Digital Printing of Enzymes on Textile Substrates as Functional Materials

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Abstract

Recently, there have been significant developments in inkjet printing for applications in various fields such as medicine, biomaterials and sensors. In this research, enzymes like horseradish peroxidase (HRP) and glucose oxidase (GOx) were directly printed by inkjet printer onto flexible textile fabric in predefined patterns to produce a functional material. The functionality of the printed enzymes (bioink) was investigated by chemical reaction after printing fresh and stored bio-ink in a digital printer. The results indicated that these enzymes can be effectively printed individually or in combination, which retains their functionality after printing. Furthermore, HRP was coupled and printed with fluorescent group, the result confirmed that the printed enzyme was still active and retained its functionality despite the printing process. Hence, the digital printing technique can be used as a novel method for producing functional textiles for advanced applications in monitoring health and security.

Keywords: Horseradish Peroxidase; Glucose Oxidase; Inkjet Printing; Functionality; Enzymes; Biomaterials

1 Introduction

Inkjet printing is a versatile technique with the potential to produce multifunctional materials at low cost. In sensing applications the advantages include the ability to retain the activity of immobilized biomolecules [1-4]. This is an attractive technique for manufacturing processes due to its precision, speed and flexibility, eliminating the additional expenses associated with other printing methods such as screen printing [2, 5-8]. It is also an eco-friendly method due to lower consumption of materials during the printing of visual effects such as tonal gradients and infinite patterns that cannot be practically achieved with screen printing [2, 7, 9, 10]. This

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technique also allows for more than one biomolecule in a small quantity to be patterned on a substrate, offering sophisticated possibilities to fabricate devices for security, or low cost diagnostic applications [11-14].

Since there is no contact between printed samples and printer head, the risk of cross contamination between the printed biomolecules is minimized [5, 15]. However, the effect of printing procedures on the activity of printed biomolecules and precision of the printed pattern [3, 12] on flexible substrate requires more investigation. Khan et al [5], Risio and Yan [7] investigated the functionality of the printed horseradish peroxidase (HRP) using a piezoelectric inkjet printer. They showed that the printed enzyme retained its functionality, which could be due to the suitable low temperature printing for bio-printing.

Other applications of inkjet printing of biomolecules include fabricating low cost bioassays and diagnostic or security devices; and packaging [5, 14-16]. A few studies have been done to address the possibility of bioassays fabrication by inkjet printing on papers and membranes [8, 14, 15, 17-19]. However, there is a little information on the application of this technique for fabrication of textile based bioassays and diagnostic devices, which can give more benefit to the users, especially in the domestic sector.

This study investigated the possibility of printing biomolecules onto flexible textile fabric. The key issues addressed in this study include printing resolution, precision and activity of the printed biomolecules [5, 13, 15]. In this research HRP and GOx were used as biomolecules. HRP was selected due to its exceptional performance including good stability, high catalytic efficiency, low cost and biocompatibility [5, 13, 20]. When HRP is incubated with colourless substrate tetramethylbenzidine (TMB), it produces a colour and luminescent derivatives, allowing visual impressions which can be viewed with a spectrometer [10, 20-22]. This principle was used to confirm the activity of the enzyme after printing onto the textile fabric. GOx was used as it is widely used for determination of free glucose in body fluids [7, 23-25].

2 Materials and Methods

2.1 Materials

2.1.1 Fabric Samples

A plain woven (square set) mercerized cotton fabric with an area density of 140 g/m² (GSM) was selected for the study. The thread density of the fabric was 33 ends and picks per cm and the yarn linear density was 40 Tex (15 Ne) both for the warp and weft. The printing substrate was prepared from the fabric by cutting into square swatches of 40 cm × 40 cm dimension, hot-pressing them to remove the creases and then attaching to a backing paper for ease of printing in the digital printer.

