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Sequential Inkjet Printing of Lysozyme and Tyrosinase on Polyamide Fabric: Sustainable Enzyme Binding on Textile Surface

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An ink containing tyrosinase catalyzes the tyrosine residues on lysozyme protein to bind it on a plasma-treated polyamide-6,6 (PA) fabric. Inkjet printing enables controlled and sequential deposition of two enzymes on PA which is necessary for proper binding. The effect of different printing sequences on crosslinking stability and enzymatic activity is presented. The lysozyme bound on the fabric shows satisfactory antimicrobial activity. The printed fabric retains about 68% of the ink activity when tyrosinase is printed before lysozyme. Further, this fabric retains about 24% of the initial activity up to four reuses. The fabric shows acceptable inhibition of bacterial growth and retains almost half of its initial activity when cold stored for a month. This work shows the potential of protein binding on textile surface using various means of sustainable technologies, namely enzyme, inkjet, and plasma.

1. Introduction

Tyrosinase (polyphenol oxidase, EC 1.14.18.1) is a copper-containing enzyme that can catalyze the oxidation of tyrosine residues found on certain proteins and convert it to *o*-quinone which can further non-enzymatically react and crosslink with amino groups.[1,2] Tyrosinase has been investigated for possible modification of proteins such as gelatin, casein, and sericin.[3] However, its potential to bind another protein on an amino based fiber surface has not been well explored, as per our best knowledge. Lysozyme (1,4-β-*N*-acetylmuramidase, EC 3.2.1.17) is a well-studied small globular protein known for its antibacterial,[4] antifungal,[5] and antiviral[6] applications. Lysozyme has been covalently bound on various fibrous materials, for example, cotton,[7] polyester (PET),[8] polyamide-6,6 (PA),[9] and wool,[10] but mostly using harsh surface binding chemicals such as glutaraldehyde. Most of these studies involved dipping the fabrics in enzyme containing solutions and lengthy incubating procedures. Amount of enzyme loading and deposition manner was less controllable. A better approach would be free from the use of harsh chemicals, minimize enzyme waste and processing time, ensure more process control, and flexibility. Lysozyme contains three tyrosine residues which tyrosinase can catalyze to covalently bind on a fibrous surface containing amino group. To the best of our knowledge, this unique approach has not been studied prior to this work, specially involving inkjet printing these two enzymes in same process. Inkjet technology ensures direct and precise deposition of biomaterials with high-resolution designs for more resource efficient and flexible-scale production compared to conventional approaches, for example, screen printing and coating. However, there are fundamental differences between the working principles and manner of enzyme deposition between these technologies, thus the obtained results might not be directly comparable.

Digital inkjet printing of enzymes on textile surfaces has great potential for applications ranging from bacterial inhibition to controlled drug delivery.[11] Textile surfaces can provide greater surface area for accommodating higher numbers of enzyme, along with a flexible, light-weight, and strong support medium, compared to flat film-like surfaces. Textiles made of synthetic fibers are of high industrial interest due to their physical strength, chemical inertness, recyclability, reusability, and applicability in various fields.[12] Several enzymes have been studied to include in an ink solution and optimize their activity after printing.[13] Synthetic textiles have been modified using several pretreatment techniques[14,15] to ensure proper ink adhesion, enzyme adsorption, and activity retention.

Plasma pretreatment of PET and PA fabrics was found to be beneficial for inkjet printing of enzymes, replacing the needs of harsh chemical based pretreatment processes.[16–19] Plasma treatment of PA can modify its topography, increase surface energy, and introduce new functional groups.[20–22] Such modification can promote better adsorption of printed enzymes through their amino end groups.[23] However, a considerable amount of enzymes were rinsed away from printed fabrics

