# Encapsulation of Lidocaine into Zein Nanofibers

A Major Qualifying Project Report Submitted to the Faculty of WORCESTER POLYTECHNIC INSTITUTE In partial fulfillment of the requirements for the Degree of Bachelor of Science

In

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By

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## Abstract

Lidocaine, a local anesthetic was encapsulated into zein nanofibers through electrospinning. The impact of process parameters on electrospinning was studied in this project. Differences on nanofiber morphology made by concentration of fiber forming solutions (20%, 25%, 30%) type of solvent, voltage (10 kV, 12 kV, 13 kV, 15 kV and 17 kV), flow rate (0.3 mL/h, 0.5 mL/h and 0.7 mL/h), and collecting distance (10 cm, 12 cm, 14 cm, 20 cm, 30 cm) on fiber morphology were examined. The optimal conditions for spinning were found to be 25% zein concentration, 70% ethanol: water (w/w), 15 kV voltage, flow rate of 0.3 mL/h, collecting distance of 10 cm, and 10 mL syringe with 0.80×22 mm needle tip, and the experiments were carried out in room temperature and 60% relative humidity. Various concentrations of the active pharmaceutical ingredient (API), lidocaine, were successfully loaded into the nanofiber. The presence and concentration of lidocaine in the nanofibers was determined through HPLC. The releasing behavior of the API was studied *in vitro* using PBS buffer followed by HPLC quantification, and *in vivo* by Skin Raman experiments. It was found that a fast and complete release of lidocaine from the nanofiber was achieved in PBS buffer (pH7.4) and released that lidocaine into the first 5 μm of human skin depth as followed by Raman experiments.

## Acknowledgements

I would like to thank Dr. Arne Gericke for being my project advisor and providing such great opportunity for me to study in Switzerland.

A special thank you to Prof. Dr. Christian Adlhart for having me in his lab, and patiently support and guide me throughout the project at ZHAW. I would also like to thank Sara Mousavi for her help on taking SEM images and teaching me to use the equipment in the lab.

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# 1.0 Introduction

## 1.1 Electrospinning

Electrospinning is one of the way to produce fibers with diameter ranges from tens to hundreds of nanometers using electric fields.[1] A syringe with polymer solution is set up on an infusion pump and connected to high voltage. When increasing the voltage and thus increasing the strength of the electrostatic field, the repulsive forces generated by the induced surface charges would overcome the surface tension of the solution, and cause a pendant drop at the tip of the needle to form a so called 'Taylor cone'. Then the charged fiber jet would erupt from the tip forward to a grounded collector. During the process of eruption, solvent evaporates from the jet and left only fibers on the collector(Figure 1) [1]. Functional electrospun fibers have great potential in industrial use due to their ability of incorporating various additives such as active pharmaceutical ingredients (APIs), antioxidants, antibacterial ingredients, and others [2]. Electrospun fibers have a high surface-to-volume ratio, which makes them a good candidate for fast releasing system as they show strong interactions with surrounding materials.

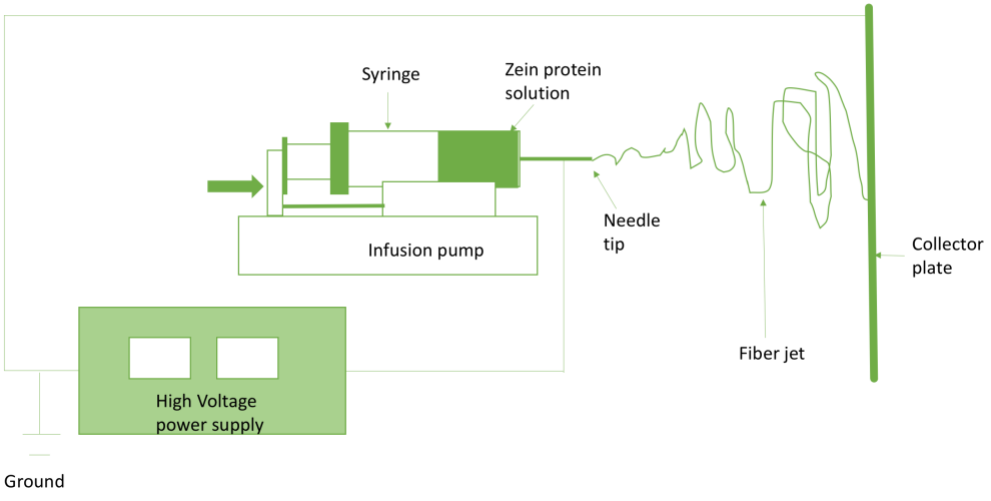



Figure 1: Schematic electrospinning setup for the production of zein protein fibers.

**1.2 Zein**

Zein is a class of prolamine protein, that is hydrophobic and can be extracted from corn. Zein is the storage protein of the corn, and is a mixture of proteins with various sizes and solubilities. Zein proteins are expressed during the developmental stage of the seed and act as storage for free amino acids [3]. This protein was classified into four structures: α, β, γ, and δ. α -Zein contributes to 70% of the four. Unlike the other three forms, it can only be extracted through aqueous alcohol. Most commercial zein products contain α -zein because the other three forms are easy to gelified. α-zein has two polypeptides, one is 19 kDa and another one is 22 kDa. Those two polypeptides were used in further experiments to determine whether the electrospinning process will interrupt the structure of zein or not. Zein can be dissolved in binary solvents with alcohol and water, such as aqueous ethanol [4].

Using zein to produce fibers was first introduced by Ostenberg in 1919. It is widely used in the food and pharmaceutical industry due to its thermal resistance and oxygen barrier properties. It is often used in coating and packaging because it can form films. Meanwhile it's biodegradable and renewable. [5] The first major commercial uses of zein was coating; it was used as a replacement of shellac in WWII, it can be used in floor coating in engine rooms of steamships because it's durable and resistant to grease. More recently, zein has been used as a replacement for polyolefin as coating for paper in food industry since it was found that zein is efficient enough to act as a barrier in fast service packaging. In 1958, Winters and Deardorff found that zein can be used for coating tablets since the coating process is rapid due to its film forming ability. It is also resistant to microbes, heat and humidity[4].

The first use of zein in producing fibers was patented in 1919 by Ostenberg, but back then, people were using either dry spinning or wet spinning to produce the fibers. The methods have been improved by many scientists. Later in 1949, Evans found that fibers can also be

produced by a two-step method[4]. Zein is mostly the byproduct of ethanol production, although Zein has great potential for commercial use in various industries, its high cost has always the chief concern for years[6].

Recently, zein was used in nanofiber production using the electrospinning process.[1] In that experiments, zein was dissolved in aqueous ethanol and used for production of nanofibers. Those fibers were used as a controlled drug release system. It was found that there are many parameters that can affect the morphology of the electrospun fibers during the process, such as concentration of fiber-forming solution, applied voltage, collecting distance, flow rate, etc.[1]. Based on the previous studies, one hypothesis was made that when other parameters are constant, higher voltage, smaller flow rate, longer collecting distance and higher concentration would give less beads on the fiber, and thus a nicer fiber.[1] Those parameters were tested in separate experiments and the morphology were measured by SEM.

### 1.3 Lidocaine

Lidocaine is a local amide anesthetic that is often used to treat ventricular tachycardia and blocks nerves, which can cause the side effects such as lower blood pressure. Lidocaine was first discovered by Nils Lofgren and Bengt Lundqvist in 1946 and became a commercial drug 2 years after its discovery. It is faster and safer than other older local anesthetics[7]. It can be used in surgical procedures such as oral surgery. When mixing with epinephrine, it can increase numbing effects and decrease bleeding. Lidocaine begins its effects within five minutes following the infiltration and can persist up to 3 hours[8]. In this study, lidocaine will be loaded into nanofibers by adding it to the fiber forming solution before electrospinning. Its releasing rate

will be monitored through a drug release profile. It is expected that by loading the drug onto the fiber, there would be changes on API's releasing behavior such as releasing rate. The drug release profile of lidocaine *in vitro* was evaluated by Glavas-Dodov. The lidocaine was encapsulated into liposomes. Over 70% of lidocaine loaded were found to be released. The concentration of gelling agent had slight effects on releasing rate. The release kinetics were found to be controlled through releasing profile data.[9]

### 1.4 Application of nanofibers in drug delivery for skin

Recently, nanofibers have been introduced to the public and attracted significant attention for their great potential in biomedical applications. They can be used for cell growth scaffolds, tissue engineering and drug delivery due to their high surface area. Various agents can also be incorporated into nanofiber such as anti-inflammatory, anti-microbial, antioxidant substances. This provides a wide range of applications of nanofibers in wound dressing, cosmetics and drug delivery. [10]

In this project, nanofibers were used as a controlled drug delivery system because of the entrapment of the drug inside the nanofiber. Electrospun fibers showed higher precision at the targeted site than other traditional methods. Meanwhile, the use of biodegradable material such as Zein is also beneficial for drug delivery. [10] Similar application can be found in the industry today such as Rivelin, a self-adhesive oral drug patch that composed of electronspun nanofibers. This patch allows better interactions between mucosal surface and biodegradable layers, helping to treat oral diseases. [11]

## 1.5 Skin Raman Spectroscopy

Raman spectroscopy is a noninvasive method to determine molecular concentration profiles inside skin. Raman spectroscopy is a vibrational spectroscopy technique that is based on inelastic scattering of light. It is possible by Raman microscopy to obtain information of molecules inside skin tissue down to hundreds of micrometers. The scattering of light is caused by the interaction between photons and molecular vibrations when samples are illuminated with monochromatic laser light. The amount of energy that is needed to excite a molecular vibration is missing in the back scattered signals which allows to obtain a vibrational spectrum of the excited molecules. The Raman signal is collected as the laser focus is scanned across the skin and moved to different depths from the skin surface. Raman spectroscopy is widely used in biological, pharmaceutical, medical and cosmetic industry. Concentration of molecules such as NMF (natural moisturizing factors) in the stratum corneum, as well as other APIs can be determined by Raman spectroscopy[12].

## 1.6 Objective of this project

In this project, the potential of electrospun zein nanofibers to be used as a drug release system was analyzed. Zein was dissolved in aqueous ethanol in an optimal concentration, along with the active pharmaceutical ingredient, lidocaine, a local anesthetic. The solution was used in the electrospinning process to produce nanofibers with diameter ranges from tens to hundreds of nanometers. The API was trapped in the nanofibers and gradually released from the fiber. The drug releasing behavior including its rate was monitored *in vitro*, by dissolving nanofibers in PBS buffer of pH 7.4 and *in vivo* by skin Raman analysis. The concentrations of the drug in the

PBS were determined using HPLC. The nanofibers can be produced as patch, directly applied to skin after moisturizing. The content of lidocaine in skin was measured. Once the efficiency of loading and releasing behavior has been studied, lidocaine-loaded biodegradable nanofibers can be used in commercial or medical practice, where lidocaine is one of the most widely used local anesthetics[13].

# 2.0 Materials and Methods

## 2.1 Materials

Zein powder was purchased from A.F. Suter & Co. Ltd, Essex, UK. It was used as received, without further purification. Lidocaine powder was purchased from Fagron Inc, MN, USA.

## 2.2 Fiber-forming solution preparation

Different concentrations (20%, 25% and 30%) of fiber-forming solutions were prepared by weight. Zein powder was dissolved in aqueous ethanol solution (ethanol/water, 70 % w/w). The solutions were stirred in room temperature for one hour.

