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Article

Study of the Enzyme Activity Change due to Inkjet Printing for Biosensor Fabrication

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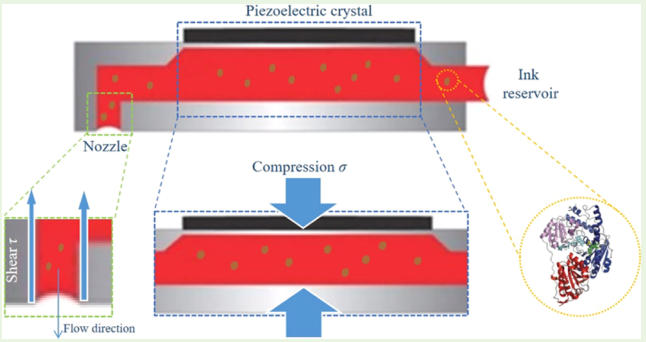
ABSTRACT: Enzymes, the most commonly used biosensing element, have a great influence on the performance of biosensors. Recently, drop-on-demand (DOD) printing technique has been widely employed for the fabrication of biosensors due to its merits of noncontact, less waste, and rapid deposition. However, enzyme printing studies were rarely conducted on the effect of printing parameters from the aspect of the pressure wave propagation mechanism. This study investigated the effects of pressure wave propagation on enzyme activity from the aspects of wave superposition, wave amplitude, resulting mechanical stress, and protein conformation change using pyruvate oxidase as the model enzyme. We found that the mechanical stress increased the activity of pyruvate oxidase during the inkjet printing process. A shear rate of 3×10^5 s^{-1} enhanced the activity by 14.10%. The enhancement mechanism was investigated, and the mechanical activation or mild proteolysis was found to change the conformation of pyruvate oxidase and improve its activity. This study is fundamental to understand the effect of both printing mechanism and induced mechanical stress on the properties of biomolecules and plays an important role in modulating the activity of other enzyme-based inks, which is crucial for the development of biosensors.



KEYWORDS: *enzyme activity, pyruvate oxidase, drop-on-demand printer, inkjet printing, mechanical stress, wave propagation, shear rate*

1. INTRODUCTION

Nowadays, biosensors are widely used in a broad range of areas such as biomedical diagnosis, point-of-care monitoring, environmental monitoring, food quality control, biomedical research, etc.[1] Enzymes as bioreceptor are extensively used to fabricate biosensors.[2] The currently developed manufacturing practice, especially inkjet printing technology, provides flexible fabrication methods for biosensors by precisely positioning a small amount of materials and takes advantages of controlling unnecessary wastage of enzymes and potential contamination.[2] Inkjet printing has attracted great attention from the research community and is becoming the most feasible technique to deposit enzymes.[3] In recent years, a number of enzyme-based glucose sensors have been developed for diabetes diagnosis.[4−7] It is believed that the inkjet printing technique will be routinely used for the development of other enzyme-based biosensors.

When printing the enzyme-contained ink, the enzymes will be affected during the drop formation process, especially by the variable pressure wave in the printhead (see Figure 1). Herein, pressure wave is the direct acoustic response induced by the displacement of piezoelectric crystal; it drives the fluid flow into the printhead chamber and drops ejection from the nozzle. Pressure waves are indirectly driven by the printing parameter settings, as the displacement of piezoelectric crystal is controlled by printing frequency, voltage, and slew rate of an electrical waveform, which decides the magnitude and rate of the volume change.[8] Drop ejection occurs through generating sufficient magnitude pressure pulses by superposition of two or more consecutive pressure waves.[9,10] Superposition of pressure waves can be complicated under different printing parameter settings. Basically, higher printing frequency means a decreased distance of adjacent waveform and more complicated wave superposition. Higher voltage results in larger wave amplitude and correspondingly higher drop velocity at the printhead nozzle. Shah et al.[11] studied the change profile of pressure in the printhead chamber and the velocity response at the printhead nozzle exit for a single pulse printing. It was found that both chamber pressure and orifice velocity varied extensively. At the time of drop ejection, the chamber pressure reached as high as 280 kPa and the velocity at the nozzle inlet reached about 4 m/s. It indicated that non-negligible mechanical stress (compression stress in the printhead