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as they were only physically adsorbed rather than being covalently bound. Therefore, this study offers a unique approach to ensure greater binding of printed enzymes on PA fabric surface within the framework of resource efficient and sustainable technology. There are multiple challenges to succeed through such an approach as addressed in this work. For a successful inkjet printing process several variables need to be optimized, for example, ink recipe formulation, printhead mechanics, and fabric surface properties for proper interaction with enzymes.[14,24,25] Often such optimization comes with a compromise on enzyme activity after printing.[14,15,24] To maintain proper activity, tyrosinase and lysozyme prefer different ink formulating materials, for example, carboxymethyl cellulose[15] and glycerol[14,24] as viscosity modifier, respectively. Therefore, two separate inks containing these enzymes were optimized for same inkjet printhead to retain adequate activity after printing. PA fabric was plasma treated to ensure proper surface activation for enzyme adsorption and ink adhesion. Effect of various enzyme printing sequence on crosslinking ability with PA and related inhibition kinetics were studied. Enzymatic activity of the printed fabrics during prolonged storage and ability to prevent bacterial growth was studied as well. The printed fabric of this study has great potential to be used for several antimicrobial applications.[11,26,27] Additionally, this fabric can be adapted for similar applications in several industries, for example, food processing, medicine, and pharmaceutical.[28] The novel approach of this work can be further studied to bind other inkjet printed enzymes and proteins on a textile surface.

2. Results and Discussion

2.1. Formulation of Inks and Printing

Ionic, rheological, and printhead properties for enzyme containing inks require proper adjustment for successful printing. The aim is to safeguard appropriate ink flow, drop formation, and subsequent ink spreading on fabric, along with conservation of adequate enzymatic activity after printing processes. These parameters need to be adjusted for each enzymatic ink and printhead combinations. Accordingly, viscosity, surface tension, ionic profiles, and printing temperature of the two prepared inks (lysozyme and tyrosinase) were optimized separately for respective enzyme concentrations as detailed in experimental section. Both enzymes were well dispersed in ink solution and appeared semi-transparent indicating low printhead nozzle blocking possibility. UV–vis spectroscopy of lysozyme containing ink showed absorbance peak at 282 nm and a shoulder-peak at 290 nm (**Figure 1**). Absorbance band in this range confirmed presence of amino acid constituents on lysozyme protein.[29] For tyrosinase containing ink, broad absorbance bands were found at 300–320 nm, thus confirming the presence of residues like tyrosine, phenylalanine, tryptophan, and histidine.[30] These results affirmed uniformity of the enzymes in ink solution and well preservation of their protein structures.

Reynolds number (*Re*), Webers number (*We*), and inverse Ohnesorge number (*Z*) of the prepared inks were used to understand their theoretical feasibility for printing. These

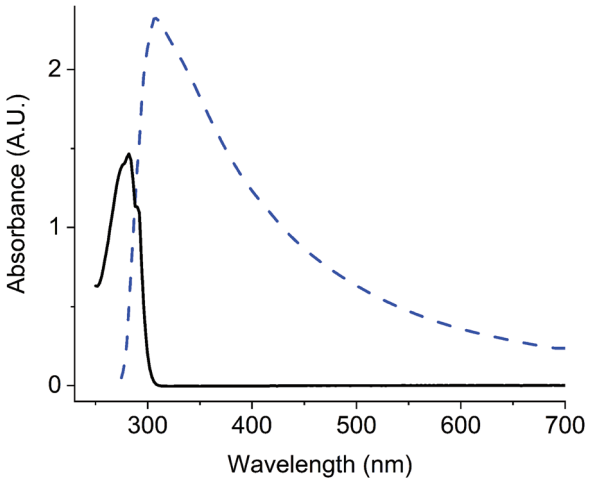

Figure 1. UV–vis absorption spectra of lysozyme (solid line) and tyrosinase (broken line) against respective ink vehicle solutions (blank sample).

dimensionless characteristics numbers were calculated (Equations (1)–(3)) from the values of print velocity (v), ink density (ρ), viscosity (η), surface tension (γ) and characteristics length as nozzle radius (r).[13,24] Calculated numbers for both inks (Z = 2.6–3.5, We = 6.8–8.0) were within the frame allowed for inkjet printing ($1< Z <10$; $We > 4$).[31] This ensured that the inks would be able to produce proper drops by overcoming the influence at air–fluid interface, thereafter ejecting the drops continuously by avoiding satellite drop formation.

