Synthesis of Electrospun Zein and Silk Fibroin/Zein Meshes with Ibuprofen for Drug Delivery to Chronic Wounds

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Abstract

Chronic wounds affect many patients today and are set to affect more due to the increasing prevalence of chronic diseases. Ibuprofen is a painkiller commonly used to mitigate pain caused by chronic wounds. However, conventional oral administration results in low absorption efficacy and short-lasting relief, causing inconvenience. Thus, this project aimed to synthesise ibuprofen-loaded electrospun nanofibre meshes for the topical delivery of ibuprofen to chronic wounds. Zein and silk fibroin (SF)/zein blend were proposed as natural polymers for the creation of the meshes. Zein meshes with ibuprofen and without ibuprofen (control) were successfully electrospun with 50% (w/v) zein solutions in 96% methanoic acid. Fibres without beading defects were produced with fibre diameters of 612.7nm (control) and 605.6nm (ibuprofen). Both meshes had tensile strength under 0.6MPa. The presence of ibuprofen was found not to significantly affect the fibre diameter and tensile properties of the meshes. Ibuprofen diffusion into phosphate-buffered saline solution (PBS) could not be determined as the control appeared to release more ibuprofen. Attempts to synthesise SF/zein meshes were unsuccessful as SF synthesised from *Bombyx Mori* cocoons failed to dissolve in methanoic acid. This study showed that ibuprofen-loaded zein electrospun meshes can be synthesised, but further work needs to be conducted to optimise electrospinning variables and mesh properties, to synthesise SF/zein meshes, and to further investigate the meshes' viability for ibuprofen delivery to chronic wounds.

1. Introduction

Chronic wounds are wounds that do not heal in an orderly set of stages and in a predictable amount of time the way most wounds do. In the United States alone, 6.5 million patients are affected by chronic wounds (Sen et al., 2009). The number of chronic wound sufferers grows yearly due to the increasing prevalence of chronic diseases such as diabetes that affect wound healing (Han & Ceilley, 2017). Ibuprofen is a commonly-used nonsteroidal anti-inflammatory drug that reduces pain and inflammation when treating chronic wounds. Conventional oral administration of ibuprofen has multiple disadvantages. Firstly, the poor water solubility of ibuprofen results in a low absorption efficacy by the body upon oral administration. Secondly, relief can only be provided for a short period of time through small

doses, which is inconvenient. Thirdly, chronic wounds require nonsteroidal anti-inflammatory drugs such as ibuprofen to be taken orally for an extended period of time. This can cause gastrointestinal, renal, and cardiovascular damage (Manoukian et al., 2017).

The topical delivery of ibuprofen has been shown to be equally effective to oral administration in the treatment of joint and soft tissue injury. It achieves high concentrations within the targeted site of action while simultaneously keeping plasma concentrations low, leading to fewer or less serious unwanted gastrointestinal, renal, and cardiovascular side effects (Manoukian et al. 2017).

Electrospinning is an effective fiber production method that uses a polymer solution and electric force. Drug-loaded electrospun meshes for a drug delivery application can be made through co-electrospinning. In co-electrospinning, the drug and polymer are combined into a solution through dissolution in a common solvent and the solution is electrospun to produce a mesh of drug-loaded nanofibers (Son et al., 2014). In particular, ibuprofen-loaded meshes have been successfully synthesised via co-electrospinning with various polymers, such as with polyvinylpyrrolidone (PVP) by Yu et al. (2011), with cellulose acetate/PVP blend by Shi et al. (2013) and with poly(lactide-co-glycolide)(PLGA)/poly(ethylene glycol)-g-chitosan (PEG-g-CHN) blend by Jiang et al. (2004).

Electrospun meshes are feasible as wound dressing and anti-inflammatory drug delivery devices as they are porous and have large surface area to volume ratios. This allows for easy diffusion of the drug into the bloodstream. Drug release is sustained over time through diffusion and fiber degradation (Jiang et al., 2004). Additionally, the pore size of the mesh can be controlled to be less than 0.2 microns, preventing bacterial access while allowing drug diffusion (Abrigo et al., 2014).