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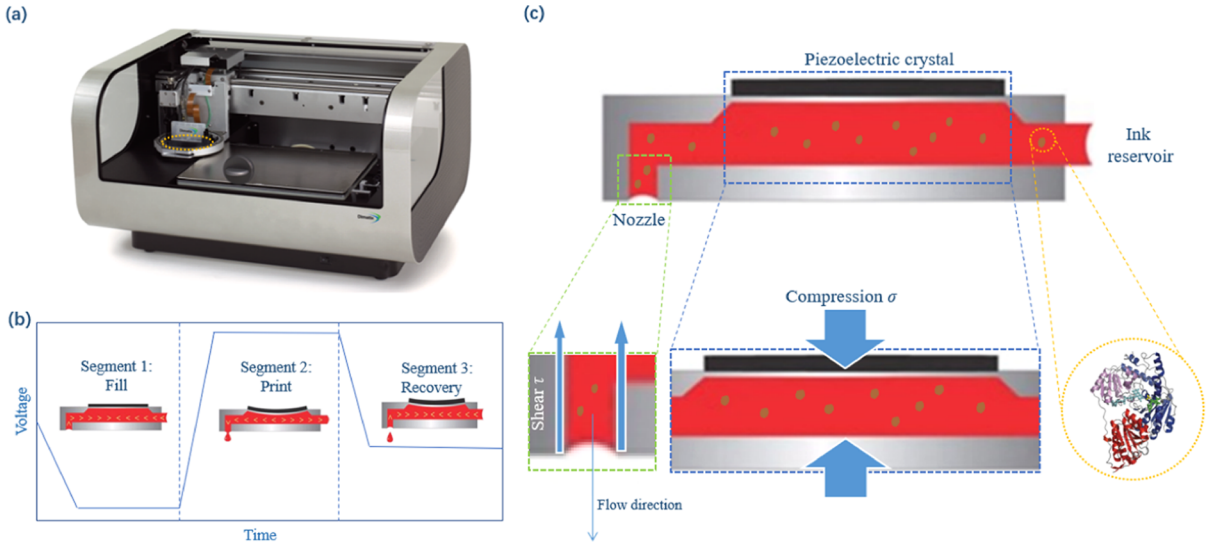

Figure 1. (a) Drop-on-demand (DOD) printer. The printhead chamber is positioned in the orange circle. (b) Schematic of the drop ejection mechanism. Piezoelectric crystal movement driven by the printing waveform and the corresponding wave propagation in the printhead chamber. The direction of pressure wave is drawn with a red arrow. Segment 1 is the ink fill period: ink is drawn from the reservoir to the printhead chamber; Segment 2 is the ink print period: ejection occurs upon the formation of enhanced pressure wave (pressure wave reflected from the reservoir combines with the positive pressure wave); Segment 3 is the recovery period; the backward going pressure wave is reflected from the nozzle. (c) Structure of the printhead chamber showing the piezoelectric crystal, nozzle, and ink reservoir. In the green dashed box is the shear stress at the nozzle, in the blue dashed box is the compression stress in the printhead chamber, and in the yellow circle is the three-dimensional conformation of pyruvate oxidase.[12]

chamber and shear stress at the nozzle) is induced by the alteration of printing parameters.

When involving multipulse printing processes, pressure and velocity change will be more complicated. Several studies investigated the dependency of the drop velocity on the printing frequency, and a wave-like velocity fluctuation was observed.[9,13] It was found that both wave amplitude and compression/shear stress (reflected by the shear rate and drop velocity) also increased with the applied voltage. Thus, it is fundamental to study the alteration of the above-mentioned printing parameters and then investigate the effect of mechanical stress on the enzyme ink.