$$Re = \frac{v\rho r}{\eta} \tag{1}$$

$$We = \frac{v^2\rho r}{\gamma} \tag{2}$$

$$Z = \frac{Re}{\sqrt{We}} \tag{3}$$

2.2. Fabric Surface Modification

Modification of PA surface through plasma treatment can result in increased surface energy and introduction of functional groups.[32] This influences wetting behavior and physical properties of the fabric. Accordingly, surface wettability of PA was improved after plasma treatment with water contact angle reduction of about 25° compared to untreated fabric. Reduction of fabric tensile strength due to plasma treatment was also nominal (≈1%). XPS results confirmed increased atomic% of oxygen (≈6%) and nitrogen (≈1.8%) on PA surface after plasma treatment. Amido carbonyls peak intensity increased (≈13%) at binding energy of 288 eV, indicating formation of carboxylic species in hydrocarbon or carbonyl groups of plasma treated PA fabrics.[15] Further, ζ-potential analysis (Figure 3) showed that isoelectric point (IEP) of plasma treated fabric moved to a lower pH value (≈3.3) than untreated fabric (≈4.3) due to increased polar species.[33] Such modified surface properties could promote adhesion of enzymes and further binding possibilities. Plasma modified PA surface can promote better adhesion with enzymes due to possible electrostatic interactions.[8]

Table 1. Fabric type and print sequence.

Sample name	Fabric treatment	1st print	2nd print
LT	Untreated	Lysozyme	Tyrosinase
pLT	Plasma	Lysozyme	Tyrosinase
TL	Untreated	Tyrosinase	Lysozyme
pTL	Plasma	Tyrosinase	Lysozyme

2.3. Enzyme Binding on Fabric and Catalytic Activity

Activity values for tyrosinase and lysozyme containing ink were 654 ± 20 units mL^{-1} and 481 ± 21 units mL^{-1} against respective substrates. As expected, none of the enzymes showed any detectable activity against the substrate of the other enzyme. After complete printing sequence all the samples (**Table 1**) were thoroughly rinsed in buffer solution to remove any unbound enzymes from PA surface. Surprisingly, none of the printed samples showed any detectable activity against L-tyrosine. It indicated that printed tyrosinase covalently bound lysozyme on PA surface by forming irreversible complexes.[34] Lysozyme protein structure consists of three tyrosine residues (Tyr20, Tyr23, and Tyr53) which can be catalyzed by tyrosinase in the presence of oxygen to form *o*-quinones.[35] These *o*-quinones might have further non-enzymatically reacted with plasma activated amino groups on PA surface to crosslink lysozyme through Maillard or Michaelis addition reaction.[1,2]

Each printed sample was subjected to check lysozyme activity against MLC. After activity assay of a freshly printed and rinsed sample (first assay), it was removed from the cuvette, well rinsed, and further checked for activity (reused). These results are presented in **Figure 2** as a percentage of lysozyme ink activity (see Table 1 for sample descriptions). Highest activity among all the samples was observed for pTL (68%) during first assay. During same assay, lowest activity was shown by LT (38%) and medium

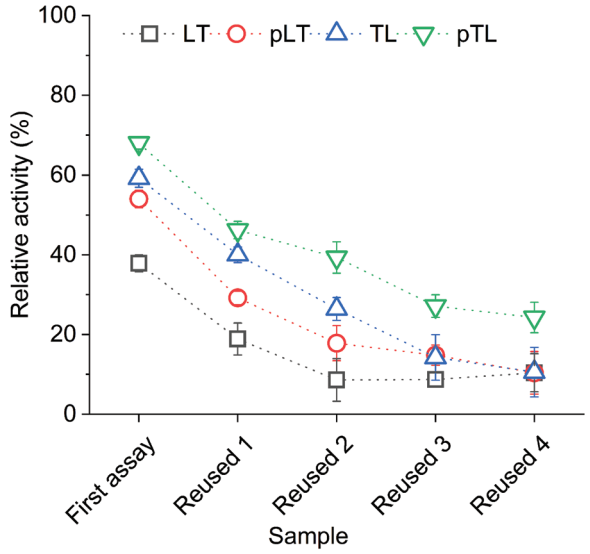

Figure 2. Lysozyme activity of variously printed polyamide-6,6 fabric samples (□–LT, ○–pLT, △–TL, and ▽–pTL; see Table 1 for details) after first assay and subsequent reuse cycles. Results are presented as a percentage of the ink activity before printing (100% activity). Error bars indicate SD ($n = 3$).

ranged activity values were observed for pLT (54%) and TL (59%). Such activity reduction could be caused by printing mechanics, fabric surface properties and diffusion limitations, and restrictions to enzyme structure mobility introduced by immobilization effect.

