DIGITAL INKJET PRINTING OF ANTIMICROBIAL LYSOZYME ON PRETREATED POLYESTER FABRIC

Tuser BISWAS, Junchun YU and Vincent NIERSTRASZ

Abstract:
Lysozyme was inkjet printed on two different polyester fabrics considering several challenges of printing enzymes on synthetic fabric surfaces. Wettability of both the fabrics were improved by alkaline pre-treatment resulting reduction in water contact angle to 60°±2 from 95°±3 and to 80°±2 from 115°±2 for thinner and coarser fabric respectively. Activity of lysozyme in the prepared ink was 9240±34 units/ml and reduced to 5946±23 units/ml as of collected after jetting process (before printing on fabric). The formulated ink was effectively inkjet printed on alkali treated polyester fabric for antimicrobial applications. Retention of higher activity of the printed fabric requires further studies on enzyme-fibre binding mechanisms and understanding protein orientation on fabric surface after printing.

Keywords: *inkjet, lysozyme, antimicrobial, digital printing*

1. Introduction

Enzymes can be immobilized on textiles to impart anti-microbial properties in a more environment-friendly manner compared to conventional biocide based solutions. Such application requires to ensure precise, flexible and contamination free immobilization method that can be offered by digital printing compared to coating or screen-printing techniques [1,2]. Drop-on-demand inkjet printing is a resource-efficient technology that can ensure these requirements [3]. Inkjet minimize use of water, energy, chemical and wastes of valuable functional materials e.g. enzymes [1]. Among two ejection mechanisms of DOD printheads i.e. thermal and piezoelectric, the latter one is preferred due to lesser possible influence on the protein structure of enzymes. Inert polymeric fibrous materials like polyester are ideal supports for such immobilization providing higher loading capacity of biomolecules and larger surface area for interaction. Use of polyester based fabric in on rise ranging from apparel and home furnishing to hygiene and medical textiles [4]. This fibre offers superior chemical, physical, and mechanical properties due to inert nature but provides challenge of printing due to hydrophobicity. Thus, it is necessary to improve wettability of polyester through a suitable pretreatment process that can facilitate proper binding of enzymes and then to retain satisfactory activity. Alkaline based pretreatment is the cheapest and most conventional process in industries that can greatly improve wettability. Lysozyme is a well-studied antimicrobial enzyme that has been grafted on polyester fabric [5] paving the way to use it for inkjet printing. This enzyme has been grafted in modified polyester and other polymeric surfaces mostly through covalent binding involving use of strong fixatives.[5,6] However, possibilities of simple adsorption of the same on pretreated fibre surfaces without use of additional chemicals are not well explored.

Challenges for inkjet printing of enzymes on fabric surface comes in multiple forms i.e. ink recipe formation, printer mechanics and fabric surface characteristics [7-11]. The ink must maintain a suitable viscosity and surface tension for effective drop ejection [12] and a feasible ionic nature for enzyme activity [13]. Then, the enzyme must be able to sustain printing temperature and shear stress generated inside a printhead [14]. Finally, influential fabric characteristics include surface structure, pore size distribution, evaporation rate and binding mechanism. By considering these parameters in this work, lysozyme was inkjet printed on two different polyesters showing satisfactory antimicrobial activity.

2. Materials and methods

Two 100% polyester plain woven fabrics with different weave density and weight per unit area i.e. thinner (150 gsm)first and coarser (290 gsm) supplied by Whaleys (Bradford) Ltd. (UK) were used. The fabrics were first washed with 2g/l non-ionic surfactant at 40°C for 30 minutes. To improve the hydrophilicity, fabrics were pre-treated with 1M sodium hydroxide solution for 30 minutes at 95°C and followed by a hot wash containing 50 mM acetic acid and drying overnight at room temperature.

Wettability of the fabrics were measured by using sessile drop method on an optical tensiometer (Attension Theta, Biolin Scientific, Sweden) with drop volume of 3 µL at room temperature. The water contact angle on three random position was measured immediately after landing of the drop on fabric surface.

Pore size of polyester surfaces was measured using a capillary flow porometer (PSM 165, TOPAS GmbH). Fabrics were first immerged into a fluorocarbon solution (16 dynes/cm) and then a gas is pressurized to pass the solution through the fabrics. The lower the pressure at which the pores empty, the higher the pore size and vice versa.

For ink preparation sodium carboxymethyl cellulose was used as viscosity modified along with a non-ionic surfactant according to required rheological parameters of the used printhead. Lysozyme in potassium phosphate buffer solution were then added to the ink modifier and well mixed before printing. Printing process was performed using a custom-made inkjet printer Urtidium B200 (VdW-Consulting, Belgium) equipped with a piezoelectric printhead Konica Minolta KM1024i. This printhead can produce drops of 13 picoliter size at a maximum frequency of 35 kHz. There are 1024 individually addressable nozzles in the print head capable of forming 360 dots-per-inch spacing.