## 2.3 Electrospinning

Fiber-forming solutions were placed in a 10 mL syringe with a 0.8*22 mm tip when the powder was fully dissolved. The syringe was set up on an infusion pump with different flow rates (0.3 mL/h, 0.5 mL/h and 0.7 mL/h). The needle tip was directly attached to the positive electrode of a high voltage direct current power supply. The optimal voltage was determined by using different voltage settings. (10 kV, 12 kV, 13 kV, 15 kV and 17 kV). As the charge built up at the needle tip where the solution was fed by the infusion pump, the zein solution was ejected toward the grounded collector plate which was placed at different distances (10 cm, 12 cm, 14

cm, 20 cm, 30 cm). During the ejection process, the solvent evaporated from the fiber jet. The fibers were then collected on an aluminum foil attached to the collector plate.

### 2.4 Drug loading

Lidocaine was loaded by adding it to the zein-only fiber forming solution before the electrospinning process. 25% zein-only fiber forming solution was first prepared as described above. Different amounts of lidocaine hydrochloride powder were then added to pre-stirred 25 % zein-only solution to form API loaded solution of different mass percent relatively to zein (4%, 10 %, 20 % and 50%)(Table 1).

Table 1:Preparation of lidocaine-loaded fiber forming solution

| Solutions | Mass of lidocaine(mg) | Mass of zein(g) | Mass percentage to solution(%) | Mass percentage(%) to zein |
|---|---|---|---|---|
| 4%-ZL | 40 | 0.932 | 1.075 | 4.3 |
| 10%-ZL | 100 | 0.932 | 2.675 | 10.7 |
| 20%-ZL | 200 | 0.932 | 5.375 | 21.5 |
| 50%-ZL | 450 | 0.932 | 12.05 | 48.2 |

The electrospinning process was carried out with the optimal parameters determined in previous experiments, which was 15-17 kV, 0.3 mL/h flow rate, and 10 cm collecting distance. Fiber containing lidocaine was collected on a plate wrapped with aluminum foil.

### 2.5 Morphology of Fibers

A scanning electron microscope (SEM), Scientific Quanta FEG 250, Thermo Fisher Scientific, OR, USA, was used to examine the morphology of the electrospun fibers. The

samples were prepared by sputtering the fibers with gold nanoparticles. Images were taken from 14 samples that were obtained using different fiber forming parameters. The diameters of nanofibers were measured using software ImageJ, a histogram was created using measurements collected from SEM images.

**2.6 Viscosity**

The viscosity of the polymer solutions was determined by a rotational viscometer Brookfield Digital Remoter Model DV-III, MA, USA. Four samples were measured to compare the possible impact of viscosity on fiber morphology. Three samples of 25% zein concentration with different lasting time and one 30 % zein concentration sample were measured.

**2.7 Fourier-transform infrared spectroscopy**

The Fourier-transform infrared (FTIR) spectroscopy measurements were carried out using a Bruker Tensor 37 FTIR spectrometer, MA, USA. Electrospun fibers, zein powder and lidocaine powder were dissolved in ethanol and let dry.

**2.8 UV-Vis**

UV-Vis spectra were determined using a SPECORD® S 600, Analytik Jena AG, Jena, Germany UV-Vis spectrometer. Zein powder and lidocaine were dissolved in methanol with the same concentration (10%); methanol was used as blank.

**2.9 HPLC**

High-performance liquid chromatography (HPLC) of the samples was performed using Agilent 1200 series instrument, CA, USA. The separation was performed using a C18 100mm*5mm column at room temperature (28 ℃) with 1 mL/min flow rate and an injection volume of 10 μL. A 65:35 (v: v) 0.05 M sodium phosphate buffer: acetonitrile was used as mobile phase; sodium phosphate buffer was adjusted to pH 6 using sodium hydroxide. The wavelength detector was set to 210 nm. Lidocaine hydrochloride standard solutions were made by dissolving lidocaine hydrochloride in acetonitrile with different concentrations. 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L standard solutions were analyzed and used to obtain a standard curve(Table 2). Standard curves were calculated using the results from 4 trials. The standard equation was found to be y= 31.911x-57.131. The r- value was found to be 0.999.(Figure 2)

Table 2 Preparation of standard lidocaine solution

| Solutions | Mass of lidocaine(mg) | Volume of Acetonitrile(mL) | Concentration(mg/L) |
|---|---|---|---|
| Std-5 | 0.5 | 100 | 5 |
| Std-10 | 1.0 | 100 | 10 |
| Std-15 | 1.5 | 100 | 15 |
| Std-20 | 2.0 | 100 | 20 |
| Std-25 | 2.5 | 100 | 25 |

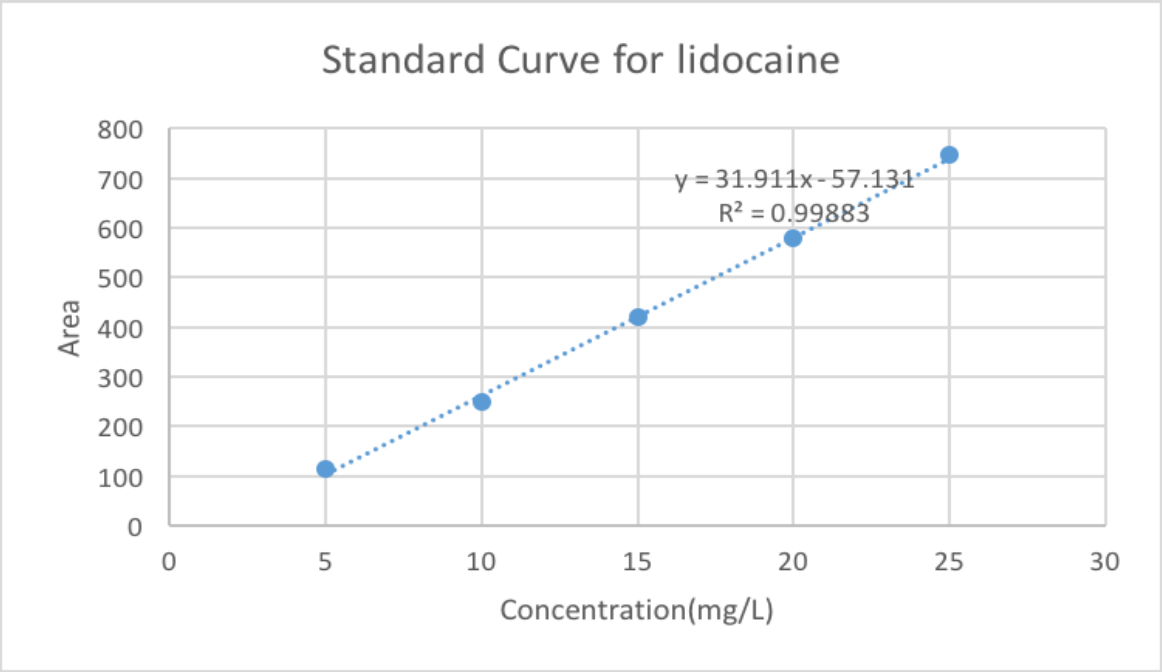



Figure 2: Standard curve for lidocaine concentration

**2.10 Optimization of Electrospinning**

During the process of electrospinning, the tip of the syringe needle became clogged by the polymer due to ethanol evaporation. This required constant cleaning at the needle tip. To solve this, a 3D-printed coaxial nozzle was used to provide more ethanol vapor at the tip to decrease the speed of clogging. Nitrogen was pumped into ethanol and the needle was surrounded by the cooling ethanol vapor by coaxial nozzle(Figure 3).

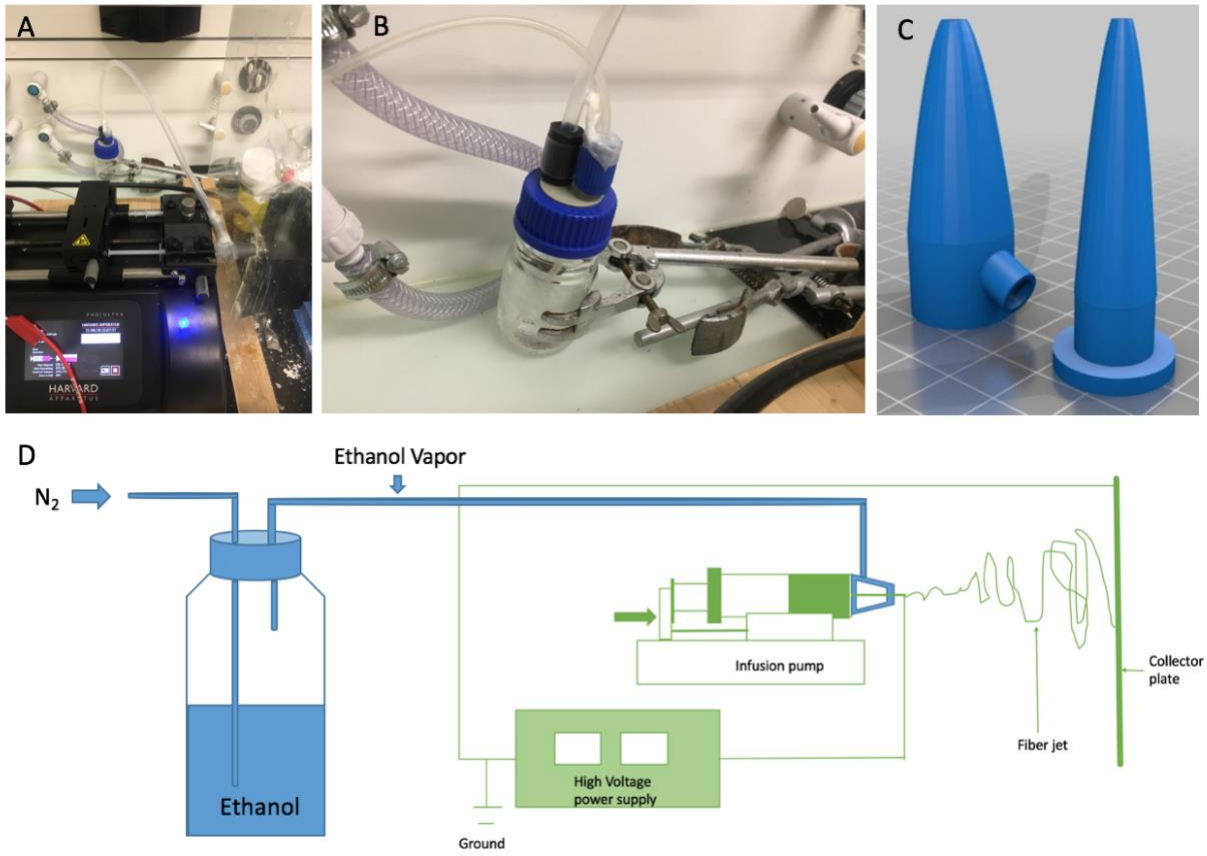



Figure 3: Coaxial nozzle schematic setup for optimization of electrospinning process. A. Experimental set up for spinning process. B: Experimental ethanol vapor set up. C: 3D printing model for coaxial nozzle. D: Schematic setup.