A piezoelectric printhead uses shear stress for ink drop ejection and this stress has been seen to influence lysozyme activity negatively.[24] Even after ink and printhead parameters optimization, an activity reduction of 10–20% could arise due to printing mechanics. Next, immobilization of lysozyme on PA fabric would change the nature of substrate interaction from a macro to microenvironment and thus affect activity. The expected covalent binding of printed enzymes with fabric surface could introduce steric hindrance and diffusion limitation. Out of the three tyrosine residues on lysozyme structure, close location of Tyr53 to aspartic acid residue (Asp52) on lysozyme active site may affect the activity as well. Additionally, enzymes in ink solution were reacting with the substrates in homogenous state, compared to the printed enzymes which were in heterogeneous state and thus could show reduced activity.

Upon reuse of the samples further reduction of activity was observed (Figure 2). Compared to first assay, activity of LT (19%) and pLT (29%) halved after one reuse, whereas TL (26%) and pTL (39%) maintained almost half of their activity up to second reuse. Upon fourth reuse cycle, pTL maintained as high as 24% of ink activity, where others went down to ≈10%. These results indicate that the ability of enzymes to actively bind with PA surface depends on received pretreatment and sequence of enzyme printing. Overall, higher activity and stronger enzyme binding were observed when tyrosinase was printed before lysozyme. Plasma treated samples showed better ability to bind enzymes than respective untreated samples. Similar plasma treatments[15] as used in this study have demonstrated to facilitate better enzyme adsorption on PA surface due to increased roughness, hydrophilicity, and electrostatic interactions.[36] Tyrosinase could have achieved proper adsorption on PA surface with higher access to oxygen content necessary for catalysis due to oxygen and nitrogen gas mediated plasma treatment compared to untreated PA. When lysozyme was printed prior tyrosinase, possibility of such adsorption and accessibility to surface oxygen was minimized and thus resulted in poor catalysis and surface binding, even if the sample (pLT) received plasma treatment. Moreover, a strong adsorption between lysozyme from first print sequence and plasma treated surface[8] might have alerted the favorable protein conformation necessary for conversion of tyrosine residues to o-quinones. Thus, highest enzyme cross-linking ability and overall activity were observed when tyrosinase was printed before lysozyme on plasma treated fabric (pTL).

Presence of well cross-linked enzymes on printed PA was further confirmed through measurement of surface ζ-potential at various pH of streaming liquid. IEP of printed sample (pTL) significantly shifted toward alkaline region (≈pH 7) compared to plasma treated and untreated samples (**Figure 3**). This shift was caused by surface charge of amino acid groups present in printed enzymes. IEP of lysozyme and tyrosinase lies in the range of pH 9–11 and pH 4.7–5, respectively. Therefore, observed IEP for printed samples indicated well presence of lysozyme protein residues. ζ-potential profile remained similar for a printed sample (pTL) after lysozyme activity assay and

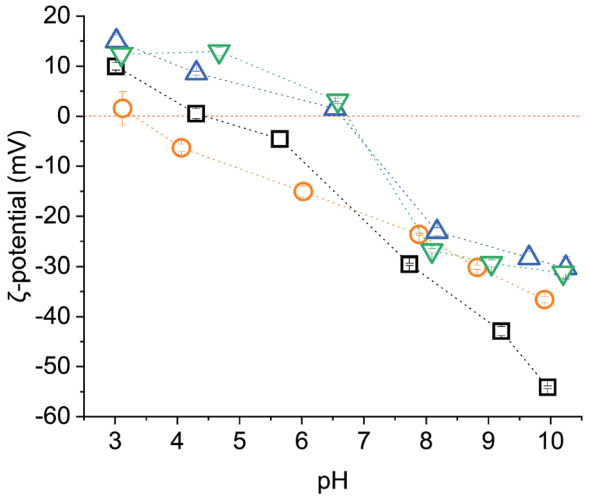

Figure 3. ζ-potential of untreated PA fabric (□), after plasma treatment (○), after enzyme printing on sample pTL (△) and after lysozyme activity assay (▽) of the same printed sample. Error bars indicate SD ($n = 3$).