Antimicrobial activity of lysozyme was studied by a Biochrom Libra S60 UV-Vis spectrophotometer against decrease of Micrococcus lysodeikticus cell concentration at 450 nm. One active unit was defined as the amount of enzyme causing a decrease in absorbance of 0.001 per minute. Activity units were calculated from the initial linear rate against a standard calibration curve. Substrate solution of 0.01 % (w/v) was prepared with 66 mM phosphate buffer adjusted to pH 6.5. Ink solution of 0.10 mL of was added to 2.5 mL of substrate solution in a cuvette with 1 cm light path. Printed fabric were placed inside a cuvette of same path length with equivalent amount of substrate solution. During activity measurement, cuvettes were equilibrated at 25°C and kept under continuous magnetic stirring using a Peltier controller unit (Evolution, Thermo Scientific, USA).

All presented data points are the mean of at least three observations. The results mentioned as ‘significantly different’ for $p<0.05$ that were obtained by the one-way analysis of variance and the Tukey test among two groups.

3. Results and discussions

Wettability of both the fabrics were improved by alkaline pre-treatment resulting reduction in water contact angle to 60°±2 from 95°±3 and to 80°±2 from 115°±2 for thinner and coarser fabric respectively. Resulted mean pore size of thinner and coarser fabric after pre-treatment was found to be 10±0.5 µm and 50±2 µm, respectively. Alkaline treatment caused hydrolytic scission of ester bonds on polyester backbone through mechanisms like exo- and endo-cleavage on different polymeric regions i.e. crystalline and amorphous [15]. It resulted in degraded products from polyester like terephthalic acid and ethylene glycol [16,17]. It also generated new hydrophilic functional groups e.g. hydroxyl and carboxyl [18,19]. Along with chemical, morphological changes brought about by the treatments that improved wettability, pore sizes and other physical properties were also modified.

Activity of lysozyme in the prepared ink was 9240±34 units/ml and reduced to 5946±23 units/ml as of collected after jetting process (before printing on fabric). This reduction is most probably due to change in lysozyme protein structure caused by shear stress inside printhead [20]. Shear stresses are produced by the jetting force of piezoelectric element for drop generation. This stress can lead to higher fluid compression rate and damage the protein structure as found for inkjet printing of other enzymes. For example, similar printing method used for peroxidase enzyme [21]. Additionally, it was found that activity loss for glucose oxidase was dependent on printer voltage and related waveform [22]. However, no such effect was found on immunoglobulins activity when compared between a single nozzle inkjet setup to manual pipetting [23].

Activity of the thinner and coarser printed fabrics were found to be 881±13 units/ml and 1515±27 units/ml, respectively. These values are significantly lower than above mentioned activity after jetting (before printing). In general, microenvironment inside a porours fabric is expected to ensure greater operational stability of enzymes against denaturation [24]. However, the same might cause reduced activity due to unwanted interaction with the fabric matrix, restricted mobility, change of protein confirmation and inaccessibility of active sites towards substrates [25]. Change of enzyme-substrate interaction from a macro to micro-environment and corresponding issues of diffusion and transportation might reduce activity after printing as well.

As indicated by above results, higher antimicrobial activity was observed for the fabric with less hydrophilicity and larger pore size. Thus showing that relatively more amount of printed enzyme was released from the this fabric surface. Reason for such activity variation could be related to manner of printed enzyme transportation inside fabric structure and their stability after adsorption. During activity assay experiments, enzymes may desorb from fabric to substrate solution depending on their adsorption stability. In such situation, printed enzymes will act similarly as enzymes in free solution [26]. Though, these desorbed enzymes would not possess similar catalysis ability as of fresh ink. This is due to the fact that protein structure of printed enzymes were already compromised during adsorption process. Then, activity can depend on the ability of printed enzymes to remain adsorbed on fabric surface brought up by the pretreatments. The fabric with lower wettability would allow comparatively limited transportation of enzyme molecules. This caused some enzymes to stay on outer surface layer with greater possibility to act on substrates. Moreover, such weakly adsorbed enzymes were able to desorb from fabric surface to substrate solution showing higher activity.

4. Conclusion

The formulated lysozyme ink can be effectively inkjet printed on alkaline pre-treated polyester fabric for antimicrobial applications. Retention of higher activity of the printed fabric requires further studies on suitable fabric pre-treatment process for inkjet printing, establishing enzyme-fibre binding mechanisms and understanding protein orientation on fabric surface.

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6. References

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