**2.11 SDS-Page**

The SDS-Page resolving gels were made of 4.8 mL acrylamide (30 % w/v)/bis-acrylamide (0.8 % w/v) solution, 5 mL tris buffer (pH 8.8), 50uL 20 % SDS, 100 µL 10 % APS, 10 µL tetramethylethylenediamine and 50 µL water. The stacking gels were made of 1.5 mL acrylamide (30 % w/v)/bis-acrylamide (0.8 % w/v) solution, 2.55 mL Tris buffer (pH 8.8), 50 µL 20 % SDS, 100 µl 10% APS, 10 µL tetramethylethylenediamine and 5.85 mL water. The resolving gel was poured between two glass plates and clipped together on a casting frame.

Bubbles were removed by the addition of isopropanol on the top of the gel. After the gel had gelified, the stacking gel was poured on top of resolving gel. The wells were formed after placing comb in gel after pouring the stacking gel. The concentration of protein in each well was made to be 2 mg/mL. 15 μg of samples was injected into each well making the final products 30 μg. The coloring buffer Coomassie was added to the gel for 1 hour and was washed off overnight. The ladder used was Dual Xtra.

**2.12 Drug Dissolution**

The lidocaine containing electrospun fibers were placed in 100 mL PBS buffer (pH7.4) and 1 mL of solution was taken every one minute for the first five minutes, and every five minutes after the first five minutes for 4 hours under constant stirring. The fibers added to PBS buffer were weighted and the amount of lidocaine present in the fiber was calculated. The concentration of the lidocaine released in the buffer was measured by HPLC.

A standard curve of lidocaine concentrations corresponding to the area on HPLC spectrum were built after the assay of 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L lidocaine standard solution as described above.

Different concentration of lidocaine in PBS buffer were tested. 20mg of nanofiber containing lidocaine were weighted and placed in 100 mL PBS buffer(pH=7.4) under constant stirring, 1 mL of solution were collected every minute for the first five minutes and every five minutes afterwards for 30 minutes. The concentration of lidocaine in each sample were calculated using the standard equation. The mass of the lidocaine in each sample taken out and remaining in the buffer were then calculated by dividing their volumes. The amount of lidocaine taken out was added to the next releasing amount. The percentage of lidocaine released was then

calculated and plugged into the graph by dividing the original mass of lidocaine that was added. (Appendix A)

Then, 53 mg of nanofiber containing lidocaine were weighted and placed in 100 mL PBS buffer (pH=7.4), 1 mL of solution were taken out at 3 minutes and every 5 minutes for 1 hour and one sample was taken at 4 hours. Collected data were analyzed using the method described above. (Appendix B)

**2.13 Human Skin Raman Test**

The releasing behavior of lidocaine loaded fiber as a patch that was directly applied to skin after moisturizing was studied using Model 3510,RiverIcon 2.5 Skin Composition Analyzer, River Diagnostics, Rotterdam, Netherlands.

In this experiment, Raman spectroscopy was used to determine the presence of lidocaine in human skin after applying the patch containing nanofibers. Lidocaine and zein reference were prepared by dissolving 2 % lidocaine 70 % aqueous ethanol (w/w), and 10 % zein powder(w/w) in ethanol. A 1 $cm^2$ patch with 2 mg nanofibers that contain 86 μg lidocaine was applied to human skin on the inside of the wrist after moisturizing for five minutes using an allergy patch. Another patch with the same size containing 0.1 mg 25 % zein only nanofibers was also tested. The patches were removed after five minutes and the same areas of skin were analyzed. The Raman intensity of lidocaine relative to keratin, a protein that is commonly seen in human skin [14] was recorded. The Raman intensity of lidocaine in zein-only fiber was measured as blank and it was subtracted from the measurements of lidocaine-loaded fibers to obtain the Raman skin depth profile. Approximately 10 Raman depth profiles were taken at different positions, and averages were used in further analysis to account for the variability of skin at the microscopic level.

# 3.0 Results

## 3.1 Impact of different parameters on nanofiber morphology

In order to study the impact of different parameters (fiber forming solution concentration, flow rate, voltage and collector distance) on nanofiber morphology, 10 samples with different production parameters were chosen and analyzed. (Table 3)

Table 3 SEM image sample parameters

| Samples | Zein Concentration (%) | Collector Distance (cm) | Voltage (kV) | Flow rate (mL/h) | Morphology/Average diameter size(nm) |
|---|---|---|---|---|---|
| $Z^{20}$-a | 20 | 12 | 17 | 0.3 | Fiber with beads, 145.62 ±44.96 |
| $Z^{20}$-b | 20 | 12 | 17 | 0.5 | Fiber with beads |
| $Z^{20}$-c | 20 | 12 | 17 | 0.7 | Fiber with beads |
| $Z^{20}$-d | 20 | 12 | 13 | 0.3 | Fiber with beads |
| $Z^{20}$-e | 20 | 12 | 15 | 0.3 | Fiber with beads |
| $Z^{20}$-f | 20 | 10 | 17 | 0.3 | Fiber with beads |
| $Z^{20}$-g | 20 | 14 | 17 | 0.3 | Fiber with beads |
| $Z^{25}$-a | 25 | 12 | 15 | 0.3 | Fiber without beads,364.01±113.22 |
| $Z^{30}$-a | 30 | 12 | 15 | 0.3 | Fiber without beads,612.49±156.55 |
| $Z^{30}$-b | 30 | 20 | 15 | 0.3 | Fiber without beads |

### 3.1.1 Impact of differences in flow rate

Flow rate for the electrospinning process is one of the parameters that may play a role in the production of different fiber morphologies. In this experiment, the concentration of fiber forming solution, voltage and distance of collector from needle tip were kept constant for 20 % zein concentration with 70 % aqueous ethanol solution (*w/w)*, 17 kV and 12 cm. A flow rate of 0.3 mL/h was determined to give fibers with less beads, and thus better results compared to 0.5mL/h and 0.7 mL/h flow rates(Figure 4). 0.3mL/h was then used as fixed parameter in further experiments to determine other optimal parameter conditions.

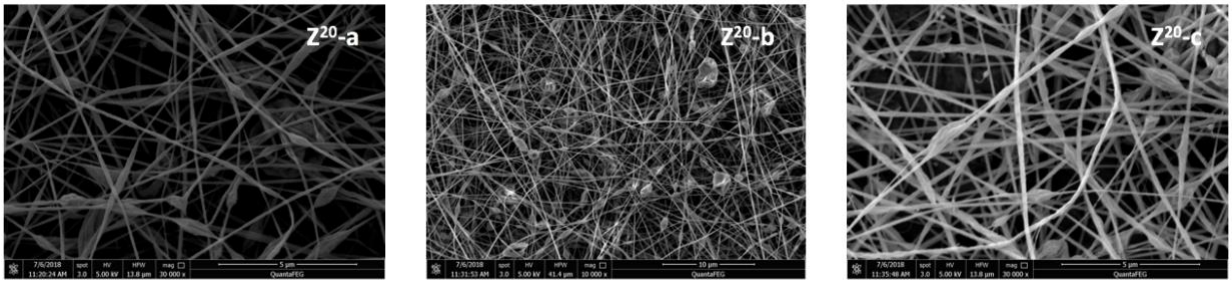



Figure 4: SEM images of zein nanofibers produced with different flow rate. a:0.3mL/h, b:0.5mL/h, c:0.7ml/h.

### 3.1.2 Impact of differences in electric field

The applied voltage in the electrospinning process is another parameter that is been studied in this experiment. Zein protein fibers forming solution were prepared with 20% Zein concentration in a 70% aqueous ethanol solution(*w/w*). The concentration of the solution, flow rate and distance of the collector from the needle tip were kept constant. The flow rate used in this experiment was 0.3mL/h, the distance was 12 cm. Differences in the morphologies of fibers using 13kV, 15kV and 17kV were examined using SEM. All three fibers showed many beads inside the fibers and with relatively the same diameter size ranging from 100-200 nm (Figure 5). Therefore, different collecting distances were then examined.

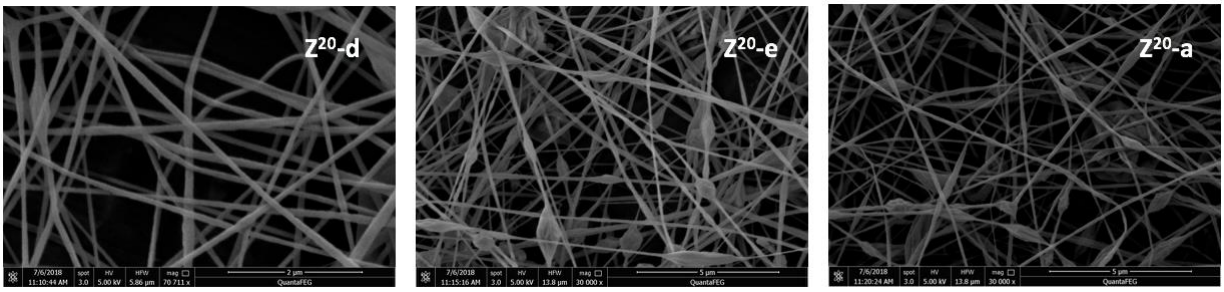



Figure 5: SEM images of nanofibers produced with different electrical field.d:13kV,e:15kV,a:17kV.

### 3.1.3 Impact of collector distance

In this experiment, voltage, flow rate and concentration of the solution were kept constant as in previous experiments. The parameters are 17kV, 0.3mL/h and 20% zein concentration with 70% aqueous ethanol solution(*w/w*). Three different distances were measured, 10 cm, 12 cm and 14 cm. Like it was observed for the experiments with varying voltages, the differences in distance did not provide much different fibers. There are beads observed inside fibers in all cases. The approximate diameter size ranged from 80-250 nm (Figure 6). After all three parameters have been examined, no obvious differences were observed in terms of reducing the number of beads. Therefore, another hypothesis was made that increasing the concentration of zein in solution will decrease the number of beads and increase the fiber size when other parameters are constant.

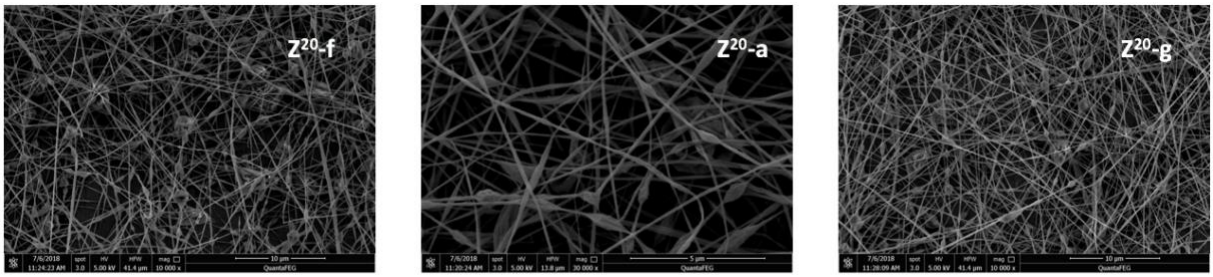



Figure 6: SEM images of zein nanofibers produced with different collector distance.f:10cm,a:12cm,g:14cm.