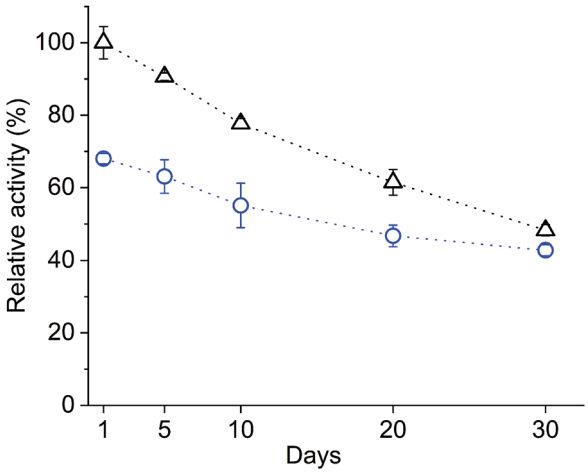

Figure 4. Lytic activity of lysozyme containing ink (Δ) and enzyme printed (O) polyamide-6,6 fabric (sample pTL, see Table 1) during storage. Error bars indicate SD ($n = 3$).

subsequent buffer rinsing. This confirmed well cross-linking of the enzymes on PA surface.

Our previous work[14] about printing lysozyme on plasma treated polyester fabric found only ≈1% activity for same ink amount of this work. In that work, 7–19% of lysozyme was desorbed from fabric during activity assay and rinsing process as no additional enzymatic or chemical approach were used for crosslinking. Thus, the results of current work demonstrate significant improvement for sustainable inkjet printing of enzymes on fabric.

2.4. Storage and Antimicrobial Activity

Prospect of using the printed fabric with adequate activity was evaluated by storing them at 4 °C for 30 days. As discussed in previous section, sample pTL showed most stable lytic activity against the substrate of lysozyme. So, lytic activity of the same printed sample was evaluated against lysozyme containing ink solution (**Figure 4**). As expected, activity of both ink and fabric reduced gradually over time. Ink and fabric lost almost half of their activity after 30 and 20 days, respectively, when compared to ink activity at day one. However, the rate of such reduction was more drastic for ink solution compared to printed fabric. Lytic activity of fabric was ≈63% and ink ≈48% after 30 days, when compared corresponding initial activity levels. Generally, irreversible changes occur to enzyme protein structure with time, hence gradual reduction in activity. Compared to ink solution, better activity performance by printed fabric over longer period of time could be caused by conformational stabilization of lysozyme protein due to cross-linking with fabric surface.[37] For same storage period of this study, previous work[14] showed only 11–14% activity retention where lysozyme was not well bound to fabric surface.

In addition to above mentioned optical density based spectroscopic results, antimicrobial activity of lysozyme was further observed through growth of bacterial colonies.[38] *Micrococcus lysodeikticus* was used as the preferred bacteria for this purpose. It is gram-positive and lysozyme can alter some of its structure involved in the maintenance of cell wall.[39] Same amount of this bacteria was incubated on a blank (containing no enzymes) and enzyme printed fabric (pTL). In **Figure 5**, a red dot represents a bacterial colony and the number of such colonies were significantly lower for the printed sample.[40,41] As mentioned in experimental section, enzymes were printed with solid rectangle design. On blank sample, colonies grew in similar pattern both inside and outside fabric area. However, on enzyme printed sample, bacterial growth was inhibited following a semi-circular pattern inside fabric area. This was achieved by lysozyme bound on printed fabric to catalyze the β-(1-4) glycoside linkages between N-acetylmuramic acid and N-acetylglucosamine in the cell wall of used bacteria.[42] Thus, antimicrobial efficacy of the printed fabric was well confirmed.

2.5. Kinetic Studies

The kinetic constants (V_{max} and K_m) of lysozyme ink and printed fabric (pTL) were calculated over a range of substrate (MLC) concentrations and the results are presented in **Table 2**. The constants were lower for printed fabric due to effects of immobilization on lysozyme protein structure, such as, mass transfer limitations. Reduction of V_{max} and $K_{m,}$ asserts covalent binding of lysozyme and related effects on enzymatic activity caused by conformational changes.[43] A lower K_m indicated better complex formation between enzyme and its substrate for fabric sample and hence reduced V_{max}. Similar results were found on a number of studies,[18,44,45] however none involved inkjet printing or using a second enzyme for covalent binding.