Several experimental studies investigated the effect of printing parameters on enzymes' activity. Nishioka et al.[14] studied the effect of displacement rate of piezoelectric crystal (by applying a fixed voltage on the piezoelement over different time ranges) on peroxidase using DOD printing. A printing frequency of 100 Hz was used to avoid the adjacent pressure wave superposition. Also, drop velocity was restricted below 0.3 m/s to avoid shear stress at the nozzle. Enzyme activity loss was found with higher compression rate. In another study, Wang et al.[6] studied the effect of printing voltage on glucose oxidase. In their study, a printing frequency of 1 kHz was applied and printing voltages of 40, 60, and 80 V were used. They suggested that the protein tertiary structure and glucose oxidase activity were not affected by shear stress at the nozzle. From the same research group, Cook et al.[15] investigated the effect of waveform slew rate on the glucose oxidase. The glucose oxidase activity was found to be slightly decreased with increased slew rate. They also repeated the experiment of varying voltage amplitudes using a higher printing frequency of 5 kHz. Different from the study of Christopher, they found that the glucose oxidase activity decreased as the printer actuation voltage increased. As the printing frequency is the major variable between the above studies, it is suspected that protein damage may occur at higher printing frequency due to increased wave superposition.

To the best of our knowledge, the effect of wave superposition on enzyme activity was rarely studied. Additionally, commercial printers usually work under resonance conditions, where pressure waves are timed to reinforce each other, resulting in even higher compression on the ink solution.[14] Current studies[6,14,15] about printing enzyme mainly focus on low-frequency printing, which cannot be transformed into the industrial fabrication practice of biosensors. Thus, it is necessary to systematically investigate the probable effect of specific mechanical stress on enzymes from the aspect of wave propagation to understand the mechanism of enzyme activity change, and it is also practical to study the change pattern of enzyme activity under different printing frequencies to facilitate the fabrication process for future biosensors.

So far, there are contradictory findings of the effect of compression stress and shear stress on the activity of different enzymes or on the three-dimensional conformation of enzyme protein:[16] on the one hand, a higher compression rate has a negative effect on enzyme activity due to the damaged peroxidase structure by compression stress;[14] on the other hand, increased compression had minimal effect on the glucose oxidase activity and corresponding protein structure.[6] In addition, the threshold of shear rate to denature the protein remains controversial.[17−20] Further investigation is imperative to understand the effect of mechanical stress on the enzyme activity and protein structure of different enzymes.

In this study, to compare the parallel printing studies of enzymes with different sizes and structures,[4−6,21−25] pyruvate oxidase was employed.[12,26−28] A piezo-driven DOD printer was used to investigate the effect of wave superposition on the activity change of pyruvate oxidase under different printing frequencies and voltages. Drop velocity was measured for each printing group. Shear rate was calculated based on the

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observed mean drop velocity to reflect the corresponding shear stress status at the nozzle. A full statistical study of enzyme activity change was presented. The protein structural changes of three different kinds of enzymes (pyruvate oxidase, glucose oxidase, and peroxidase) were examined before and after printing.

2. METHODS AND EXPERIMENTS

2.1. Materials. Pyruvate oxidase from microorganisms (PyOD, E.C.1.2.3.3) was purchased from Toyobo (New York). Peroxidase from horseradish (E.C. 1.11.1.7), glucose oxidase from *Aspergillus niger* (GOx, E.C.1.1.3.4), bovine serum albumin (BSA), Triton X-100 and glycerol were obtained from Sigma-Aldrich. 4-Aminoantipyrine, *n*-ethyl-*n*-(2-hydroxy-3-sulfopropyl)-*m*-toluidine (EHSPT), thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), tetrasodium salt hydrate (EDTA), and $MgSO_4$ were purchased from TCI. All other reagents used were of analytical grade and used as received. All solutions were prepared in double-distilled water. Citrate buffer (ionic strength: 0.02 M, pH 5.7) and phosphate buffer (ionic strength: 0.04 M, pH 5.9) were prepared according to normal lab procedures.

2.2. Methods. *2.2.1. Bio-Ink Preparation.* Pyruvate oxidase was prepared in citrate buffer. Ink formula was developed to satisfy the rheological requirement and to stabilize the enzyme. According to manufacturer's data supplied with the printhead, the suitable viscosity and surface tension range for dispensing fluids are 2−30 cP and 28−42 dyne/cm, respectively. Additives were used to modify the physical properties of the ink. Specifically, 20% w/v glycerol was added to adjust the viscosity. To modify the ink surface tension, 0.075% Triton X-100 was included. Bovine serum albumin (BSA, 0.05% w/v) was added as an enzyme stabilizer and sacrificial agent to minimize protein absorption on the polypropylene bag of the ink cartridge.[29] The density of the ink was calculated by weighing the mass divided by its volume. Physical properties of the ink are shown in Table 1.

