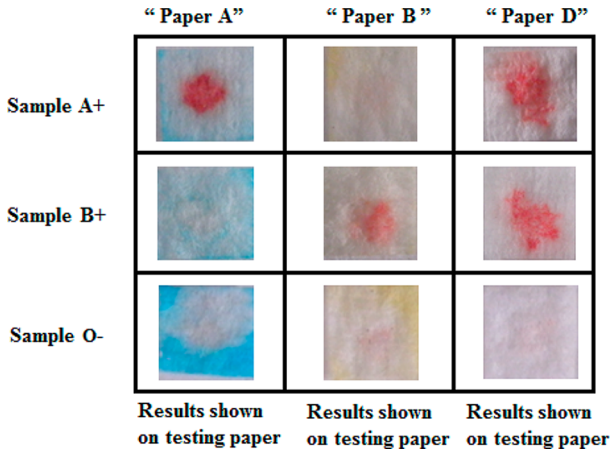


Figure 8. Testing of blood types using testing papers treated with 2 × 3.5 μL of commercial antibodies. Paper A, Paper B, and Paper D, all showing expected results (1 μL blood sample, plasma treatment).

required. We also demonstrated a real bioactive paper application of blood typing paper. Plasma treatment of antibody loaded papers greatly increases the blood penetration rate into the paper and at the same time leaves a sufficient level of antibody activity to agglutinate RBCs that carry the corresponding antigens. Plasma treatment of the blood typing papers shows that this method may be further developed into a fabrication step in mass production of bioactive paper diagnostics where devices must have a high level of wettability and sufficient bioactivity. This study shows that the sequence of plasma treatment and the introduction of bioactive reagents onto paper may no longer be an engineering restriction in sensor production. These two fabrication steps can be implemented in any sequence to suit the engineering of the best production efficiency.

■ ASSOCIATED CONTENT

Supporting Information

Four figures: (1) Figure S1. A schematic of an ideal blood typing testing-result-matrix of antibody-specific agglutination of RBCs on testing paper; (2) Figure S2. A schematic of the process for evaluating the protection of HRP paper by BSA from plasma treatment; (3) Figure S3. The protective action of BSA on HRP paper. (a) Untreated HRP-BSA paper; (b) Plasma (50 W, 20 s) treated HRP-BSA paper; (4) Figure S4. The stability of HRP paper (a) and HRP-BSA paper (b) under plasma treatment (50 W). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +61 3 99053447. E-mail: wei.shen@monash.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is supported by Australian Research Council Grant (ARC DP1094179 and LP110200973). Authors thank Haemokinesis for its support through ARC Linkage Project. The authors would like to specially thank Dr. E. Perkins in the Department of Chemical Engineering, Monash University for proofreading the manuscript. J.T., L.L., and M.L. thank Monash University Research and Graduate School and the Faculty of Engineering for their postgraduate research scholarships. P.J. is grateful for the research grants from the Thailand Research Fund (TRF) through the Royal Golden Jubilee PhD Program (RGJ) and Center of Excellence for Innovation in Chemistry (PERCH−CIC), Commission on Higher Education, Ministry of Education.

■ REFERENCES

(1) Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1318−1320.
(2) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E. *Anal. Chem.* **2010**, *82*, 3−10.
(3) Li, X.; Tian, J.; Nguyen, T.; Shen, W. *Anal. Chem.* **2008**, *80*, 9131−9134.
(4) Pelton, R. *TrAC, Trends Anal. Chem.* **2009**, *28*, 925−942.
(5) Tian, J.; Shen, W. *Chem. Commun.* **2011**, *47*, 1583−1585.
(6) Li, M.; Tian, J.; Al-Tamimi, M.; Shen, W. *Angew. Chem., Int. Ed.* **2012**, *51*, 5497−5501.
(7) Mukhopadhyay, R. *Chem. World* **2010**, *7*, 50−53.
(8) McMaster University Daily News. http://dailynews.mcmaster.ca/article/bioactive-paper-closer-to-commercialization/ (accessed Nov 5, 2012).
(9) Paper Money Web site. http://www.globalpapermoney.org/bioactive-paper-research-receives-added-funding-for-bioactive-paper-development-cms-5380 (accessed Nov 5, 2012).
(10) Jarujamrus, P.; Tian, J.; Li, X.; Siripinyanond, A.; Shiowatana, J.; Shen, W. *Analyst* **2012**, *137*, 2205−2210.
(11) Al-Tamimi, M.; Shen, W.; Zeineddine, R.; Tran, H.; Garnier, G. *Anal. Chem.* **2011**, *84*, 1661−1668.
(12) Pykönen, M.; Silvaani, H.; Preston, J.; Fardim, P.; Toivakka, M. *Colloids Surf., A* **2009**, *352*, 103−112.
(13) Abe, K.; Suzuki, K.; Citterio, D. *Anal. Chem.* **2008**, *80*, 6928−6934.
(14) Li, X.; Tian, J.; Shen, W. *ACS Appl. Mater. Interfaces* **2010**, *2*, 1−6.
(15) Tian, J.; Kannangara, D.; Li, X.; Shen, W. *Lab Chip* **2010**, *10*, 2258−2264.
(16) von Keudell, A.; Awakowicz, P.; Benedikt, J.; Raballand, V.; Yanguas-Gil, A.; Opretzka, J.; Flötgen, C.; Reuter, R.; Byelykh, L.; Halfmann, H.; Stapelmann, K.; Denis, B.; Wunderlich, J.; Muranyi, P.; Rossi, F.; Kylián, O.; Hasiwa, N.; Ruiz, A.; Rauscher, H.; Sirghi, L.; Comoy, E.; Dehen, C.; Challier, L.; Deslys, J. P. *Plasma Processes Polym.* **2010**, *7*, 327−352.
(17) Rauscher, H.; Kylián, O.; Benedikt, J.; von Keudell, A.; Rossi, F. *ChemPhysChem* **2010**, *11*, 1382−1389.
(18) Fumagalli, F.; Kylián, O.; Amato, L.; Hanuš, J.; Rossi, F. *J. Phys. D: Appl. Phys.* **2012**, *45*, 135203.
(19) Birmingham, J. G.; Hammerstrom, D. J. *IEEE Trans. Plasma Sci.* **2000**, *28*, 51−55.
(20) Rossi, F.; Kylián, O.; Hasiwa, M. *Plasma Processes Polym.* **2006**, *3*, 431−442.
(21) Eremin, A. N.; Budnikova, L. P.; Sviridov, O. V.; Metelitsa, D. I. *Appl. Biochem. Microbiol.* **2002**, *38*, 151−158.
(22) Gronczewska, J.; Biegniewska, A.; Ziętara, M. S.; Skorkowski, E. F. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2004**, *137*, 307−311.
(23) Marini, I.; Moschini, R.; Corso, A.; Mura, U. *Cell. Mol. Life Sci.* **2005**, *62*, 3092−3099.
(24) D'Souza, S. E.; Altekar, W.; D'Souza, S. F. *World J. Microbiol. Biotechnol.* **1997**, *13*, 561−564.
(25) Kylián, O.; Benedikt, J.; Sirghi, L.; Reuter, R.; Rauscher, H.; von Keudell, A.; Rossi, F. *Plasma Processes Polym.* **2009**, *6*, 255−261.