2.1.2 Chemicals

Horseradish Peroxidase (HRP, Catalogue: 77332, lyophilized, powder, beige ~150 μmol mg⁻¹ min⁻¹); Glucose Oxide (GOx, G6125, Type II, ≥15,000 units/g solid) and phosphate buffer solution (PBS, with concentration of 50 mM phosphate (×10) and pH value of 7.4) were used in
this study. All these chemicals were purchased from Sigma-Aldrich, Australia and were used as received for the preparation of bioink solutions.

2.2 Methods

2.2.1 Preparation of Bioink

Four different bioink solutions were prepared by dissolving various amounts of HRP and GOx as follows: (1) 0.1 mg/mL HRP solution; (2) 1 mg/mL HRP solution; (3) 1 mg/mL GOx; and (4) 1 mg/mL HRP solution with 1 mg/mL GOx. All solutions were made to total of 50 mL using phosphate buffer solution. The bioink solutions were mixed carefully to prevent enzyme deactivation.

In all the experiments fresh bioink solutions were used to print on fabric except for one experiment in which the solution (1) was kept at room temperature in the cartridge for 24 hours before printing on the substrate. This experiment was performed to analyse the functionality of the bioink after storing it for 24 hours at room temperature.

A solution of 0.02% Triton X-100, 2.5 g/L TMB and 0.1% H$_2$O$_2$ was prepared and used to identify the enzyme activity of the printed HRP [15, 23-25], which developed a blue colour. Another solution was prepared with 10 mg/mL glucose and dianisidine (reduced) by dissolving in diluted PBS for the detection of printed GOx enzyme activity [23-25] by developing brown colour.

2.2.2 Enzyme Printing

Printing experiments were carried out using a Sherpa digital printer fitted with a piezoelectric inkjet print head. The print head was driven by a controller (Jet Drive), using EFI software to control the movement of the printing platform below the print head in X-Y direction. Single or double/multiple enzymes were printed onto the textile substrates. The text (in this case RMIT and CSIRO) was printed using Arial font with font size of 180. The cartilages were filled with relevant enzymes for each experiment and prepared paper was fed to the printer. Single or mixed bioink was printed from one cartilage, while two enzymes or combination was printed from two separate cartilages. To print from separate cartilages, first bioink was printed on the fitted paper, then the same printed paper was fed to the printer and the second enzyme was printed on the paper and at the same spot as the first one.

2.2.3 Fluorescent Printing

HRP enzyme was coupled to fluorescent group to produce the effect of fluorescence. The tagged fluorescent dye allowed enzyme to be tracked by UV-light. Fluorescein Isothiocyanate (FITC) was used as a fluorescent substance. Fluorescent HRP enzyme was prepared by dissolving 2 mg of HRP enzyme in 2 mL of conjugating buffer (0.1 M carbonate/bicarbonate buffer, pH, 9.2). About 0.5 mg, FITC was dissolved in 0.28 mL conjugating buffer. This solution was covered with aluminum foil to prevent degradation. Then in the final step, FITC-Buffer solution was mixed with 1 mL HRP-buffer solution, reacted for 2 h at room temperature and the excess FITC was removed by gel filtration. In gel filtration, PBS buffer solution was added four times into the gel.
tube to get gel half way down before final solution was added. The separated liquid of yellow colour was collected in small test tube and covered with aluminum foil. The collected solution had an active HRP enzyme attached to the fluorescent group.

### 3 Results and Discussion

TMB and HRP reaction takes 20 minutes to complete, [20-23] therefore, it is important to wait for 20 minutes after printing the fabric with TMB to get the full colour intensity for fabric sample comparisons. Two reactions were involved to develop the colour from the enzymes as shown in the Scheme 1 [(a) and (b)]. In the first step, colourless TMB was converted into Quinone iminium double cation radical of TMB (blue in colour). In the second stage, glucose was converted into gluconic acid with the production of H₂O₂. Subsequently, the H₂O₂ helped in the oxidation process for the development of the colour.