Table 1. Physical Properties of the Ink at Room Temperature

viscosity (cP)	surface tension (dynes/cm)	density (g/cm^3)
3.26	32.6	1.02

2.2.2. Printing Experiments. The level, slew rate, and duration of the waveform that defines the displacement of a piezoelectric actuator will highly influence the droplet formation process and the fluid mechanical stress (Figure 1b,c). A printing waveform was optimized based on drop formation requirements of a stable round droplet and no satellite drop (see the Supporting Video: stable drop). The waveform settings were same in all experiments.

The velocity of the droplet in flight was determined by stroboscopic images captured by a CCD camera. The average drop velocity was calculated by measuring the spacing between droplets and the printing frequency. The shear rate ε during drop generation, as a reflection of shear stress at the nozzle, is given by the equation

$$\varepsilon = v/r \quad (1)$$

where v and r are the drop velocity and radius, respectively.[30] The shear rates of printing voltages of 24−36 V at a 2 V interval with printing frequencies of 5, 6, 7, and 8 kHz were calculated and are plotted in Figure S1.

Ink was directly printed from a 21.5 μm diameter printhead onto a clean storage pan to get approximately 120 μL of the printed sample. Pipetted ink was used as the unprinted control group against the printed sample to ensure that any observed effects can be solely attributed to the printing process. All printed ink groups were gathered and stored in a refrigerator immediately after printing. All printing groups were repeated in triplicate, similar to other enzyme printing experiments.[25]

To obtain satisfied printing performance for precise positioning of the ink and avoid the occurrence of jetting issue (see Figure S2), only the printing setting that drives the droplet as a round drop at the 1000 μm mark at the drop watcher window will be retained to analyze the enzyme activity. After screening the printing performance, the performed experiments with different printing parameters were finalized and are listed in Table 2. Specifically, a printing frequency of 4−10 kHz with an interval of 2 kHz (groups 1−4, first series) at a constant voltage of 23.6 V (most stable droplet ejecting performance was observed at 23.6 V) were chosen to investigate the wave superposition effect in the printhead cartridge. A printing voltage of 24−36 V with an interval of 4 V (groups 5−8, second series) at a frequency of 6 kHz (based on the timed meniscus oscillation) was used to investigate the effect of wave amplitude on the enzyme activity.

Table 2. Printing Parameters Used in the Experiments

name	number	printing frequency (kHz)	printing voltage (V)
control	0	none	none
first series	1	4	23.6
	2	6	23.6
	3	8	23.6
	4	10	23.6
second series	5	6	24
	6	6	28
	7	6	32
	8	6	36

The selection of printing voltage for three kinds of enzymes was based on the overall drop formation performance (see the Supporting Video: stable drop and unstable drop). The printing parameters of the stable drop and unstable drop video are 6 kHz 24 V and 6 kHz 32 V, respectively, using peroxidase ink. From the stable drop video, round and stable droplets ejecting from 0 to 1200 μm mark from all nozzles are seen clearly. This ensures the precise and stable deposition of the ink. For unstable drop video, droplets from all nozzles were found broken to pieces for the 100 μm mark. Due to the requirement of printing performance, we only choose 24 and 28 V at 6 kHz for all three kinds of the enzyme ink after careful screening of the droplet quality.

2.2.3. Determination of Enzyme Activity. Pyruvate oxidase activity was determined using a modified method reported by Sedewitz.[31] The principle of this assay relies on the oxidative coupling among H_2O_2, 4-aminoantipyrine, and EHSPT. 4-Aminoantipyrine solution (0.15%, w/v, 0.2 mL), 0.2 mL EHSPT solution (0.3%, w/v), 0.2 mL TPP solution (3 mM), 0.2 mL FAD solution (0.15 mM), 0.2 mL EDTA solution (15 mM), 0.2 mL $MgSO_4$ solution (0.15 mM), and 0.3 mL peroxidase (50 U/mL) were added into 1 mL phosphate buffer (0.15 M). The obtained buffer was mixed with a 0.5 mL pyruvate solution (0.3 M) and equilibrated at 37 °C for 5 min, resulting in the working solution. Then, 0.1 mL of diluted pyruvate oxidase ink solution (0.1−0.5 U/mL) was added to the aforementioned working solution and mixed by gentle inversion. The absorbance of the resultant quinoneimine dye at 550 nm was immediately recorded for 4 min. Measurements were carried out against the reagent blank, containing all of the components of the ink but the enzyme. The extinction coefficient of the quinoneimine dye under the assay conditions was 36.88 cm^3/μmol.[32] Enzyme activity experiments were conducted on all of the printed samples and unprinted control group.