### 3.1.4 Impact of fiber forming solution concentration

Three different concentrations of zein protein in 70 % aqueous ethanol solution (*w/w*) were tested. The flow rate was fixed to be 0.3 mL/h, the voltage was 15 kV and the collecting distance was 12cm. Solutions with concentration of 25 % and 30 % zein showed significant improvement in reducing the number of beads. Very little beads were observed on those fibers(Figure 5). Meanwhile, solution with 30% concentration produced fibers with larger diameter, ranging from 400-750 nm, whereas a concentration of 25% yielded a fiber diameter size ranging from 150-350 nm. 30 % Zein solution with 20 cm collecting distance also showed decrease in fiber diameter compare to the experiments with 10 cm collection distance (Figure 7-10).

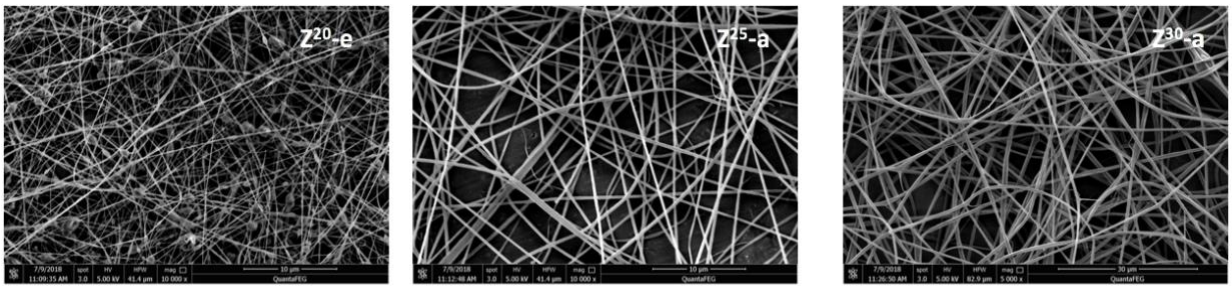



Figure 7: SEM images of zein nanofibers produced with different fiber forming solution concentration.

Table 4 Viscosity of the Zein Solution

| Sample | Viscosity |
|---|---|
| 25% Zein with 70% Ethanol/water (w/w) (day 1) | 93.51±0.29 |
| 30% Zein with 70% Ethanol/water (w/w) (day 1) | 206.21±0.61 |

As the concentration of zein increased, the viscosity of fiber-forming solution increased. The viscosity also depends on the temperature of the measuring time. The 25 % solutions were

prepared and measured three times on different days. The viscosity measurements clearly showed changes in the values throughout the time course of the experiment (Table 4). Nanofibers with 30% Zein concentration showed larger diameter size in SEM images, suggesting that higher viscosity could lead to production of nanofibers with larger diameter size. (Figure 8-10)

### 3.2 Impact of different parameters on nanofiber diameter size range

Three samples, $Z^{20}$-e, $Z^{25}$-a and $Z^{30}$-a were chosen and 100 measurements of diameter on SEM nanofiber images were taken through ImageJ. Based on the measurements, a histogram of diameter size ranges was obtained (see Figure 8-10).

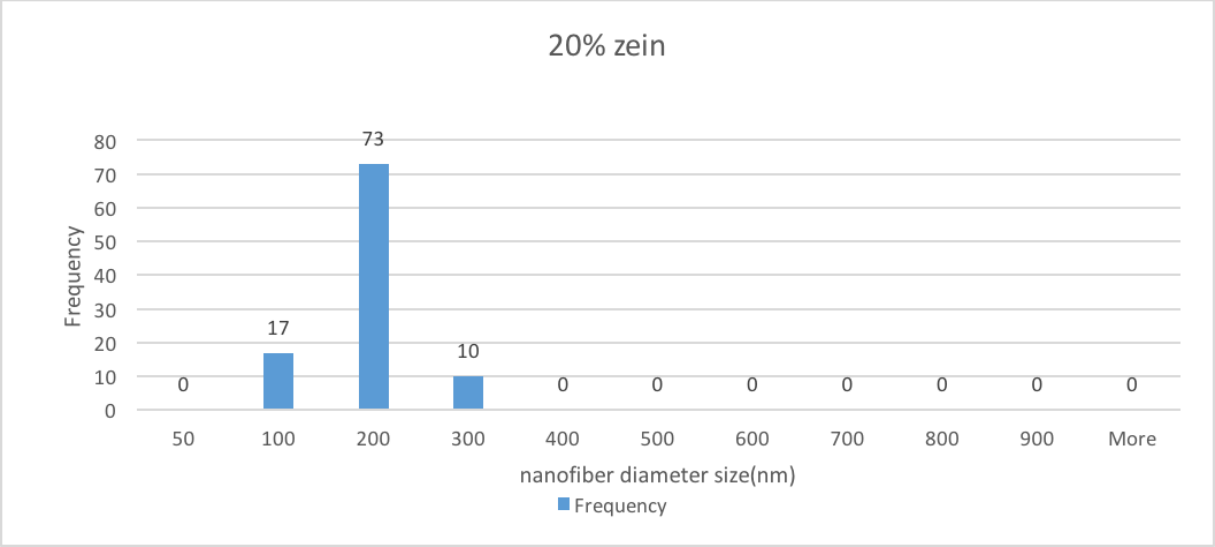



Figure 8: Diameter size range for zein nanofibers with 20 % concentration. The diameters ranged from 100-300 nm.

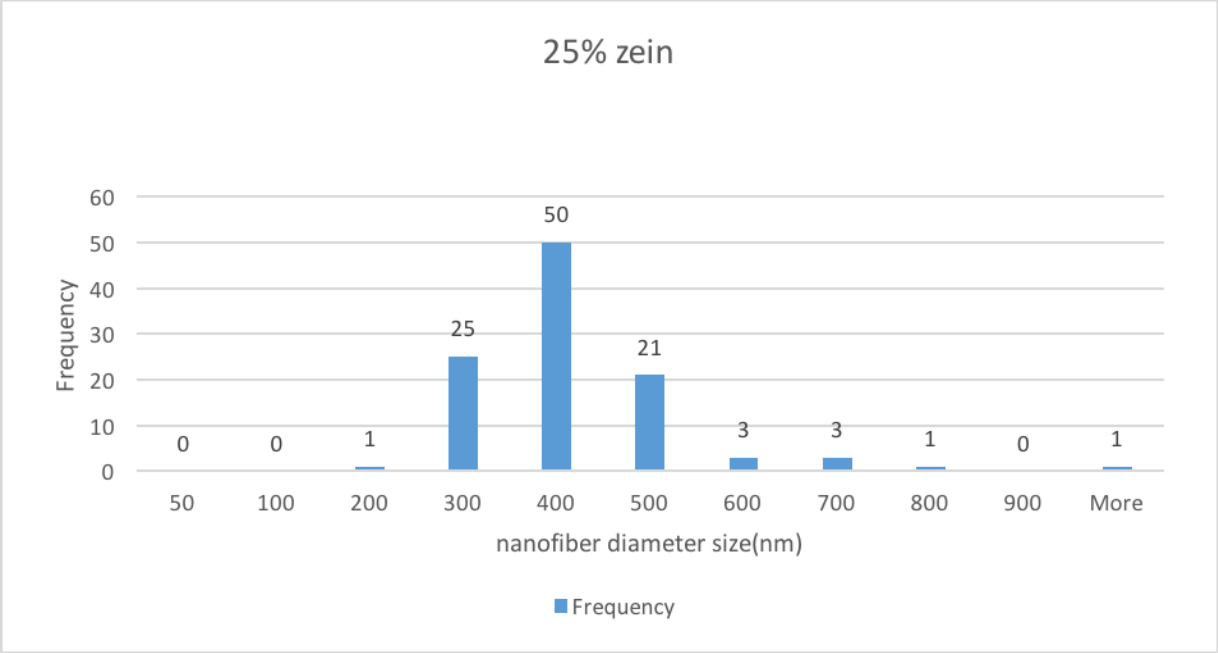



Figure 9: Diameter size range for zein nanofibers with 25 % concentration. The diameters ranged from around 200-700 nm.

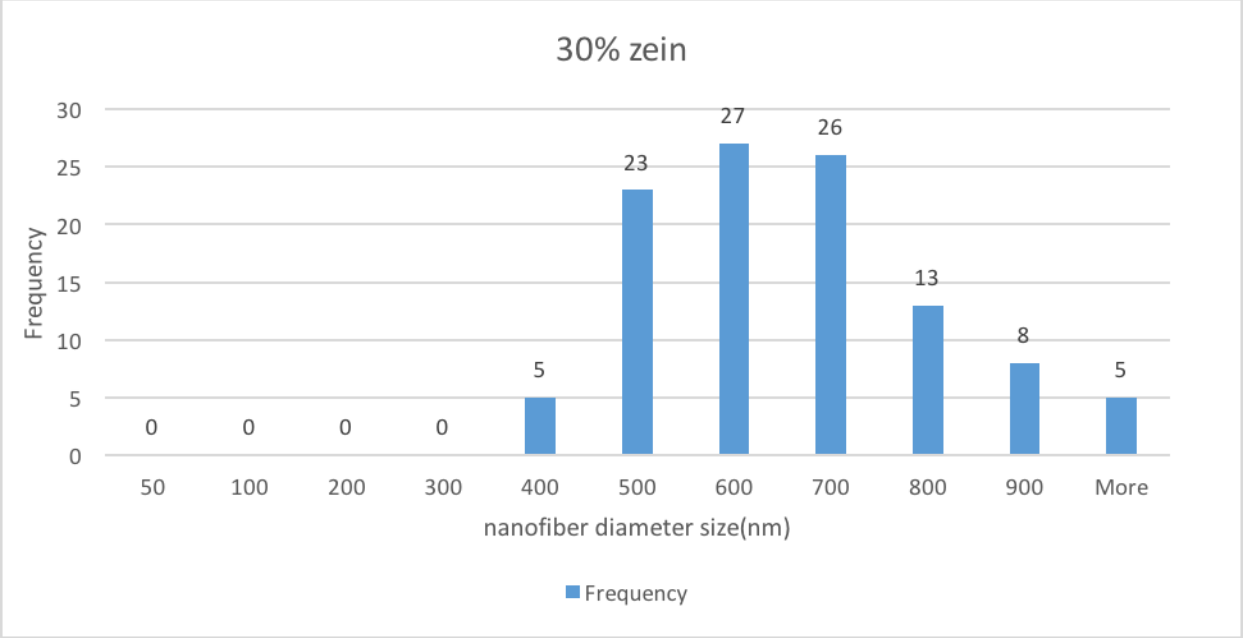



Figure 10: Diameter size range for zein nanofibers with 30 % concentration. The diameters ranged from 400-900 nm.

The histograms of three samples, $Z^{20}$-e, $Z^{25}$-a and $Z^{30}$-a showed that, as the concentration of zein in nanofiber forming solution increased, the approximate diameter size also increased. The 20 % zein concentration solution generally produced fibers with diameters of 100nm to 200

nm, whereas 30% zein concertation nanofibers forming solution produced fibers with diameters from 400 nm to 900 nm.

### 3.3 Morphology of lidocaine-loaded nanofibers

Three different amounts of lidocaine (10%, 25 %, and 50 % relative to zein) were then added to 25 % zein fiber-forming solution and mixed well. Fibers were produced under optimal conditions determined in the previous experiments, which was 15 kV, 0.3 mL/h flow rate, 10cm collecting distance and 0.8*22 mm needle tip. All fibers showed similar diameter sizes, ranging from 200 nm to 500 nm, no obvious beads were observed in the SEM images(Figure 11). Thus, the morphology of the nanofiber was not influenced by loading of drug.

