Synthesis and Characterization of Electrospun Polyvinylidene Fluoride-based (PVDF) Scaffolds for Renal Bioengineering

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Abstract. With the continuous search for alternative treatment for End Stage Renal Disease (ESRD), the use of synthetic scaffolds as a prospect is becoming a forefront in regenerative medicine. This study aimed to fabricate and evaluate the effects of varying electrospinning parameters (the applied voltage, spinning distance and flowrate) to the fiber diameter and pore size of the scaffolds produced using polyvinylidene fluoride (PVDF). Electrospun scaffolds were subjected to scanning electron microscopy (SEM) and strain apparatus to assess structural and tensile properties. Results showed that applied voltage, spinning distance, and flowrate directly affect the overall pore size and fiber diameter of the electrospun scaffolds, as well as resulting in a direct effect to the tensile strength of the electrospun scaffold. The optimal values for the parameters in fabricating a PVDF electrospun scaffold would be a voltage of 20kV, a spinning distance of 100mm and a flowrate of 0.5ml/h. Results also show that spinning distance and flowrate have statistically more impact on the outcome of the fiber diameters, while applied voltage and spinning have statistically more impact on the outcome of the pore sizes. Moreover, the electrospun scaffolds acquired thinner fiber diameters and smaller pore sizes when compared to that of the native kidney tissue. Nonetheless, the promising mechanical integrity of the electrospun PVDF-based scaffolds offer a potential approach to addressing ESRD.

1. Introduction

End-Stage Renal Disease (ESRD) or kidney failure is an under-recognized medical crisis. This is a condition wherein the kidney fails to perform its function such as filtering toxic wastes in the body and balancing bodily fluids. This requires an urgent need to develop strategies and technologies that can address this crisis.

The development of biomimetic tissue replacements is an emerging strategy that can potentially solve the continuing crisis on tissue or organ shortage. Among the methods used in the fabrication of tissue replacements, electrospinning has gained attention due to its capacity to create scaffolds from various natural and synthetic polymers that can mimic the extracellular matrix (ECM) or microenvironment found in
biological tissues [1-3]. The use of natural polymers promotes better biocompatibility; however, these polymers have poor mechanical integrity [4]. On the other hand, the use of synthetic polymers appears superior in terms of mechanical properties and great processing flexibility [5,6]. Synthetic polymers such as polyvinylidene fluoride (PVDF), polylactic acid (PLA), and poly-vinyl alcohol (PVA) have been used in the electrospinning process for biomedical applications because of their durability, stability, and good mechanical properties [7-9].

PVDF is a thermoplastic that is a result of the polymerization of vinylidene difluoride. PVDF has gained substantial use in medical applications such as implants, generation of soft tissue and nerves, suture material, and bio membranes. PVDF is used due to its desirable biocompatibility, biostability and satisfactory mechanical strength, with minimal cellular and tissue response. Additionally, due to its ruggedness and chemically inert characteristics, PVDF has been widely used as a medical coating for different medical devices, as well as multi filament application as vascular grafts, ligaments, and artificial corneas [7]. Thus, the use of polymers with these properties especially intended for renal bioengineering could be an advantageous approach [10,11].

The properties of scaffolds produced from electrospinning are determined by different parameters such as spinning distance, applied voltage, and flowrate. The manipulation of these parameters affects the structural and mechanical properties of the electrospun scaffolds. However, the effect of electrospinning parameters to the structural and mechanical properties of PVDF-based scaffolds intended for renal bioengineering has rarely been studied directly. To gain an understanding of the factors that influence the properties of electrospun scaffolds, this study determined the effects of the changes in the electrospinning parameters (spinning distance, applied voltage, and flowrate) to the structural and mechanical properties (fiber diameter, pore size, and tensile strength) of the scaffolds produced using PVDF. Through better understanding of the effects of the electrospinning parameters, fabricated electrospun scaffolds will be better optimized to provide better cell environment.