2.2.4. Circular Dichroism (CD). The selected printing parameter settings (control group, groups 5 and 6) were analyzed separately. BSA was excluded in all of the samples for the consideration of potential mixed spectrum with the target protein. Spectra from 195 to 260 nm were recorded at room temperature using 0.1 mm path length quartz cuvettes at a protein concentration of 0.75 mg/mL after the printing process. Ten consecutive measurements of printed samples were performed as suggested by the technician, and the average spectra were recorded. The observed ellipticities [mdeg] were converted into molar ellipticity [θ] for further protein secondary structural calculation and analysis.

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2.2.5. Characterization and Apparatus. An SNB-1 viscosimeter (Karoth Shanghai, China) was used to measure the viscosity at room temperature under steady shear at 12 rpm. A BZY101 Automatic Surface Tensiometer (Vetus Anhui, China) was used to characterize the surface tension. Printing experiments were carried out using a Fujifilm DIMATIX Materials Printer Dmp-2831 (FUJIFILM Dimatix, Inc.) with the customized ink cartridge DMC-11610. The driving waveform of printhead piezoelement was controlled through Graphical User Interface application software (DMP-2800). A Cary 100 UV−visible spectrophotometer (Agilent) was used to conduct enzyme activity experiments. Circular dichroism assays were carried out using a Jasco J-810 spectrometer (Jasco).

2.2.6. Data Analysis. All statistical analyses were conducted in GraphPad Prism 8.0. The Shapiro−Wilk test was used to investigate the normal distribution status of all data sets. Repeated measures one-way analysis of variance (ANOVA) was used for multiple comparisons. Tukey's post hoc comparisons were performed to determine whether there were significant differences between groups. Regression analysis was performed for different data groups. All results are expressed as the average and standard error of the mean for three replicates (if not mentioned otherwise). A $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effects of Wave Superposition under Different Printing Frequencies. Four printing frequencies (first series) were used to investigate the effect of pressure wave superposition on enzymes. The increase in enzyme activity and shear rate response are depicted in Figure 2. An evident

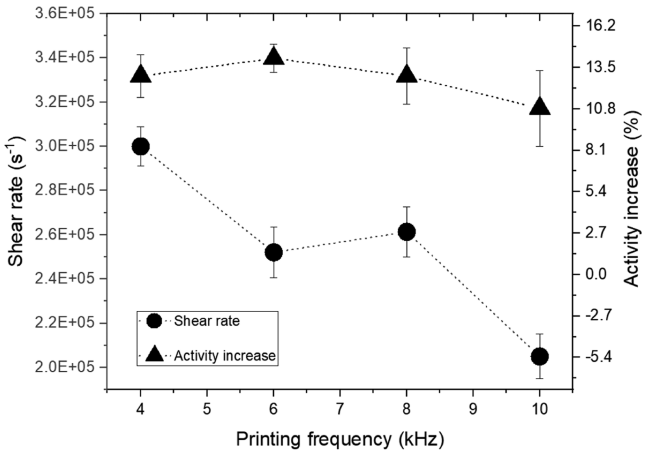

Figure 2. Increased percentage of the enzyme activity and the shear rate after printing under 4, 6, 8, and 10 kHz at 23.6 V (first series). The unprinted enzyme ink was the control group. Enzyme activity was calculated based on the pyruvate oxidase activity assay. Increased percentage of enzyme activity was calculated compared to that of the unprinted control group. Shear rate was deducted from the droplet velocity. Results represent mean ± SEM, $n = 3$.

improvement was observed after printing compared with that of the unprinted control group ($p < 0.05$). The quantified enhancement of the pyruvate oxidase activity ranged from 10.84 to 14.10%. It is deduced that the protein conformation was changed during the printing process. Thus, the regions inside the enzyme protein were exposed and the accessibility of substrate was enhanced resulting in an improvement of the enzyme activity. The enhancement phenomenon of the enzyme activity was also reported by other researchers through ultrasound treatment[33,34] or single-molecule fluorescence-magnetic tweezer microscopy.[35] To further verify the assumption of the potential protein structural change, characterization of the protein secondary structure after printing was performed and is presented in Section 3.3.