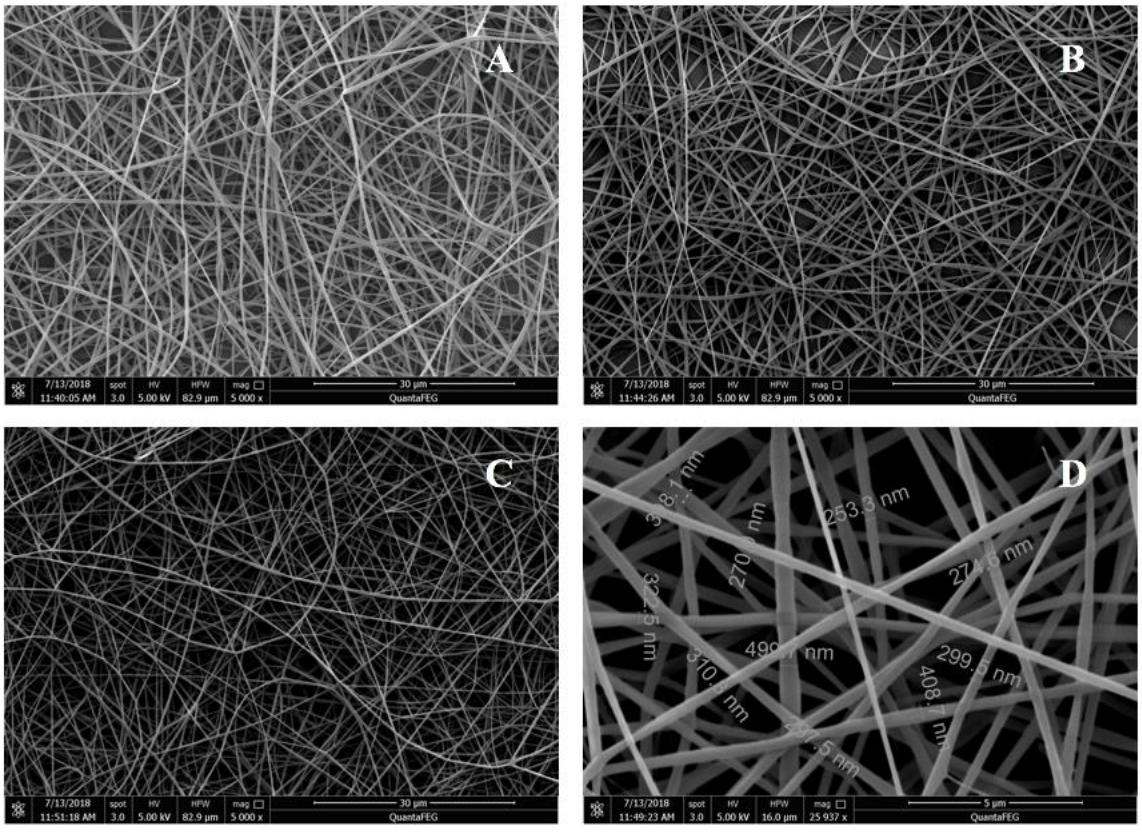



Figure 11: SEM images of lidocaine-loaded electrospun zein nanofiber with flow rate of 0.3mL/h, 15kV, 25% Zein concentration with different amount of lidocaine relative to zein. A: 10% lidocaine; B: 20%; C: 50%. All fibers showed approximately same diameter ranged from 200nm-500nm.

### 3.5 Presence of lidocaine in nanofiber (HPLC)

The presence of lidocaine in the respective nanofibers was tested using high-performance liquid chromatography (HPLC). A standard HPLC method for determination of lidocaine was developed using known concentrations from 5 mg/L to 25 mg/L; 70% methanol to 30% water (w: w) was used for these experiments. Electrospun fibers using fiber forming solutions containing lidocaine were dissolved in ethanol and assayed using HPLC. Lidocaine shows a strong absorbance at a wavelength of 195 nm, and elutes between 1 to 2 minutes. After testing the fiber samples, a standard lidocaine solution of 25 mg/L was added to the original sample. Same peak showed on spectrum with lowered intensity, indicating the lidocaine was successfully loaded to the fiber with concentration higher than 25 mg/L. The area under the peak of the fiber-only sample was 1427, after diluting the sample with standard lidocaine solution, the area under the peak decreased to 968 (mAU*s) (Figure 12.).The concentration of lidocaine in electrospun fiber is found to be 91.119mg/L by plugging the area into the standard curve. After being diluted with standard lidocaine solution, the final concentration of sample with both fibers and standard solution was found to be 61.725 mg/L.

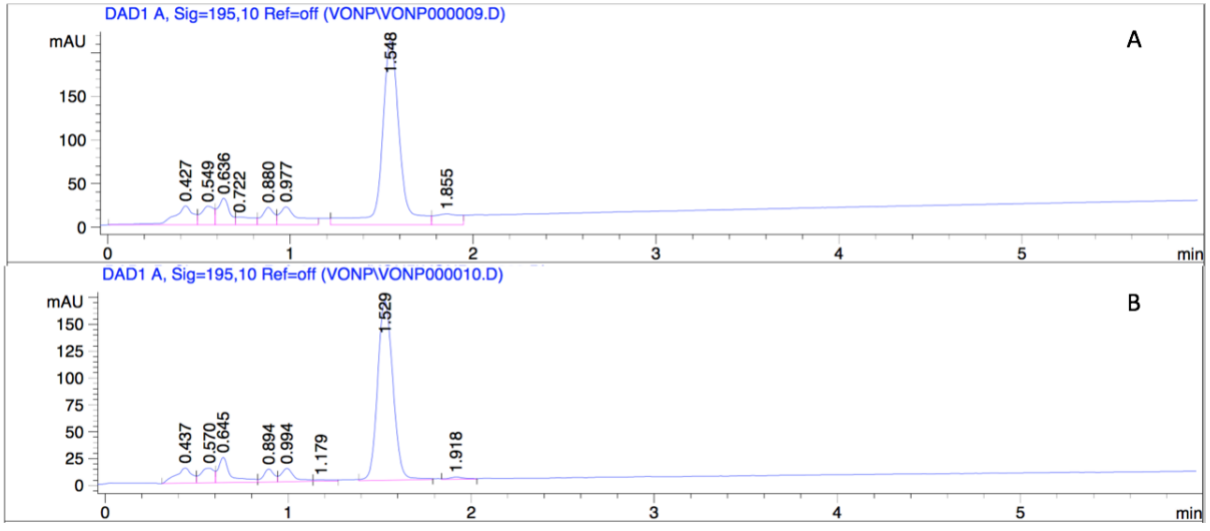



Figure 12: High-performance liquid chromatography (HPLC) results of lidocaine-loaded fiber/fiber with standard solution. A: lidocaine-loaded fiber dissolved in ethanol; B: lidocaine-loaded fiber dissolved in ethanol, diluted with 25mg/L lidocaine standard solution. Lidocaine eluted around 1.5 min, the intensity of the peak changed after addition of standard solution, indicating lidocaine is present in the fiber, absorbs at 195nm.

**Fourier-transform infrared spectroscopy and UV-Vis spectroscopy**

The presence of lidocaine in the nanofiber was also studied using Fourier-transform infrared spectroscopy (FTIR) and UV-Vis spectroscopy. However, both methods showed very little difference to sufficiently prove that lidocaine was loaded onto the fiber(Figure 13).

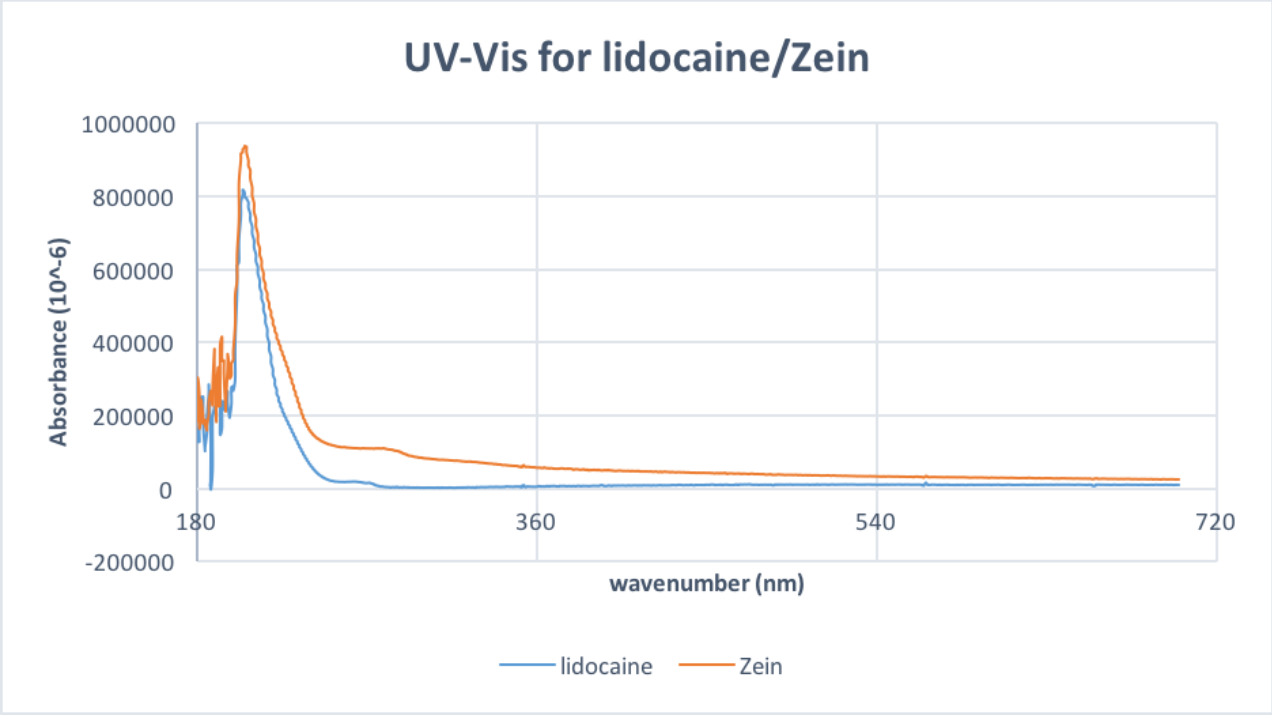



Figure 13: UV-Vis spectrum of lidocaine/zein. The dissolved zein sample was represented by orange line and lidocaine sample was represented by the blue line.