Fibers made from natural polymers can exhibit enhanced biocompatibility, bioactivity, and biodegradability, which are properties needed in biomaterials engineering. Of these proteins, keratin, collagen, SF, elastin, zein, and soy are some of the most common used in fiber fabrication, including electrospinning (DeFrates et al., 2018).

Zein is a prolamin and is the principal storage protein of corn (*Zea mays*). In comparison to animal proteins, zein is economical and readily available, being a co-product when corn is processed for food or fuel. It is biodegradable and has low immunogenicity. (Vogt et al., 2018). Zein has an amphiphilic polymeric nature, which makes it easily joinable with both hydrophilic and hydrophobic polymers in order to produce a compatible material with better

properties than the individual components. Unfortunately, zein has poor mechanical strength (Vogt et al., 2018).

Bombyx Mori silk fibroin has great flexibility, durability and excellent mechanical strength. Being a natural protein fiber, it possesses a structure very similar to human skin with smooth, breathable, soft, non-itching and antistatic characteristics (Lu et al., 2014). This makes silk suitable for biomedical applications. *B. Mori* silk consists of silk fibroin (SF) and sericin at a ratio of around 70:30. The large quantity of glycine in SF creates high tensile strength, which is essential for medical applications (Ude et al., 2014). On the other hand, sericin causes an allergic reaction in about 10% of samples in vertebrate models, and was shown to have some degree of cell toxicity when used in high quantities (Aramwit et al., 2014). Thus, pure SF is obtained with sericin removed through a degumming process.

While Huang et al. (2013) synthesised ibuprofen-loaded zein meshes through coaxial electrospinning, no studies have attempted to synthesise such meshes using co-electrospinning. SF/zein electrospun mesh without a drug was successfully synthesised by Yao et al. (2009) and shown to have significantly improved tensile strength as compared to pure zein electrospun mesh. However, no studies to date have investigated the electrospinning of ibuprofen-loaded SF/zein meshes for the delivery of any drug.

2. Objectives and Hypotheses

2.1. Objectives

1. To synthesise zein nanofibre meshes with and without ibuprofen via electrospinning.

2. To prepare silk fibroin from *Bombyx Mori* cocoons and synthesise SF/zein nanofibre meshes in a 1:2 (w/w) ratio with and without ibuprofen via electrospinning.

3. To investigate the a) fibre diameter, b) tensile strength, and c) ibuprofen release kinetics in phosphate-buffered saline solution (PBS) for all electrospun meshes synthesised.

2.2. Hypotheses

1. Zein nanofibre meshes with and without ibuprofen can be synthesised via electrospinning.

2. Silk fibroin can be prepared from *Bombyx Mori* cocoons. Silk fibroin/zein (1:2 w/w) nanofibre meshes with and without ibuprofen can be synthesised via electrospinning.

3a. Electrospun meshes with and without ibuprofen have similar fibre diameters and tensile strengths.

3b. Ibuprofen-loaded electrospun meshes release ibuprofen into PBS when immersed into PBS.

3c. Silk fibroin/zein electrospun meshes have smaller fibre diameters and higher tensile strengths than zein electrospun meshes.

3. Materials and Methods

3.1. Materials

Zein (product Z3625, M_w unspecified) and ≥96% methanoic acid were purchased from Sigma-Aldrich. *Bombyx Mori* cocoons were purchased from TheYarnTreeUSA on Etsy. (±)-Ibuprofen was purchased from Cayman Chemical, and dialysis flasks and cassettes were purchased from Thermo Fisher Scientific.

3.2. Unsuccessful Preparation of Silk Fibroin

5.0g of Bombyx Mori cocoons were boiled in 500ml of 0.0200M sodium carbonate solution for 30 minutes, then rinsed in deionised water for 20 minutes three times. Boiled and rinsed cocoon pieces were then dried and stored. Multiple boiled pieces of cocoon pieces were put together in batches and heated in an oven at 60.0°C in 9.30M lithium bromide solution (1g cocoons: 4ml LiBr) for 4 hours. The SF-LiBr solution obtained was dialysed against deionised water (MWCO:3500) until lithium bromide was removed. The aqueous SF solution was centrifuged at 6000 rpm for 20 minutes, then poured through a filtration mesh to remove insoluble impurities. The SF solution was then lyophilised to obtain solid SF.