Although significant improvement was observed between printing groups and control groups, less effect was found between the different printing frequencies ($p > 0.05$). This suggests that pressure wave superposition through the variance of printing frequency has less effect on the pyruvate oxidase activity. An explanation is that low wave amplitude will constrain the compression wave due to the pursuit of a stable ejection performance in the first series; thus, superposition of multiple compression waves hardly produces sufficient mechanical stress on pyruvate oxidase.[14] To further verify this hypothesis and investigate the role of increased wave amplitude, a second series of printing voltage were executed and are given in Section 3.2.

The correlation between shear rate and printing frequencies was plotted to establish the relationship between shear rate and enzyme activity. Although the activities varied from 10.84 to 14.10% with shear rates from 2.05×10^5 to 3×10^5 s^{-1}, no correlation was identified ($p = 0.3894$) between shear rate and enzyme activity for the first series. It is deduced, in our case, that wave superposition was not significant enough to intensify the shear force on the enzyme ink.

3.2. Effects of Wave Amplitude under Different Printing Voltages. As wave amplitude is proportional to the printing voltage, different printing voltages were used to investigate the effects of wave amplitude on pyruvate oxidase activity (second series). Statistical differences ($p < 0.05$) were observed for the enzyme activity increase among printing groups in the second series (Figure 3). It is verified that the

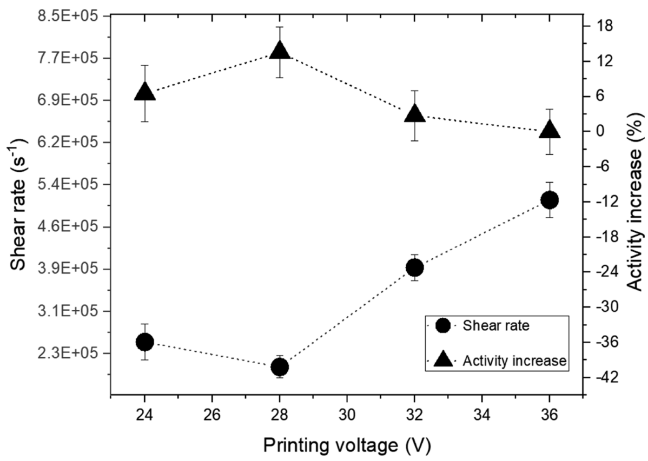

Figure 3. Increased percentage of the enzyme activity and the shear rate after printing under 24, 28, 32, and 36 V at 6 kHz (second series). The unprinted enzyme ink was the control group. Enzyme activity was calculated based on the pyruvate oxidase activity assay. Increased percentage of the enzyme activity was calculated compared to that of the unprinted control group. Shear rate was deducted from the droplet velocity. Results represent mean ± SEM, $n = 3$.

wave amplitude had significant effects on the catalytic activity of pyruvate oxidase. Less improvement was obtained when the voltage was further increased to 32 V or even 36 V because large wave amplitude was induced by high fluid shear, resulting in partial enzyme denaturation.[17] Under these circumstances, activity enhancement brought by the printing process was mitigated by the enzyme structural deformation. Normally,