2. Materials and methods

2.1 Preparation of polymeric solution
PVDF solution was prepared by mixing polyvinylidene fluoride pellets in, N-dimethylformamide (DMF) to obtain a 13% w/v polymer concentration. The solution was magnetically stirred at room temperature until complete dissolution of the PVDF pellets. Porcine kidney samples were used as controls.

2.2 Preparation of electrospinner
The INOVENSO NE300 Series electrospinner was used in fabricating the PVDF-based scaffolds as shown in Figure 1. The 5ml PVDF (13% w/v) solution was placed in a 10cc syringe which was connected to a single electrospinning nozzle using polyethylene tubing. A drum collector was used and kept at ca constant 500rpm was covered with aluminum foil for collection of the electrospun scaffolds. This is to distribute the fibers evenly as opposed to using a stationary plate. The electrospinning environment was kept at constant room temperature of 22 °C. Table 1 shows the parameters manipulated in the study.

The values displayed in Table 1 were based on the previous electrospinning experiments done using PVA and PLA solutions. The ranges and extremes for PVDF were determined to provide suitable scaffold samples. Every solution was electrospun for 15 minutes, resulting to a total of 8 runs. For each run, the electrospinning nozzle and tubing were cleaned with warm water and left to dry completely before proceeding to the next run. This is to ensure that no build up occurs on the electrospinning nozzle and tubing.
which could affect the overall flowrate of the solution. Similarly, the aluminum foil covering of the collector plate was replaced for every sample.

| Voltage (kV) | Spinning Distance (mm) | Flowrate (mL/h) |
|-------------|------------------------|-----------------|
| 20          | 100                    | 0.5             |
|             | 150                    | 0.9             |
| 25          | 100                    | 0.5             |
|             | 150                    | 0.9             |

2.3 Evaluation of the structural properties of the PVDF-based scaffolds

The morphology and microstructure of the electrospun nanofiber mats were analyzed using scanning electron microscopy (SEM). Each of the 8 samples were subjected to SEM imaging. Samples were sputtered with gold using a fine coater machine to create a conductive layer before mounting the sample on the specimen stage. Magnifications of X2000 and X5000 were used to magnify the samples as seen in Figure 2. For each sample, SEM images from three different areas of the sample were taken and labeled A, B, and C, as shown in Table 2 and Table 3. This is to test the homogeneity of the samples for their fiber diameters and pore sizes, resulting to a total of 36 SEM images. The resulting SEM images were further examined using ImageJ to determine the average pore size and fiber diameter of the scaffolds [12]. The average fiber diameter and pore size for each sample were obtained by taking the measurements of 30 fibers and pores, respectively.

2.4 Evaluation of the tensile property of the PVDF-based scaffolds

The PASCO Stress/Strain Apparatus was utilized to test the tensile property of the scaffolds. A scaffold was attached to the apparatus’ clamps. One clamp was connected to the lever arm while the other was connected to the crank. The crank was operated manually and was turned clockwise to stretch the scaffold. Depending on how much pull the scaffold had, the lever arm would push onto the force sensor which in turn would send a reading to DataStudio. The software then recorded the amount of force applied to the sensor with respect to how much distance the sample had been pulled by cranking. Any breakage of the samples was also recorded. Once the crank had reached its capacity, the sample was removed, and the crank was turned counterclockwise to return the clamp to its original position. A new sample was placed on the clamps and the test was done again. The same process was repeated for each run.

3. Results and discussion

3.1 Evaluation of the structural properties of the PVDF-based scaffolds

For the fiber diameter, the electrospinning setup which obtained the highest average fiber diameter is at 20 kV, 100 mm, and 0.5 mL/h while the lowest average fiber diameter was obtained at 20 kV, 150 mm, and 0.9 mL/h.
Preliminary testing showed that PVDF when electrospun with voltages higher than 30kV, it is observed that there is matting of the nanofibers as seen in Figure 1. This is the result of the electrospun solution not having enough time to form as fibers during its transition from the nozzle to the collector. There have been no observed beading formations between 20kV and 30kV of voltage applied, suggesting that PVDF as a solution has good relaxation time or good extensional viscosity. However, higher applied voltage resulted in even lesser uniformity which starts resulting in large bead formations. It was also observed that the higher the voltage being applied, the less uniformity in fibers there was.