Stability of enzyme activity under higher operational temperature is an important indication of its practical usability.[46] An enzyme printed fabric having high thermal stability would be suitable for industrial applications. Therefore, inactivation rate constant (k_i) and half-life ($t_{1/2}$) of lysozyme containing ink

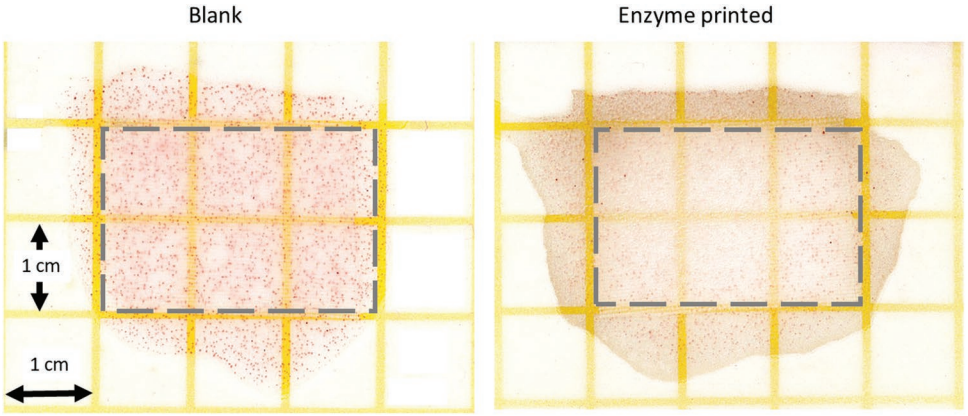

Figure 5. Antimicrobial effect of enzyme printed polyamide-6,6 fabric. Fabrics were placed inside the grey dashed area.

and a fabric sample (pTL) were studied against MLC substrate and the results are presented in **Table 3**. Lysozyme in ink solution and fabric, both were stable to heat treatment up to 85 °C ($k_i \approx 0.08$ to 0.1 h^{-1}), but started to denature near 95 °C ($k_i \approx 0.2$ to 0.6 h^{-1}). Similarly, half-life for ink and fabric at 85 °C (both ≈6.4 h) was drastically reduced near 95 °C (≈1.1 and 2.6 h, respectively). As evident from these results, inactivation rate was significantly lower and hence, half-life value was significantly higher for fabric compared to ink near 95 °C.

Lysozyme in buffer solution can experience heat denaturation above 70 °C when incubated for about 30 min; however, this limiting temperature may be increased by addition of proper additives.[47] Glycerol, used as viscosity modifier in ink formulation, may increase thermal stability of lysozyme.[48] Structural stabilization of lysozyme brought by covalent binding on PA fabric would further improve its resistance to heat denaturation as shown in other works.[49,50] However, to the best of our knowledge, none of the previous studies involved inkjet printing and use of a second enzyme for such covalent binding. The ultimate irreversible denaturation of lysozyme structure near 95 °C could occur due to inter- and intra-molecular exchange of disulfide bonds, deamidation of asparagine residues (near pH 6.5), and precipitation formation due to protein aggregation.[51] Effect of pH on printed tyrosinase and lysozyme has been studied in our previous works.[14,15,24] Optimum pH (≈6) of tyrosinase containing ink and fabric was similar to its activity in buffer solution.[15] However, lysozyme containing ink was preferred to print near pH 6, instead of printing near pH 9.[14,24] Before printing, lysozyme had highest activity near pH 9. Although, after printing the activity significantly reduced for pH ≈9, compared to pH ≈6. Main reason for such behavior was vulnerability of lysozyme protein structure to inkjet printing force near pH 9 at optimized ionic strength of the ink.

Table 2. Kinetic parameters of lysozyme containing ink and enzyme printed polyamide-6,6 fabric.

	V_{max} [Units mg^{-1} protein]	K_m [mM]
Lysozyme ink	1221 ± 37	0.277 ± 0.013
Fabric (pTL)	764 ± 3	0.223 ± 0.002

Data represent mean ± SD, $n = 3$.