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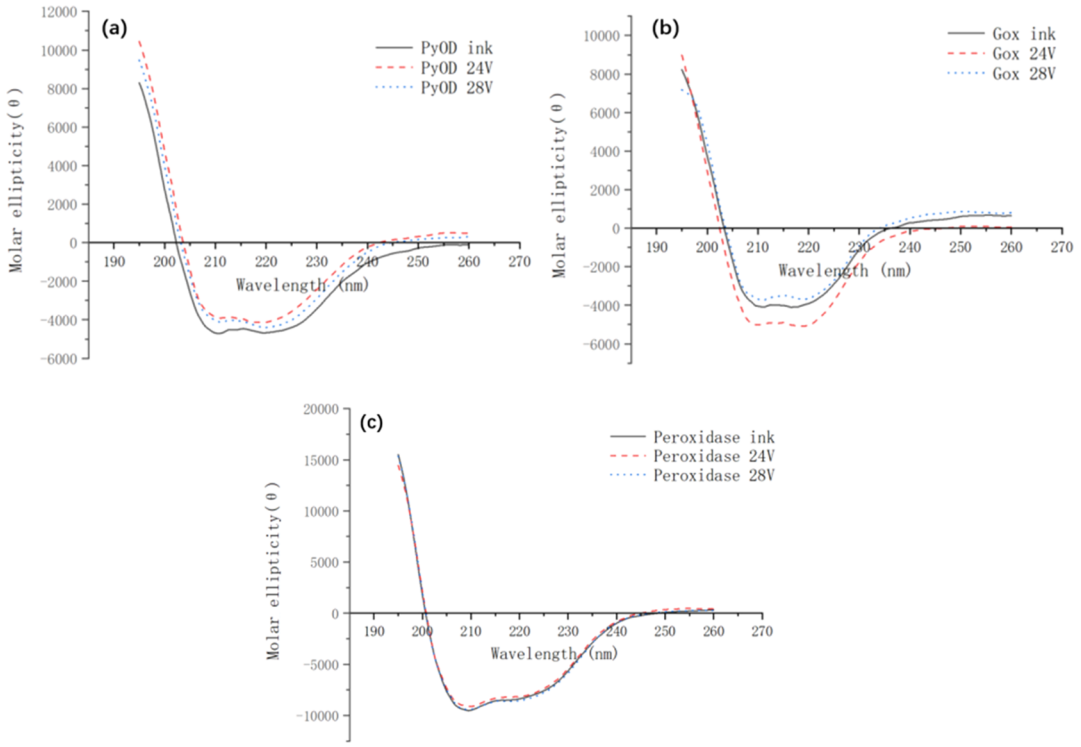

Figure 4. Circular dichroism spectra of the unprinted protein sample and samples printed at 24 and 28 V (n = 10). A printing frequency of 6 kHz was used. (a) Pyruvate oxidase, (b) glucose oxidase, and (c) peroxidase.

higher printing voltage is suggested in the inkjet printing process since higher voltage will prominently increase efficiency. However, excessive mechanical force induced by high wave amplitude can be detrimental to the enzymes.

A negative correlation was found between shear rate and enzyme activity (second series, $r = -0.59$, $p < 0.05$): a lowest shear rate of 2.52×10^6 s^{-1} increased the activity by 13.55% ($p < 0.05$), whereas a highest shear rate of 5.11×10^6 s^{-1} can only enhance the activity by no more than 5%. This is because mechanical activation dominated the change in the situation of lower shear force. However, denaturation occurred when higher shear rate was reached.[2] The effects of enzyme denaturation were also found by other researchers through fluid shear on bovine insulin[20] and lysozyme.[36] To further verify the assumption of enzyme structural change, circular dichroism was performed and is presented in Section 3.3.

3.3. Secondary Structure of Protein after Printing. To verify the hypothesis that mechanical stress will induce a change in the protein's secondary structure, circular dichroism was performed to characterize the enzyme's secondary structure before and after printing. Glucose oxidase and peroxidase were also printed using the same ink formula for comparison. Figure 4 shows the CD spectra of different enzyme inks exposed to shear rates driven by different printing voltages. The percentages of different secondary structures were calculated and are listed in the Supporting Information (see Table S1).