3.2.1. Problems

The first batch of SF unexpectedly solidified and was insoluble in 96% methanoic acid. The second batch solidified when stored over the weekend after dialysis and before it could be centrifuged. In later batches, SF-LiBr solution was also centrifuged and filtered as well as diluted before dialysis, preventing solidification during dialysis. When solid SF was obtained from freeze-drying as planned, a low yield of SF was obtained and the solid SF was also completely insoluble in 96% methanoic acid, despite overnight magnetic stirring and sonication. Thus, only zein electrospun meshes could be synthesised.

3.3. Electrospinning

3.3.1. Preparatory Steps

23-gauge needles with outer diameters of 0.6mm were filed to flatten the sharp tips. For each electrospinning attempt, the same square plastic board was covered with a fresh piece of aluminium foil and affixed to the fume hood wall to act as a collector.

1.60g, 2.20g, 2.80g, 3.40g, and 4.00g of zein were dissolved into separate portions of 5.0ml of 96% methanoic acid with magnetic stirring for 24 hours. Using these solutions, electrospinning was carried out for short periods similarly to as described in the following section. Potential difference applied, needle tip-collector distance, and flow rate of the solution were adjusted to find a suitable set of parameters. Samples were taken by pressing microscope slides attached to the end of a wooden ruler against the collector.

After the samples were viewed under an optical microscope and electrospinning conditions were observed, a concentration of 3.50g zein/5.0ml methanoic acid was chosen for the fabrication of the meshes. This was found to be 50% (w/v) by measuring solution volume.

3.3.2. Electrospinning of Zein Meshes

A solution for the control mesh without ibuprofen was created by dissolving 10.50g of zein into 15.0ml of methanoic acid, while a solution for the ibuprofen-loaded mesh was created by dissolving 10.50g of zein and 0.375g of ibuprofen into 15.0ml of methanoic acid. The solutions were carefully drawn into a 20ml syringe and air was pushed out. A flattened 23-gauge needle was screwed onto the syringe. The syringe was secured into a syringe pump with the needle tip positioned 9.0cm away from the collector. The live wire of the high-voltage DC power supply was attached to the needle near its tip while the ground wire was attached to the collector.

After the syringe pump was turned on at 4ml h^{-1} and the DC power supply was turned on at 10kV, a charged jet of zein solution was sprayed from the needle tip. Methanoic acid evaporated mid-air and zein nanofibres were deposited onto the collector plate. Electrospinning was carried out for 4 hours. A zein nanofibre mesh was formed on the collector, which was carefully scraped off using a spatula. Two meshes were produced, one without ibuprofen (control) and one with ibuprofen (drug-loaded).

3.3.3. Problems during Electrospinning

While fibres were successfully formed, optimal electrospinning was not achieved with the zein solutions. Droplets of solution periodically formed and dripped from the needle tip. These were caught on paper towels. Deposits of solidified zein solution and small amounts of fibres periodically formed at the needle tip, obstructing the solution from being spun. The needle tip was regularly wiped using paper towels attached to a non-conducting wooden ruler to clear blockages.

During the electrospinning of the ibuprofen-loaded mesh, the needle repeatedly jammed at approximate 45-minute intervals, causing no solution to be pushed out by the syringe pump. The jammed needle was replaced with a fresh needle whenever this occurred. This problem did not occur with the control mesh.

3.4. Mesh Morphology

A 2.0cm square was cut from each mesh with a scalpel and sent for SEM imaging. The qualitative morphological characteristics of the mesh were observed. 200 fibre diameter measurements were taken for each mesh using the ImageJ software and used to calculate the average fibre diameters.

3.5. Tensile Testing

Three 3.0cm*1.0cm rectangular strips were cut from each mesh with a scalpel. Thickness of each piece was measured using a micrometer. The samples were placed onto a Kelvin® Material Tester (product number 841845) and pulled until they broke. The distance pulled and tension force measured were automatically measured and logged onto a computer multiple times per second. The initial tested lengths of the samples and the final lengths at breakage were recorded, allowing percentage elongation at break to be calculated.