3. Conclusion

This study aimed to bind an enzyme on synthetic fabric using less chemical extensive and more sustainable technologies such as inkjet printing and plasma treatment. Thereby, two enzymes (lysozyme and tyrosinase) were inkjet printed on polyamide fabric in different sequences. Lysozyme was bound on the polyamide surface being catalyzed by tyrosinase. Surface binding stability and antimicrobial activity from lysozyme was greater when tyrosinase was printed first in sequence on plasma pretreated fabric. The printed fabric retained satisfactory activity up to four reuse and a month of cold storage. Here printed polyamide fabric shows great potential for antimicrobial related applications. The unique enzyme binding strategy presented in this study can be used for immobilization of other proteins and biomaterials on textiles using inkjet printing.

4. Experimental Section

Materials: A plain weave polyamide 6,6 fabric (PA) with a weight of 118 g m^{-2} was used as support material for printing and it was kindly provided by FOV Fabrics AB (Sweden). Lysozymes from chicken egg white (E.C. 3.2.1.17) and *Micrococcus lysodeikticus* (ATCC 4698) cell substrate for activity assays were purchased from Alfa-Aesar (Germany) and Sigma-Aldrich (Germany), respectively. Tyrosinase (EC 1.14.18.1) from mushroom *Agaricus bisporus* was purchased from Worthington Biochemical Corporation (USA). All other chemicals were of analytical grade and obtained from Merck/Sigma-Aldrich.

Fabric Treatment and Characterization: An atmospheric pressure glow discharge equipment (PLATEX 600, Grinp, Italy) was used for plasma treatment of the fabric surface after washing with a non-ionic surfactant (1% w/w of Triton-X 100) for 30 min at 50 °C and drying. Plasma treatment was achieved by using a combination of oxygen and nitrogen as feed

Table 3. Thermal stability data of lysozyme containing ink and enzyme printed polyamide-6,6 fabric.

	Ink		Fabric	
Temperature [°C]	k_i [h^{-1}]	$t_{1/2}$ [h]	k_i [h^{-1}]	$t_{1/2}$ [h]
75	0.087 ± 0.002	7.93 ± 0.15	0.087 ± 0.001	8.0 ± 0.06
85	0.108 ± 0.002	6.39 ± 0.09	0.109 ± 0.006	6.39 ± 0.32
95	0.643 ± 0.005	1.08 ± 0.01	0.265 ± 0.002	2.62 ± 0.02

Data represent mean ± SD, $n = 3$.

gases (1 L min^{-1} of each) at electrical power of 1.5 kW, fabric feed speed of 1 m min^{-1}, and inter-electrode distance of 1.5 mm. Helium gas (1.5 L min^{-1}) was used to create an inert environment immediately before each treatment.

Fabric wettability was measured through water contact angles (θ) by using the sessile drop method on an optical tensiometer (Attension Theta, Biolin Scientific). θ was measured on three random positions immediately after dropping 3 µL of water at room temperature. Tensile strength to rupture the fabrics was measured according to ISO 13934/1 standard using a semi-automatic electronic strength tester (Tensolab, Mesdan). X-ray photoelectron spectroscopy (XPS) was performed on a PHI 5000 VersaProbe-III instrument equipped with a monochromated aluminum source with a photon energy of 1486.6 eV and beam size diameter of 100 µm at 15 kV.

Ink Preparation and Printing: Two separate inks containing tyrosinase and lysozyme were prepared. Ink recipes consisted of four constituents, that is, buffer solution, viscosity modifier, surfactant, and enzyme. Each recipe was optimized for the used printhead following strategies of the previous works.[14,15] Briefly, lysozyme and tyrosinase containing inks had glycerol ($M_w \approx 92$) and carboxymethyl cellulose (CMC, $M_w \approx 90\,000$) as viscosity modifier with protein concentration of 0.05 and 1 mg mL^{-1}, respectively. For both inks, Triton-X 100 was used as a non-ionic surfactant and pH was adjusted to 7. Inks had viscosity of 6–8 mPa s at 20 °C, shear rate 10 000 s^{-1}, and surface tension of 31–34 mN m^{-1}.