The negative bands at 208, 216, and 222 nm presented in all of the spectra indicated that α-helixes and β-sheets existed in the enzyme structures. For pyruvate oxidase, the α-helix content decreased prominently with the increase in the printing voltage/shear rate. Especially for the group printed at 6 kHz, 28 V, a decreased percentage of 31.9% was identified compared to that of the unprinted group. However, the β-sheet content of pyruvate oxidase barely changed in the 6 kHz, 24 V and increased by 10.5% compared to that of the control group. This showed that the β-sheet structure was more stable than α-helix for pyruvate oxidase. In pyruvate oxidase, six parallel β-sheets were surrounded by α-helixes and the active sites were buried in the region interface and uncovered by the C-terminal domain.[12,28] It can be interpreted that decrease in the α-helix content at the region interface in the printing process facilitates the entry of substrate, which improved the catalytic activity of pyruvate oxidase. When considering further that the proteolysis occurred during the printing process, higher improvement was obtained.[37,38] Thus, according to the results shown in Figure 3b, the change in the protein secondary structure and potential proteolysis under pressure wave has a positive effect on the activity of pyruvate oxidase.

The secondary structures of both glucose oxidase and peroxidase had a smaller change in the overall percentage (≤12%) after printing compared to that of pyruvate oxidase (~31%), as shown in Table 3. For glucose oxidase, the content of β-sheet was less than 5% for both printing groups, indicating that the glucose oxidase secondary structure was stable under pressure wave, which maintained its activity after printing. Similar conclusions were obtained by other researchers about the glucose printing and sensor fabrication process.[7,39]

Table 3. Relative Secondary Structural Percentage Change Compared to Unprinted Enzyme Ink

	6 kHz 24 V		6 kHz 28 V	
enzyme	α-helix	β-sheet	α-helix	β-sheet
pyruvate oxidase	72.3%	100.5%	68.1%	110.5%
glucose oxidase	111.8%	95.8%	89.8%	102.3%
peroxidase	101.6%	103.2%	103.5%	100.5%

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For peroxidase, a minimal protein unfolding phenomenon was observed from the increased percentage (≤5%) of α-helixes and β-sheets for both printing conditions. The stable performance of peroxidase's secondary structure suggests its potential application of inkjet printing peroxidase sensor.

It is suggested that the change difference of the secondary structure between pyruvate oxidase and other enzymes results from the shape, size, and structural stability of the enzyme. Glucose oxidase is composed of two identical subunits: each subunit has 587 amino acids and has a molecular weight of 66.7 kDa.[40] Peroxidase has 309 amino acids with a molecular weight of 44 kDa.[41] Pyruvate oxidase consists of four identical subunits (603 amino acids) with a molecular weight of 265 kDa.[12,27] According to the circular dichroism result, we deduced that the secondary structure of the enzyme with a larger size, more amino acids, and more subunits was more unstable during the printing procedure. As pyruvate oxidase activity increased due to the instability nature of the printing procedure, the activity−stability trade-off phenomenon on pyruvate oxidase is surprisingly favorable in the inkjet printing process.[42] The change difference in the secondary structure between different enzymes suggests that printing parameters should be properly regulated to modulate the pressure wave and to obtain optimum enzyme activity performance for specific enzyme printing.

4. CONCLUSIONS

In this study, we investigated the effect of wave propagation on the activity of pyruvate oxidase. This is the first study to investigate the change in enzyme activity based on the printing mechanism in the bioprinting process. Pyruvate oxidase activity was positively influenced by the pressure wave-induced mechanical forces and the change in protein conformation induced by mechanical activation or mild proteolysis. It is found that the printing frequency had less effect on the variation of pyruvate oxidase catalytic ability since a limited change of wave superposition was induced; the mechanical stress induced by printing voltage had a significant effect on the catalytic efficiency of pyruvate oxidase, and low shear stress tended to create higher enhancement for the activity. That is because partial denaturation and mechanical activation of the enzymes occurred simultaneously when higher mechanical stress was induced. A comparison of CD spectra results on different enzymes revealed that the protein structural change varied due to the property of the specific protein.

The results of this paper are favorable for the fabrication of future biosensors to increase their sensitivity and stability. The ink formula and printing parameters need to be adjusted based on not only the requirement of drop formation but also the performance of the enzyme ink. The practice of correlating the pressure wave propagation and the corresponding mechanical stress with the change in the enzyme activity is meaningful for other research studies of enzyme printing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsbiomaterials.0c01515.

Shear rate at different printing parameters; jetting issues during printing; and secondary structural percentage of pyruvate oxidase, glucose oxidase, and peroxidase (PDF)

Stable drop video (MP4)

Unstable drop video (MP4)

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Notes

The authors declare no competing financial interest.

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