### 3.6 SDS-page

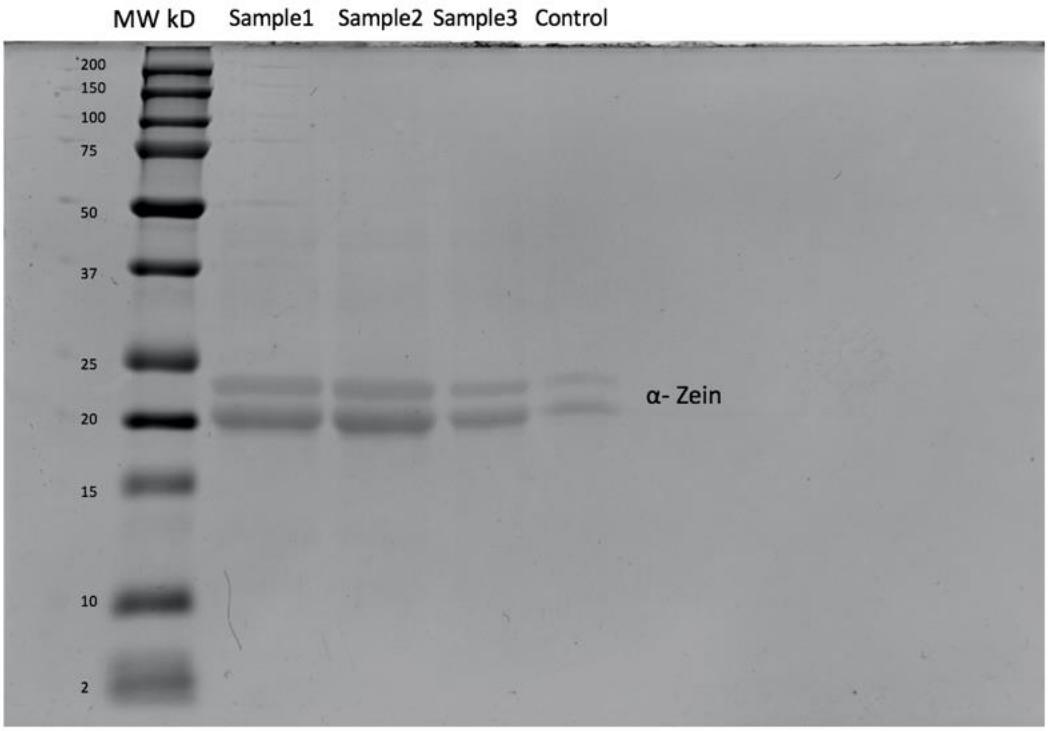



Figure 14: SDS-Page of electrospun fiber. Sample 1: 25% zein fiber without lidocaine; Sample 2: 4% lidocaine-loaded 25% zein fiber. Sample 3: 50% lidocaine loaded 25% zein fiber. Control: zein powder dissolved in ethanol. All samples shoed bands at 19kDa and 22kDa.

The electrospun fibers were observed to be ivory, since the zein powder has yellow-orange color. It was investigated whether the electrospinning process could break the structure of zein. Thus, an SDS-page gel electrophoresis experiment was performed on the fiber to determine whether the protein is still complete. The α-zein contains two polypeptides: 19 kDa and 22 kDa [1], which was shown by the SDS-page gel results (Figure 14). Therefore, the α-zein was still present after the electrospinning process.

### 3.7 Drug Dissolution

0.858 mg lidocaine was released in PBS buffer at 100% in the first five minutes, the released percentage was 96% at 3 minutes. (Figure 15)

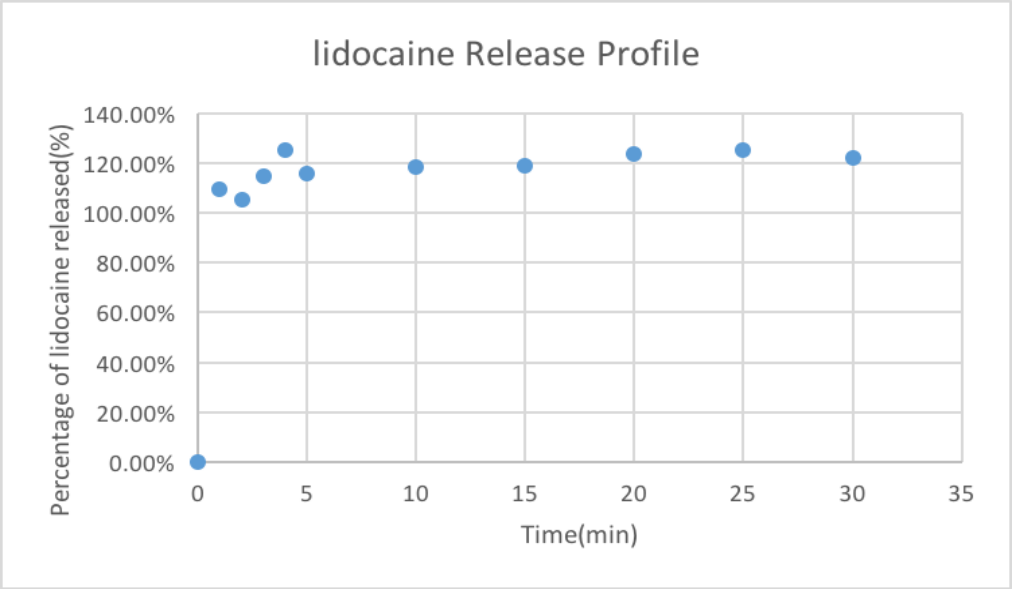



Figure 15: Releasing profile of 0.858mg-lidocaine loaded fiber in PBS buffer

Since the lidocaine was released 100% in the first 5 minutes, samples with more lidocaine concentration and shorter collecting intervals were examined. The release profile was built using the same method. This dissolution showed a fast and complete drug release at 1 minute. (Figure 16).

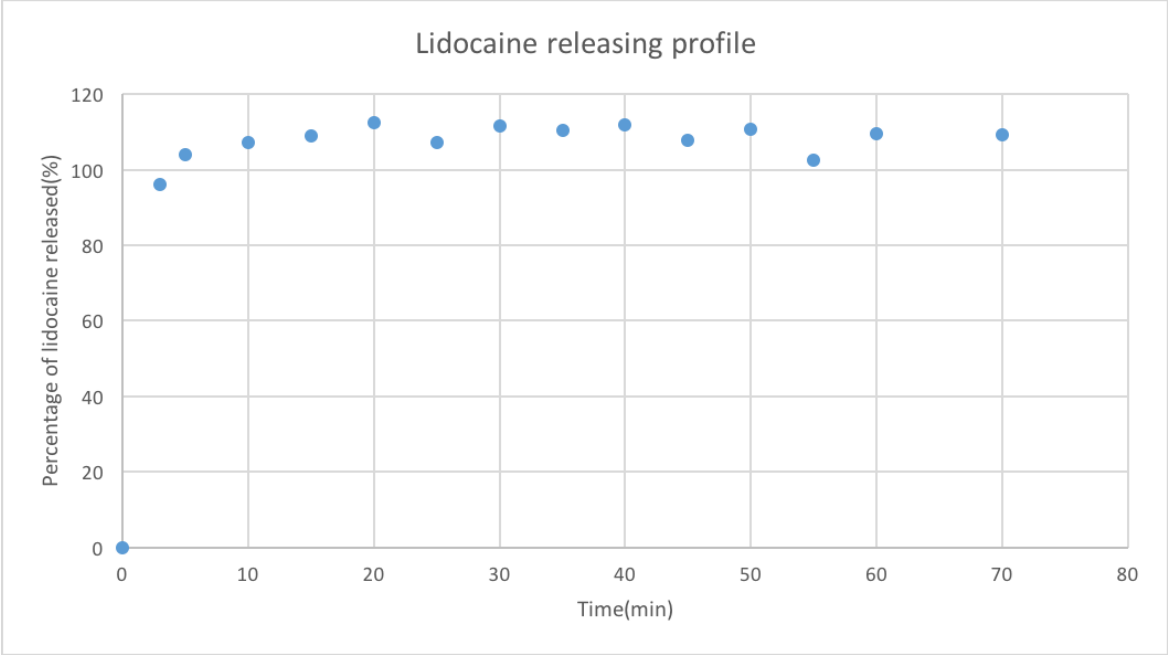



Figure 16: Releasing profile of 2.82mg-lidocaine loaded fiber in PBS buffer.

### 3. 8 Skin Raman Test

2% lidocaine aqueous ethanol solution and 10 % Zein solution were used as reference. Zein-only fiber patch was applied to human skin after moisturizing the skin for 5 minutes, the amount of lidocaine in skin was measured and recorded. The data were analyzed using keratin as reference, a protein that can be found in human skin. The measurement of lidocaine in skin at 10 different positions with different depths in skin were recorded (Appendix C) and plugged into a profile and used as blank. (Appendix D).

After obtaining the relative lidocaine amount in skin after applying zein-only fiber patch as blank, measurements of lidocaine in skin after applying a Zein-lidocaine fiber patch were recorded (Appendix E) and analyzed (Appendix F).

The two skin depth profile were then combined and compared (Appendix G). A drug profile was obtained by subtracting zein-only profile (blank) from lidocaine loaded profile (Figure 17). The drug profile showed that there was evidence of lidocaine present in the first 5μm in skin depth. Although the releasing efficiency needs further studies, these experiments proved that 4% lidocaine encapsulated in zein nanofiber was able to release in vivo by directly applying as a patch after moisturizing the skin.

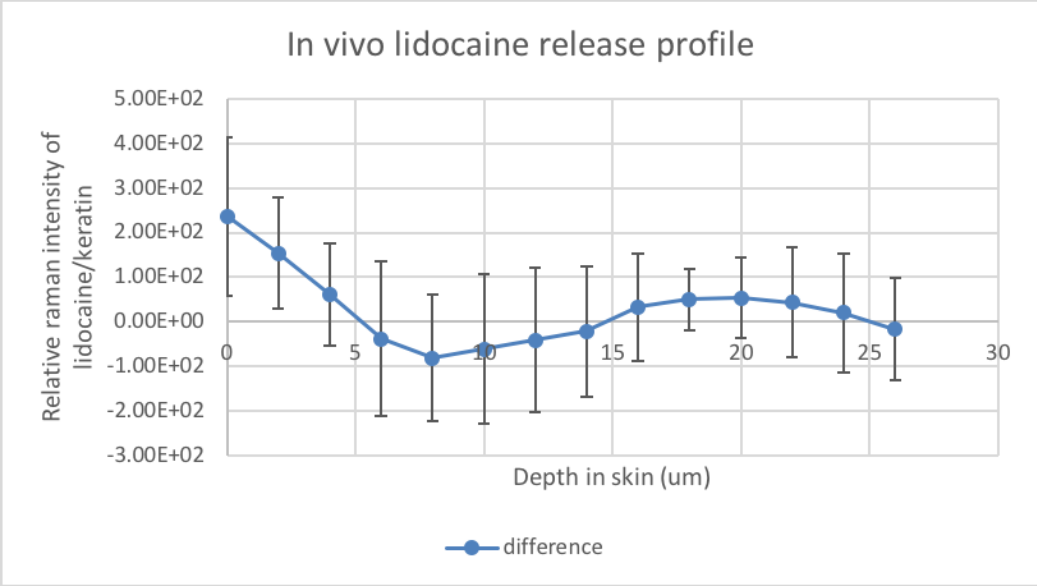



Figure 17 : In vivo skin depth lidocaine profile after applying zein-lidocaine patch for five minutes.

## 4.0 Discussion

Lidocaine was successfully encapsulated into nanofibers, and in vitro releasing profiles in PBS showed high efficiency in loading and releasing API's. It is a fast and complete release in the first minute. This patch can be used as an instant anesthetics product. The next step could be decreasing its releasing rate so it can have a greater potential in pharmaceutical industries as a long-term painkiller. Other APIs such as ibuprofen were successfully added to zein electrospinning nanofibers and showed sustained drug release through *In vitro* dissolution tests by Huang and his group. They also used modified coaxial electrospinning process to preventing clogging. Unelectrospinnable solvent, DMF, was used as sheath fluid. [15]

The presence of lidocaine was determined by HPLC and there is no obvious change in the morphology of the fibers based upon the SEM images. The *in vivo* release profiles were studied through Raman skin test on human skin. Lidocaine was found in skin by directly applying patches containing nanofibers after moisturizing. Further studies are needed for the *in vivo* skin test for quantification.