![Reaction Scheme](image_url)

Scheme 1: Reactions involved in developing colour, indicating the activity of the printed enzyme: (a) HRP and (b) GOx [1, 20]

The effect of reaction time on the absorbance of TMB substrate was studied. Fig. 1 shows the absorbance versus reaction time of the printing chemicals. The figure indicates increased absorption of TMB over time. It is clear from this figure that maximum colour intensity appeared 15-20 minutes after the start of the reaction. This result of our experiments with reaction completed in 15-20 minutes after spraying TMB onto the substrate and the high colour intensity is in agreement with earlier findings.

In this part of the experiment the stability of the enzymes at room temperature was investigated by printing the HRP kept in the cartridge (at room temperature) for 24 hours. It was found that the enzyme was stable even after 24 hours after putting into the cartridge. Both the printed fabric samples (fresh and stored for 24 hours) showed similar levels of depth and brightness of the
printed pattern as shown in Fig. 2, which indicated activity of printed enzyme with ink stored for 24 hours. Fig. 2 (a) exhibited the activity of the printed fresh HRP on the cotton fabric. Enzyme activity and stability were evaluated using TMB solution sprayed on to the printed pattern. In the presence of H$_2$O$_2$, the oxidation of the chromogenic TMB catalyzed by the printed HRP produced blue colour as per Fig. 2 (a). The appearance of blue colour, on the pattern indicated active enzyme post printing.

In production settings, enzymes could be retained in the cartridges for a few hours at room temperature before being printed onto the substrate. Fig. 2 (b) demonstrated the functionality of printed HRP which was retained in the cartridge for 24 hours before printing.

Therefore, the stability of enzyme at room temperature is very critical. The activity of the printed HRP was evaluated by spraying TMB solution onto printed pattern and identify blue colour as it appeared, Fig. 2 (b). Development of blue colour indicated the stability of enzyme at room temperature for at least 24 hours and minimal change occurred in the level of enzyme activity as demonstrated by the proximity of colour intensity in both samples.

Enzyme mixture, i.e. GOx/HRP was also printed on cotton fabric. The mixture (solution number 4) was placed in one cartridge and printed simultaneously onto the cotton fabric. Then the functionality of the printed solution was determined by spraying glucose/dianisidine solution onto the printed pattern. The results shows the developed brown colour which indicates that the enzymes are still active even after mixing and printing processes.

In another experiment two or three biochemicals were printed at one spot by printing them separately from deferent cartilages. Each enzyme solution (solutions 2 and 3) was placed in
separate cartridges and printed at the same spot. The functionality of the printed enzyme was determined by spraying glucose/dianisidine solution onto the printed pattern. The brown colour formed, indicating activity of HRP/GOx mixture. This method can be used for enzymes that are not compatible in the same mixture.

Fig. 3 shows printed HR enzyme with fluorescence group has high fluorescence under UV light.

These results indicate that the inkjet printer technology can be an economical and easy way to produce a textile based bioassays or security devices. This technique offers printing multiple bio-inks simultaneously or separately while presenting high resolution printed patterns in micro scales. Another advantage of this technique is to lock enzymes into the fabric to avoid enzyme molecule movement and produce preciser printed patterns [5, 13]. Furthermore, the fluorescence materials can be successfully printed onto textile fabric by using this technique.

4 Conclusion

In recent years much research has been directed towards the application of inkjet printing for production of paper-based bioassays and diagnostic devices, because it is cheaper than other standard techniques and can be easily scaled up with high precision and speed for commercial production. However, there is little information on producing textile-based bioassays and diagnostic devices with this technique. In this study, the possibility of printing bioink on textile fabric has been investigated. The inkjet printer was used to avoid destruction of the bioink from heat and shear force during the printing procedure. The results indicated a strong possibility of printing enzymes onto the flexible textile fabric without affecting their functionality. The results also revealed that the enzymes can be kept at room temperature without losing their functionality. Another advantage is that the printed pattern on flexible textile fabric was high on both resolution and precision, demonstrating the possibility of the application of these textile-based assays in detection of toxic chemicals in work environment or for security passes. Constructing commercial garments with these sensors could be an economical alternative to security cards to increase the safety of people exposed to hazardous chemicals or atmospheres. However, more research is required to optimize the printing conditions, durability and precision.
References