![Figure 1. (a) SEM image of electrospun PVDF at 30kV. (b) SEM image of electrospun PVDF at 35kV](image1)

There were no indications that the gold sputtering on the samples were too thick as there were no observed obscurities in the finer details of the SEM images as seen in Figure 2. The high contrast observed in the images also indicate that the gold sputtered layer was not too thin. Overall, there were no observed hindrances in the SEM images due to the gold sputtering process.

![Figure 2. Electrospun samples under SEM Imaging marked and numbered and measured with ImageJ.](image2)

Results showed that electrospun scaffolds at 25 kV mostly obtained average fiber diameters that are lower than those electrospun at 20 kV suggesting that the increased applied voltage led to the decrease in average fiber diameters. An exception, however, even with increased high voltage, is the average fiber diameters obtained between those with 150 mm spinning distance and 0.9 mL/h flowrate which are 0.531
μm and 0.576 μm, as seen in Table 2 respectively. This is probably because the increased flowrate resulted to increased volume of the polymer solution in the Taylor cone formed during electrospinning. This increase in the volume of polymer solution during electrospinning caused the overflowing of the polymeric solution from the nozzle.

It was also be observed that the electrospinning setups with 100 mm spinning distance obtained higher average fiber diameters as compared to those with 150 mm spinning distance. The increased distance caused the stretching of the fibers during electrospinning leading to thinner fibers.

Table 2. Average fiber diameter of the PVDF-based scaffolds

| Parameters | Flowrate (mL/h) | A       | B       | C       | Average Fiber Diameter (μm) |
|------------|----------------|---------|---------|---------|-----------------------------|
| Voltage (kV) | Spinning Distance (mm) |         |         |         |                            |
| 20         | 100            | 0.5     | 0.866   | 1.001   | 1.039                       | 0.969                       |
|            |                 | 0.9     | 0.597   | 0.658   | 0.839                       | 0.698                       |
|            | 150            | 0.5     | 0.771   | 0.589   | 0.660                       | 0.674                       |
|            |                 | 0.9     | 0.522   | 0.548   | 0.523                       | 0.531                       |
| 25         | 100            | 0.5     | 0.736   | 0.787   | 1.052                       | 0.858                       |
|            |                 | 0.9     | 0.590   | 0.587   | 0.708                       | 0.628                       |
|            | 150            | 0.5     | 0.585   | 0.694   | 0.598                       | 0.625                       |
|            |                 | 0.9     | 0.535   | 0.574   | 0.618                       | 0.576                       |

Table 3. Three-way ANOVA of the effects of electrospinning parameters to fiber diameter

| parameters | SS        | df | MS        | F         | p-value (Alpha 0.05) | p eta-sq |
|------------|-----------|----|-----------|-----------|----------------------|----------|
| A          | 0.012105042 | 1  | 0.012105042 | 1.360690366 | 0.260510917          | 0.078377665 |
| B          | 0.211500375 | 1  | 0.211500375 | 23.77410426 | 0.000168243          | 0.597728213 |
| C          | 0.178365042 | 1  | 0.178365042 | 20.04946373 | 0.000380734          | 0.556165381 |
| A x B      | 0.011310042 | 1  | 0.011310042 | 1.27132687 | 0.276143418          | 0.073609103 |
| A x C      | 0.006970042 | 1  | 0.006970042 | 0.783480867 | 0.389187559          | 0.046681667 |
| B x C      | 0.034884375 | 1  | 0.034884375 | 3.921244907 | 0.065143915          | 0.196837342 |
| A x B x C  | 0.000876042 | 1  | 0.000876042 | 0.098473139 | 0.757723881          | 0.006116924 |
| Within     | 0.14234     | 16 | 0.00889625 |           |                      |           |
| Total      | 0.598350958 | 23 | 0.026015259 |          |                      |           |

a (A) Applied Voltage, (B) Spinning Distance, (C) Flowrate

For the pore size, the results demonstrated that electrospun scaffolds with higher average fiber diameters also obtained higher average pore sizes. This is most likely due to the low entanglement or accumulation between the fibers which increased the pore size of the electrospun scaffolds.