In regards to the electrospinning process, all samples with 20% zein concentration have beads inside the fiber. 25% zein solution and 30% solution gave fibers without beads. Due to the higher concentration, the 30% zein solution produced thicker fibers with diameters ranging from 400 nm to 750 nm. 25% zein solution produced fibers with diameters ranging from 150 nm to 350 nm. Longer collecting distances also result in decreasing fiber diameter sizes. Although there is no direct evidence showing the correlation between viscosity and fiber morphology, it is hypothesized that increasing viscosity may lead to larger diameters, since the comparison of the average fiber diameter size for 30% and 25% zein fiber showed larger diameters for the higher

zein concentration. By adding a coaxial nozzle, the time that the tip was clogged was elongated from 10-15 seconds up to around 120 seconds. Although the clogging issue was not completely solved, decreasing the surrounding temperature and providing more solvent improved the electrospinning process. More modifications can be done on this apparatus to achieve better results in the future including introducing a poor volatile organic solvent as a sheath fluid as Yu *et al* reported.[16]

## 5.0 References


[1]S. Torres-Giner, E. Gimenez, J.M. Lagaron. “Characterization of the morphology and thermal properties of Zein Prolamine nanostructures obtained by electrospinning”. Food Hydrocolloids, June 2008.

[2] Fatma Kayaci, Tamer Uyar. “Electrospun zein nanofibers incorporating cyclodextrins”. Carbohydrate Polymers, September 2012, 90(1):558-568.

[3] Song R, Llaca V, Linton E, Messing J. "Sequence, regulation, and evolution of the maize 22-kD alpha zein gene family". *Genome Res*. November 2001, 11 (11): 1817–25.

[4] Lawton, John W. "Zein: A History of Processing and Use". American Association of Cereal Chemists, November 1, 2002.

[5] Y. Li, L.-T. Lim, Y. Kakuda. “Electrospun Zein Fibers as Carriers to Stabilize (−)-Epigallocatechin Gallate”. Journal of Food Science, 03 April 2009.

[6] Core, Jim. “Corn Protein Could Reduce Ethanol Production Costs”. April 15, 2002, United States Department of Agriculture Agricultural Research Service.

[7] Scriabine, Alexander. “Discovery and development of major drugs currently in use”. *Pharmaceutical Innovation: Revolutionizing Human Health*. Philadelphia: Chemical Heritage Press. Archived Sept 08, 2017.

[8] "Lidocaine Hydrochloride (Local)". The American Society of Health-System Pharmacists. Archived from the original on 2015-09-06. Retrieved Aug 26, 2015.

[9] M.Glavas-Dodov, K.Goracinova, K.Mladenovska, E.Fredro-Kumbaradzi, "Release profile of lidocaine HCl from topical liposomal gel formulation". International Journal of Pharmaceutics, 2002, 242,381-384.

[10] Pallavi Kamble, Bhakti Sadarani, Anuradha Majumdar, Sukhwinder Bhullar. "Nanofiber based drug delivery systems for skin: A promising therapeutic approach". Journal of Drug Delivery Science and Technology, July 2017, 124-133.

[11] H.E. Colley, Z. Said, M.E. Santocildes-Romero, etc, "Pre-clinical evaluation of novel mucoadhesive bilayer patches for local delivery of clobetasol-17-propionate to the oral mucosa." Biomaterials, 2018,178, 134-146.

[12]Peter J. Caspers, Gerald W. Lucassen, Elizabeth A. Carter, Hajo A. Bruining, and Gerwin J. Puppels, "In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles". Journal of Investigative dermatology, 2001 Mar;116(3):434-42.

[13]Daniel E Becker, Kenneth L Reed. "Local Anesthetics: Review of Pharmacological Considerations". Anesthesia Progress, 2012 Summer; 59(2): 90–102.

[14] ChunSik Choe, Johannes Schleusener, Jürgen Lademann and Maxim E. Darvin,“ Keratin-water-NMF interaction as a three layer model in the human stratum corneum using in vivo confocal Raman microscopy”. Scientific Reports, 2017 Nov;15900(7).

[15] Weidong Huang, Tao Zou, Shengfang Li, etc. “Drug-Loaded Zein Nanofibers Prepared Using a Modified Coaxial Electrospinning Process”. AAPS PharmSciTech. 2013 Jun; 14(2): 675–681.

[16] Yu DG, Yang JM, Li L, Lu P, Zhu LM. “Obtaining finer polymer nanofibers using two different electrospinning processes”. Fiber Polym. 2012;13:450–455.

## Appendix A: Releasing data of 0.858 mg-lidocaine loaded fiber

| Time(min) | Area | Concentration(mg/L) | Mass of lidocaine in buffer(mg) | Mass of lidocaine taken out(mg) | Mass of lidocaine released(mg) | Percentage |
|---|---|---|---|---|---|---|
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00% |
| 1.00 | 243.30 | 9.41 | 0.94 | 0.01 | 0.94 | 109.73% |
| 2.00 | 231.54 | 9.05 | 0.90 | 0.01 | 0.90 | 105.48% |
| 3.00 | 257.79 | 9.87 | 0.97 | 0.01 | 0.99 | 114.87% |
| 4.00 | 286.71 | 10.78 | 1.05 | 0.01 | 1.07 | 125.12% |
| 5.00 | 260.47 | 9.95 | 0.96 | 0.01 | 0.99 | 115.92% |
| 10.00 | 267.19 | 10.16 | 0.97 | 0.01 | 1.01 | 118.25% |
| 15.00 | 269.16 | 10.23 | 0.96 | 0.01 | 1.02 | 118.93% |
| 20.00 | 282.91 | 10.66 | 0.99 | 0.01 | 1.06 | 123.59% |
| 25.00 | 287.33 | 10.79 | 0.99 | 0.01 | 1.07 | 125.08% |
| 30.00 | 278.60 | 10.52 | 0.96 | 0.01 | 1.05 | 122.18% |
| 180.00 | 297.42 | 11.11 | 1.00 | 0.01 | 1.10 | 128.37% |

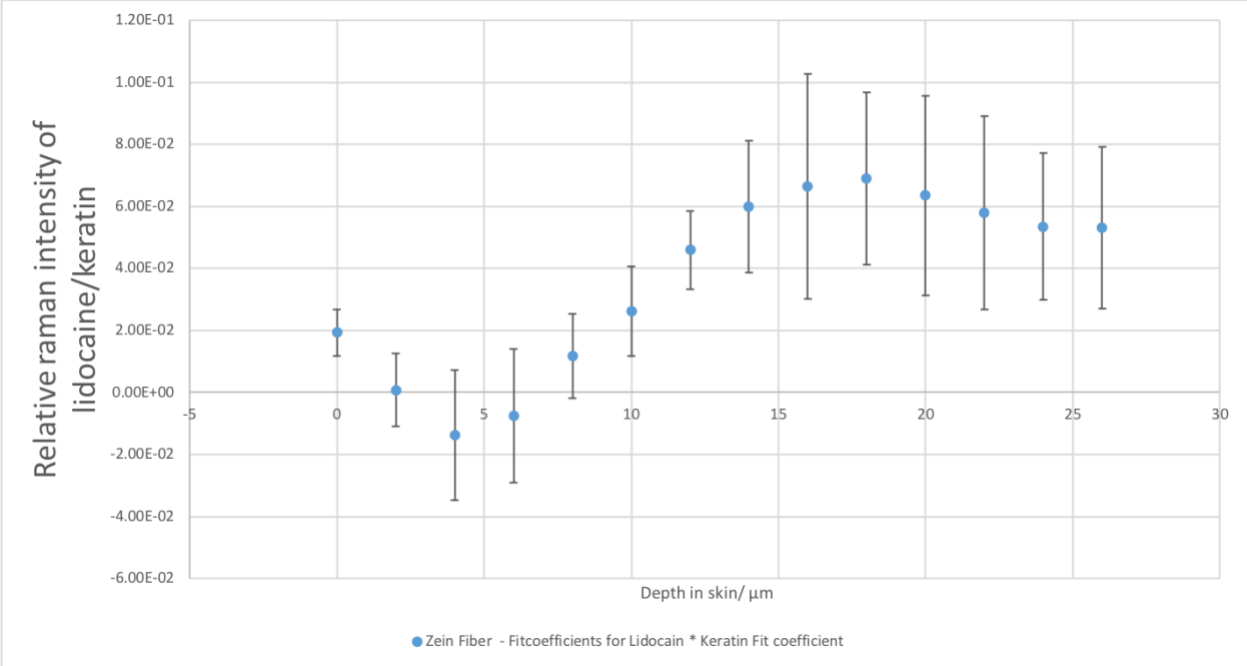
## Appendix B: Releasing Data of 2.282mg-Lidocaine Loaded Fiber

| Time(min) | Area | Concentration (mg/L) | Mass of lidocaine in buffer(mg) | Mass of lidocaine taken out(mg) | Mass of lidocaine released(mg) | Percentage (%) |
|---|---|---|---|---|---|---|
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 3.00 | 641.51 | 21.89 | 2.19 | 0.02 | 2.19 | 95.94 |
| 5.00 | 701.03 | 23.76 | 2.35 | 0.02 | 2.37 | 104.03 |
| 10.00 | 723.98 | 24.48 | 2.40 | 0.02 | 2.44 | 107.12 |
| 15.00 | 738.57 | 24.93 | 2.42 | 0.02 | 2.49 | 109.06 |
| 20.00 | 764.47 | 25.75 | 2.47 | 0.03 | 2.57 | 112.48 |
| 25.00 | 723.80 | 24.47 | 2.32 | 0.02 | 2.45 | 107.17 |
| 30.00 | 758.30 | 25.55 | 2.40 | 0.03 | 2.55 | 111.63 |
| 35.00 | 749.28 | 25.27 | 2.35 | 0.03 | 2.52 | 110.47 |
| 40.00 | 760.39 | 25.62 | 2.36 | 0.03 | 2.55 | 111.88 |
| 45.00 | 728.30 | 24.61 | 2.24 | 0.02 | 2.46 | 107.87 |
| 50.00 | 751.00 | 25.32 | 2.28 | 0.03 | 2.53 | 110.67 |
| 55.00 | 692.82 | 23.50 | 2.09 | 0.02 | 2.34 | 102.60 |
| 60.00 | 757.82 | 25.54 | 2.25 | 0.03 | 2.50 | 109.42 |
| 70.00 | 766.27 | 25.80 | 2.24 | 0.03 | 2.50 | 109.35 |
| 280.00 | 714.26 | 24.17 | 2.08 | 0.02 | 2.33 | 102.12 |