A drop-on-demand piezoelectric inkjet printhead (Dimatix Sapphire QS-256/80, Fujifilm, USA) with 100 dots-per-inch resolution was used for printing. It was mounted on a custom-made printing platform manufactured by Xennia technology. The printhead was set to a temperature of 30 °C. Inks were printed on fabric samples as a solid rectangle on an area of 6 cm × 2 cm. Samples were printed with 50 µL of each ink in an alternating sequence as specified in Table 1.

Protein Quantification: After complete print sequence, samples were thoroughly rinsed in phosphate buffer (50 mM) solution to remove any unfixed proteins from fabric surface. The number of proteins released from printed fabric to buffer solution was counted by bicinchoninic acid (BCA) assay. Briefly, a working solution was made by adding 50 parts of reagent A (sodium carbonate, sodium bicarbonate, bicinchoninic acid, and sodium tartrate in 0.1 M sodium hydroxide) and 1 part of reagent B (cupric sulfate). Then, 0.1 mL of buffer containing proteins were added to 2 mL of working solution and incubated for 30 min at 37 °C before cooling to room temperature. The concentration of proteins was measured spectroscopically by the corresponding absorbance at 562 nm against a constructed standard curve.

Enzyme Activity Assay: Printed fabrics were dried at room temperature for two hours after rinsing process to proceed for activity assays. A standard ultraviolet–visible (UV–vis) spectrophotometer cuvette (polymethyl metacrylate, 4.5 mL) was used for assays of ink and a modified system by combining two standard cuvettes was used for fabric samples. Thus any unwanted interaction between UV–vis light and fabric samples was avoided. Both cuvette systems corresponded to equivalent amounts of enzyme and substrate solution. Phosphate buffer (0.5 M) at pH 7 and 25 °C was used for assay of both enzyme types. Lytic activity of lysozyme was measured against 1×10^{-2}% w/v substrate solution at 450 nm and one active unit was defined as the amount of enzyme causing a decrease in absorbance of 1×10^{-3} per minute. Tyrosinase activity was measured against 1×10^{-3} M L-tyrosine substrate at 280 nm and one active unit was defined as the amount of enzyme causing an increase in absorbance of 1×10^{-3} per minute. The activity was calculated from the initial linear rate against a standard calibration curve covering a range of protein concentrations.

Thermal stability kinetics were measured by incubating the printed fabrics and 50 µL of ink samples at temperatures ranging from 75 to 95 °C for an hour. At selected intervals, samples were removed and cooled down to room temperature prior subjecting to assay solution. The inactivation rate constant (k_i) for each incubation temperature was calculated from a plot of logarithmic residual activity against duration and half-life ($t_{1/2}$) was calculated as $0.693/k_i$.[52] Michaelis–Menten constant (K_m) and maximum rate of the reaction (V_{max}) for lysozyme were measured from initial reaction rates against 0.125–1 × 10^{-2}% w/v substrate concentrations at 25 °C and pH 7. K_m and V_{max} values were calculated from Lineweaver–Burk plots.

Zeta Potential: Zeta (ζ) potential and isoelectric point of the fabrics were measured using a SurPASS electrokinetic analyzer (Anton Paar, Austria). A pair of fabrics of same sample with an area of 10 × 20 mm^2 each was placed in the clamping cell to be separated by a spacer during formation of a streaming channel. A background electrolyte of 1 mM KCl solution was used and the pH was adjusted in the range of 3–10 with HCl (0.2 M) and NaOH (0.2 M). Streaming potential method and the Helmholtz–Smoluchowski equation were used to determine ζ-potential.[53]

Antimicrobial Assay: Growth of bacterial substrate cell on printed fabrics was evaluated using sample ready 3M Petrifilm aerobic count plates. Each plate contains water-soluble gelling agent, indicator dye, and nutrients which are necessary for bacterial growth. Plates were processed and incubated following instruction of manufacturer.[38] Briefly, a fabric sample in the plate were wetted with 1 mL of substrate solution (pH 7) and incubated at 30 °C for 72 h.

Statistical Analysis: The OriginLab software was used for data and statistical analysis. Error bars in all data represent standard deviations (± SD). The number of samples (n) are presented in the figure and table legend of each data. Results mentioned as "significantly different" ($p < 0.05$) were obtained by the one-way analysis of variance and the Tukey test among two groups.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

antimicrobial activity, antibacterial effect, digital printing, nylon, plasma, polyphenol oxidase

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