3.6. Ibuprofen Release in Phosphate-Buffered Saline Solution

A calibration curve at 265nm was obtained by taking 9 readings of ibuprofen dissolved into PBS at concentrations ranging from 0.00625mg/ml to 0.500mg/ml. A standard solution was created in a 10ml volumetric flask by dissolving 5.00mg of ibuprofen into 10.0ml of PBS. Solutions of lower concentration were then prepared by dilution with PBS, using a micropipette of tip size 200–1000µl to transfer small amounts of solution.

Three 2.0cm squares were cut out from each mesh with a scalpel. The six mesh squares were immersed into 10ml of PBS each. At 15-minute intervals, each solution was used to fill a cuvette which was read at 265nm in the spectrophotometer. The cuvette samples were then immediately poured back into their respective solutions.

4. Results and Discussion

4.1. Unsuccessful Preparation of SIlk Fibroin

Aqueous silk fibroin gels, or solidifies, when the conformation of SF peptide chains changes from random coil (Silk I) to β-sheets (Silk II) (Ayub et al., 1993). Unexpectedly, the initial batches of aqueous SF solution gelled in less than 1 week, during or directly after dialysis. This contradicted Rockwood et al. (2011) whose studies found that SF solutions can usually be stored at 4°C for at least a month. A dry solid was obtained after lyophilisation of the solidified SF.

In later batches, the aqueous SF solution obtained after dialysis could not be continuously freeze-dried over 48-72 hours as described by Rockwood et al. (2011), and was instead freeze-dried over two weeks. The long duration was likely responsible for β-sheet formation.

Solid SF in random coil conformation is soluble in methanoic acid. However, the SF obtained from both the unsuccessful dialysis and the two-week freeze drying were completely insoluble in 96% methanoic acid, despite overnight stirring and the use of sonication. This further suggests that the conformation of SF had changed to β-sheets.

4.2. Problems during Electrospinning Process

In electrospinning with ideal conditions, a droplet of polymer solution is held at the tip of the needle by surface tension while a thin jet of solution is ejected from the droplet due to electrostatic repulsion caused by a high charge density in the solution at the tip. The droplet forms a Taylor Cone shape, which is constantly maintained at the needle tip. However, solution drippage and absence of a Taylor Cone occurred during electrospinning in this study, likely due to surface tension being too low as compared to the potential difference applied.

Needle blockages with the zein-ibuprofen solution were thought to have occurred because zein and ibuprofen interact by forming hydrogen bonds (Huang et al., 2013), increasing the viscosity of the solution.

4.3. Mesh Morphology

For both solutions, meshes with fibres of good quality were produced. Beading defects were not observed, though some fibres had non-uniform diameters and deviations from a circular shape at some points. The majority of fibres were straight. This suggests that the electrospinning parameters were somewhat suitable despite the aforementioned problems.