# Appendix C: Raman spectroscopy data of lidocaine in zein-only fiber(blank)

| Depth(micron) | Position 1 | Position 2 | Position 3 | Position 4 | Position 5 | Position 6 | Position 7 | Position 8 | Position 9 | Position 10 | Mean Value | | Standard deviation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| -10 | | 8.00E+02 | 1.05E+03 | -1.09E+01 | 6.11E+02 | 1.46E+03 | 3.74E+02 | 1.63E+02 | 1.16E+03 | 5.36E+02 | | 7.01E+02 | 5.11E+02 |
| -8 | | 2.09E+02 | 1.11E+03 | -8.03E+00 | 6.12E+02 | 2.43E+02 | 5.52E+02 | 3.22E+02 | 9.95E+02 | 7.01E+02 | | 5.05E+02 | 3.92E+02 |
| -6 | 2.36E+02 | 2.87E+01 | 5.30E+02 | 2.33E+02 | 4.70E+02 | 3.67E+02 | 522.4 | 4.70E+02 | 6.73E+02 | 6.02E+02 | -1.22E+02 | 3.92E+02 | 1.97E+02 |
| -4 | -1.10E+02 | 1.63E+02 | 3.80E+02 | 2.97E+02 | 4.71E+01 | 4.09E+02 | 349.14 | 1.14E+02 | 5.04E+02 | 2.60E+02 | 6.13E+01 | 2.39E+02 | 1.98E+02 |
| -2 | 130.13 | 180.27 | 1.51E+02 | 291.02 | 125.04 | 2.29E+02 | 246.24 | 86.109 | 2.82E+02 | 217.09 | 108.64 | 1.91E+02 | 7.37E+01 |
| 0 | 168.14 | 93.434 | 97.91 | 158.48 | 50.469 | 119.38 | 101.61 | -60.456 | 192.4 | 60.103 | 4.2592 | 1.02E+02 | 7.51E+01 |
| 2 | -30.533 | 19.71 | 27.927 | 47.443 | -34.707 | 39.691 | -24.003 | -91.349 | 59.792 | 97.055 | -43.284 | 1.55E+00 | 4.95E+01 |
| 4 | -139.2 | 8.8829 | -35.41 | 22.312 | -131.52 | -40.097 | 44.928 | -162.49 | 11.011 | 94.544 | -20.831 | -4.68E+01 | 7.82E+01 |
| 6 | -110.98 | 92.725 | -15.333 | 35.448 | -148.28 | -70.514 | 52.122 | -218.19 | 170.75 | -69.16 | 63.007 | -2.36E+01 | 1.24E+02 |
| 8 | 44.678 | 130.07 | 15.281 | 85.669 | -70.733 | -65.145 | 86.393 | -61.956 | 160.59 | 87.562 | 65.659 | 3.61E+01 | 8.74E+01 |
| 10 | 73.05 | 180.87 | 89.19 | 183.96 | -25.932 | 27.349 | 90.432 | -20.686 | 17.544 | 172.61 | 145.45 | 6.84E+01 | 7.75E+01 |
| 12 | 223.18 | 217.55 | 138.59 | 177.31 | 40.972 | 46.745 | 96.556 | 127.98 | 41.531 | 179.13 | 243.07 | 1.23E+02 | 7.25E+01 |
| 14 | 256.26 | 214.86 | 146.4 | 163.24 | 158.66 | 58.067 | 127.98 | 213.23 | 158.93 | 226.61 | 283.24 | 1.66E+02 | 5.74E+01 |
| 16 | 227.32 | 179.3 | 159.02 | 182.01 | 200.28 | 98.452 | 119.51 | 283.9 | 260.42 | 288.12 | 339.19 | 1.90E+02 | 6.10E+01 |
| 18 | 201.18 | 199.91 | 194.12 | 191.46 | 197.25 | 203 | 163.43 | 238.25 | 318.97 | 236.42 | 244.45 | 2.12E+02 | 4.44E+01 |
| 20 | 81.152 | 191.99 | 167.72 | 239.46 | 164.27 | 300.68 | 246.08 | 214.86 | 310.14 | 225.54 | 196.6 | 2.13E+02 | 7.17E+01 |
| 22 | 83.543 | 179.41 | 73.072 | 241.84 | 139.9 | 394.04 | 301.98 | 248.72 | 247.27 | 302.82 | 165.33 | 2.12E+02 | 1.04E+02 |
| 24 | 129.38 | 159.98 | 15.066 | 194.82 | 132.24 | 385.57 | 338.11 | 275.66 | 230.81 | 370.26 | 172.31 | 2.07E+02 | 1.15E+02 |
| 26 | 201.37 | 128.37 | 99.141 | 165.04 | 126.44 | 354.76 | 341.94 | 284.84 | 251.56 | 250.65 | 136.7 | 2.17E+02 | 9.57E+01 |

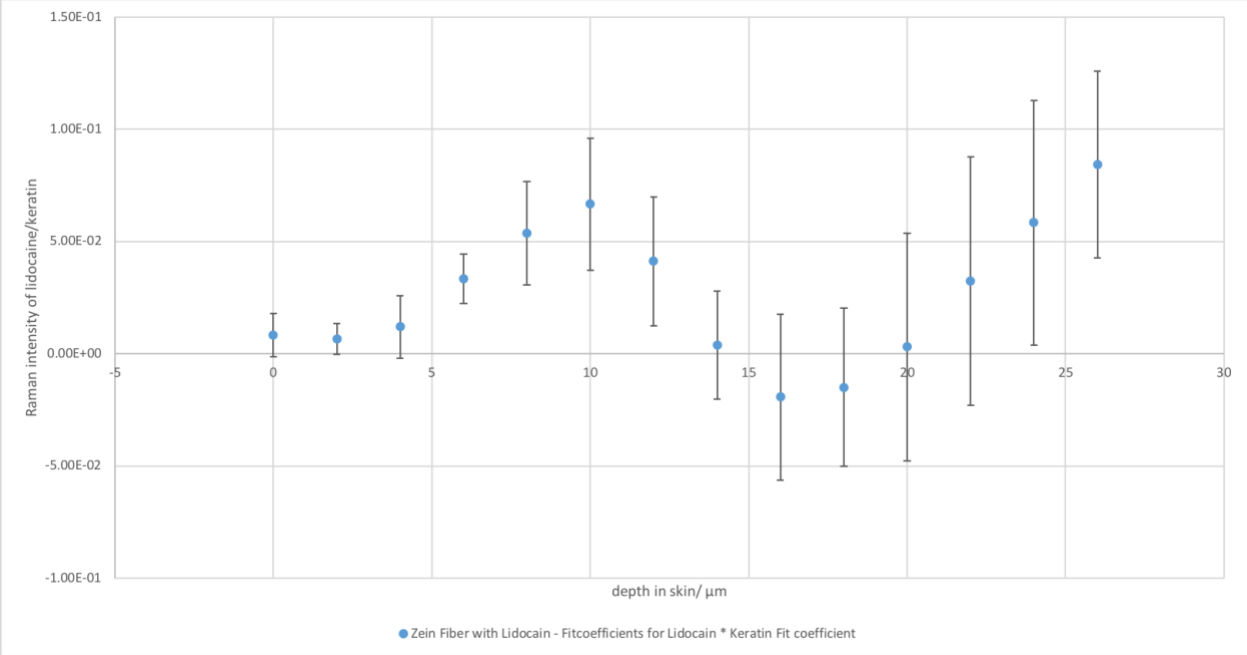
## Appendix D: In vivo Skin Depth profile of lidocaine in zein-only fiber(blank)



# Appendix E: Raman Spectroscopy Data of Zein-lidocaine Fiber

| Depth(micron) | Position 1 | Position 2 | Position 3 | Position 4 | Position 5 | Position 6 | Position 7 | Position 8 | Position 9 | Mean value | Standard deviation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| -10 | | | 7.33E+02 | 4.75E+02 | 1.59E+03 | 1.25E+03 | 4.26E+02 | | | 8.94E+02 | 5.07E+02 |
| -8 | | 4.56E+02 | 3.62E+02 | 2.89E+02 | 3.80E+02 | 9.43E+02 | 3.35E+02 | -1.77E+02 | | 3.70E+02 | 3.27E+02 |
| -6 | 2.68E+02 | -2.82E+01 | 7.00E+02 | 4.79E+02 | 5.83E+02 | 8.82E+02 | 1.83E+02 | -2.33E+02 | 1.20E+02 | 3.28E+02 | 3.61E+02 |
| -4 | 6.41E+02 | 2.31E+02 | 4.48E+02 | 5.13E+02 | 6.82E+02 | 4.80E+02 | 6.79E+02 | 1.15E+03 | 4.34E+02 | 5.84E+02 | 2.56E+02 |
| -2 | 646.2 | 392.3 | 417.03 | 374.92 | 383.25 | 288.74 | 659.93 | 8.74E+02 | 1.73E+02 | 4.68E+02 | 2.17E+02 |
| 0 | 501.59 | 197.33 | 295.84 | 264.03 | 196.43 | 183.58 | 445.82 | 651.8 | 315.84 | 3.39E+02 | 1.61E+02 |
| 2 | 182.14 | -10.421 | 54.741 | 113.44 | 84.483 | 143.51 | 291.81 | 355.04 | 178.55 | 1.55E+02 | 1.14E+02 |
| 4 | -66.666 | -110.29 | -1.5984 | 35.693 | -57.589 | 155.74 | 105.48 | 28.384 | 46.315 | 1.51E+01 | 8.48E+01 |
| 6 | -111.08 | -214.58 | 16.527 | 18.235 | -101.66 | 152.63 | -0.75489 | -229.18 | -84.434 | -6.16E+01 | 1.22E+02 |
| 8 | -121.79 | -135 | -0.61531 | 102.21 | -29.806 | 116.44 | 3.0971 | -176.74 | -157.49 | -4.44E+01 | 1.10E+02 |
| 10 | -110.82 | -132.5 | 46.886 | 242.92 | 44.518 | 173.45 | 101.2 | -138.29 | -159.94 | 7.49E+00 | 1.49E+02 |
| 12 | -50.422 | -53.39 | 128.31 | 248.14 | 131.83 | 288.26 | 188.17 | -45.135 | -92.807 | 8.26E+01 | 1.45E+02 |
| 14 | 21.892 | 57.821 | 228.32 | 295.78 | 199.97 | 288.78 | 253.61 | -67.685 | 27.995 | 1.45E+02 | 1.35E+02 |
| 16 | 123.55 | 191.24 | 285.43 | 356.56 | 251.15 | 335.45 | 265.97 | 46.811 | 146.75 | 2.23E+02 | 1.03E+02 |
| 18 | 208.48 | 299.44 | 254.7 | 336.23 | 246.31 | 325.46 | 277.78 | 186.64 | 221.38 | 2.62E+02 | 5.21E+01 |
| 20 | 238.35 | 255.34 | 227.73 | 350.2 | 189.02 | 314.05 | 300.93 | 308.06 | 206.45 | 2.66E+02 | 5.50E+01 |
| 22 | 226.44 | 218.35 | 238.34 | 324.47 | 136.63 | 247.37 | 275 | 370.86 | 260.9 | 2.55E+02 | 6.63E+01 |
| 24 | 203.8 | 206 | 225.34 | 251.2 | 102.14 | 181.93 | 273.6 | 344.23 | 260.61 | 2.28E+02 | 6.73E+01 |
| 26 | 181.51 | 210.99 | 171.25 | 195.93 | 85.03 | 164.18 | 294.41 | 284.98 | 218.3 | 2.01E+02 | 6.35E+01 |

# Appendix F: In vivo Skin Depth profile of lidocaine in lidocaine loaded fiber



## Appendix G: In vivo skin depth profile of zein only and lidocaine loaded fiber