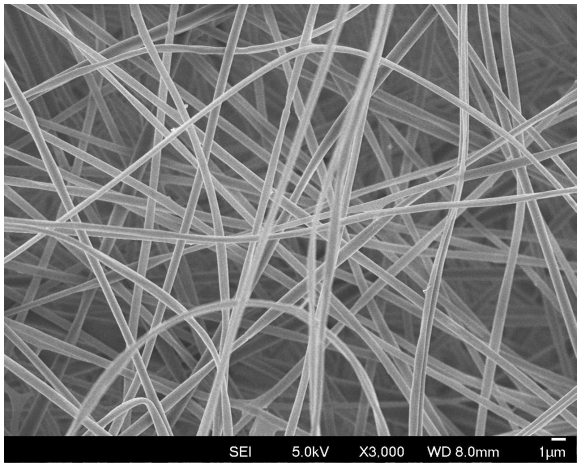

Figure 1: Ibuprofen mesh at 3000x magnification

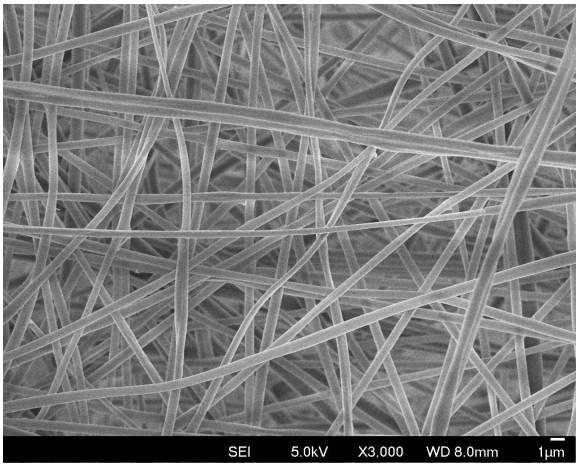

Figure 2: Control mesh at 3000x magnification

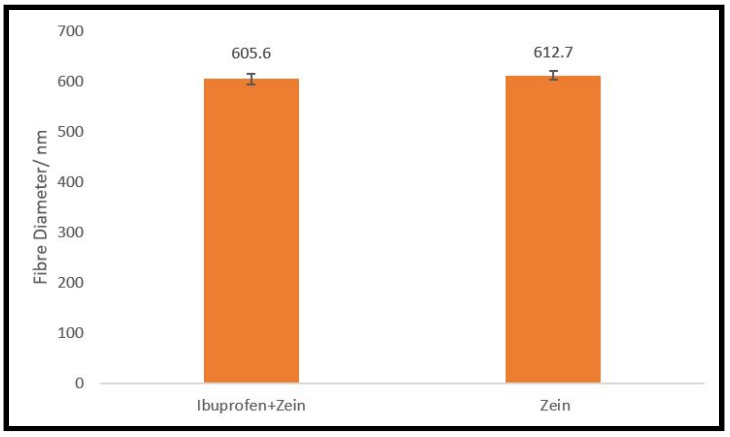

Figure 3: Mean fibre diameter of meshes

The ibuprofen mesh had a mean fibre diameter of 605.6nm, while the zein mesh had a mean fibre diameter of 612.7nm. The T-test showed a p-value of 0.600, which is greater than 0.05. Thus, no significant difference was observed between the mean fibre diameters of the two meshes. Nevertheless, the ibuprofen mesh had a greater standard deviation of fibre diameters and more curved fibres than the control mesh, suggesting that the presence of ibuprofen reduces the stability of the electrospinning process.

The fibre diameters were very different from zein meshes synthesised by Yao et al. (2009) (around 410 nm) using the same concentration in methanoic acid. This could be attributed to the reported use of zein with M_w=35,000 by Yao et al. (2009). This is highly atypical as two polypeptides with M_w=19,800 and 22,000 make up 75-80% of zein by mass (Rosentrater & Evers, 2018). Information about M_w of zein used in this study was not provided by the distributor (Sigma-Aldrich). In addition, electrospinning parameters used were different.

4.4. Tensile Testing

A maximum tension of 0 was recorded for all six samples. The machine had a minimum reading of 0.1 pound-force, which corresponds to 0.6 MPa for mesh with a thickness of 0.07mm (the thinnest thickness reading for the six samples) and a width of 10mm. Thus, zein electrospun meshes with and without ibuprofen synthesised in this study likely both have tensile strengths less than 0.6 MPa.

The poor tensile strength was due to multiple factors. Firstly, zein is known to have poor tensile strength, which was the rationale for proposing to synthesise a mesh with silk fibroin added to zein. Secondly, electrospinning of both meshes was intermittent due to needle tip obstructions and also needle jamming for the ibuprofen mesh. Thus, fewer bonds were formed between zein nanofibres as fibres that landed earlier on the collector more often dried completely before more fibres landed onto them. Thirdly, tiny droplets of zein-methanoic acid solution were sometimes sprayed onto the collector, possibly causing the redissolution of some zein nanofibres, resulting in many weak points.

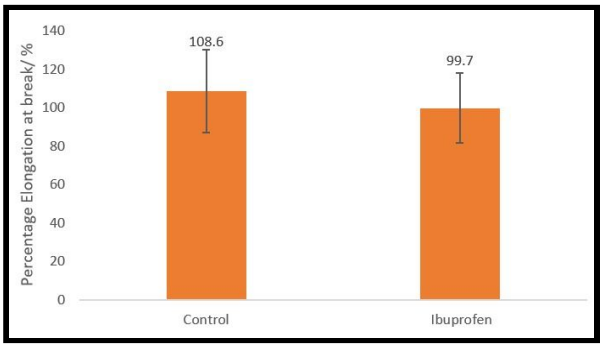

Figure 4: Elongation of meshes at break.

Both the control and ibuprofen meshes had much higher elongation at break than the electrospun zein nanofibre meshes synthesised by Yao et al. (2008) (43%). This further suggests that meshes synthesised in this study have fewer cross-linkages between nanofibres, resulting in weaker cohesive forces holding the mesh together.

4.5. Ibuprofen Release in Phosphate-Buffered Saline Solution

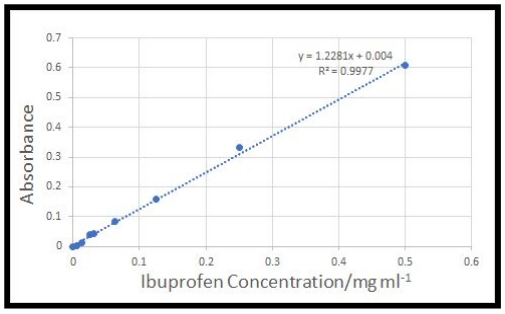

Figure 5: Calibration curve of ibuprofen in PBS.

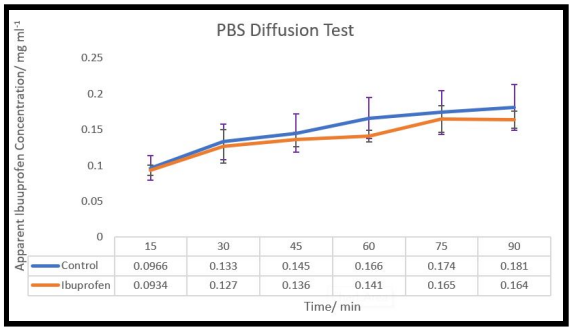

Figure 6: Apparent concentrations of ibuprofen in PBS at 15-minute intervals after 2cm mesh squares were immersed.

The results of the diffusion test seemed to suggest that the control mesh, which contained no ibuprofen, released more ibuprofen into PBS than the ibuprofen mesh at any given point of time. Thus, ibuprofen diffusion kinetics into PBS could not be determined. This contradicted the literature review where Jiang et al. (2004) successfully used UV-spectrophotometry at 264nm to characterise ibuprofen release from electrospun meshes.

The source of error is suspected to be that some zein droplets were sprayed onto the collector during electrospinning. These dried onto the mesh and may have dissolved into PBS upon immersion, severely interfering with the spectrophotometer readings. The control mesh was thought to have higher readings due to its greater thickness. Additionally, the concentration of ibuprofen used (approximately 1.79% w/v in zein-methanoic acid solution) was likely too low for its effect to be observed.

5. Conclusion and Recommendations for future work

Zein nanofibre meshes with and without ibuprofen could be synthesised via electrospinning. SF/zein nanofibre meshes could not be synthesised as the preparation of SF from *Bombyx Mori* cocoons was unsuccessful. Electrospun zein meshes with and without ibuprofen were found to have similar fibre diameter and tensile properties, with mean fibre diameters of 605.6nm (ibuprofen mesh) and 612.7nm (control mesh). Both meshes had poor tensile strength. It could not be determined whether the ibuprofen-loaded mesh released ibuprofen when immersed into PBS. This project demonstrated that electrospun zein meshes loaded with ibuprofen can be synthesised, but was unable to determine if they are a viable alternative to conventional oral administration of ibuprofen.

Further work should be conducted to improve electrospinning parameters such that better meshes can be produced, without spraying of solution droplets onto the mesh and with more linkages between nanofibres. Work should also be conducted to produce SF and synthesise SF/zein electrospun meshes. Furthermore, triplicates of zein meshes with and without ibuprofen should be produced and tested to increase the statistical significance of results. (While three samples were taken from each mesh for tests, triplicates for each mesh were not produced due to time constraints caused by the pandemic.) Greater concentrations of ibuprofen could be used. Crosslinking techniques could be utilised to improve the tensile strength of meshes, though it would have to be ensured that ibuprofen in the meshes does not react with a crosslinking agent used or decompose upon heating to merge fibres.

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