The ANOVA (Analysis of Variance) in Table 3 shows that that “B” spinning distance and “C” flowrate have the most effect on fiber diameter. The same can be said for “B x C” where if both of the parameters spinning distance and flowrate were to be changed and applied voltage were to be held constant, it would show that it would statistically have the most effect on the outcome of the fiber diameter.

Pore size remained consistent with changed in parameters showing that more than half of the samples fell in the range of between 1.98μm to 2.40μm as observed in Table 4. The most significant of change is found within the parameters of 20kV at a spinning distance of 100mm, wherein in this extreme the average pore size is at its highest. A large increase from 2.383μm to 2.875μm is observed. Difference is further observed with flowrate, suggesting that within the 20kV applied voltage and 100mm voltage range, flowrate
determines the variance showing that a flowrate of 0.5 mL/h significantly produces larger pore sizes than 0.9 mL/h. The ANOVA for the pore size as seen in Table 5 show that the “A” applied voltage and “B” spinning distance have statistically more impact on the pore sizes of the scaffold than the flowrate. The same can be said for “A x B”, where changes in both applied voltage and spinning distance while flowrate is held constant, statistically will show more impact on the outcome on the scaffold’s pore sizes.

### Table 4. Average pore size of the PVDF-based scaffolds

| Parameters | Pore size (μm) | Voltage (kV) | Spinning Distance (mm) | Flowrate (mL/h) | A | B | C | Average Pore Size (μm) |
|------------|---------------|--------------|------------------------|-----------------|---|---|---|-----------------------|
|            |               | 20           | 100                    | 0.5             | 2.692 | 2.813 | 2.850 | 2.875 |
|            |               |              | 0.9                    | 2.289 | 2.692 | 2.169 | 2.383 |
|            |               | 150          | 0.5                    | 1.896 | 1.989 | 2.003 | 1.963 |
|            |               |              | 0.9                    | 2.166 | 1.809 | 1.780 | 1.918 |
|            |               | 25           | 100                    | 0.5             | 2.155 | 2.071 | 2.157 | 2.127 |
|            |               |              | 0.9                    | 1.606 | 2.216 | 1.805 | 1.876 |
|            |               | 150          | 0.5                    | 2.285 | 1.635 | 2.385 | 2.102 |
|            |               |              | 0.9                    | 2.117 | 2.136 | 2.215 | 2.156 |

### Table 5. Three-way ANOVA of the effects of electrospinning parameters to pore size

|          | SS           | df | MS             | F             | p-value (Alpha 0.05) | p eta-sq |
|----------|--------------|----|----------------|---------------|----------------------|----------|
| A        | 0.233051042  | 1  | 0.233051042    | 4.670408354   | 0.046192573          | 0.225946594 |
| B        | 0.400158375  | 1  | 0.400158375    | 8.019286265   | 0.01202436           | 0.333868633 |
| C        | 0.155365042  | 1  | 0.155365042    | 3.113559087   | 0.096721493          | 0.162897924 |
| A x B    | 0.891276042  | 1  | 0.891276042    | 17.86142229   | 0.000642234          | 0.527485885 |
| A x C    | 0.023126042  | 1  | 0.023126042    | 0.463452373   | 0.050748322          | 0.028150376 |
| B x C    | 0.165170042  | 1  | 0.165170042    | 3.310054042   | 0.087620362          | 0.171416094 |
| A x B x C| 0.000975375  | 1  | 0.000975375    | 0.019546789   | 0.000555063          | 0.001220184 |
| Within   | 0.798392     | 16 | 0.0498995      |               |                      |          |
| Total    | 2.667513958  | 23 | 0.115978868    |               |                      |          |

a (A) Applied Voltage, (B) Spinning Distance, (C) Flowrate

Another observation is seen with regards to fiber diameter in Table 2 of PVDF scaffolds within the 20kV applied voltage and 100mm spinning distance range. The largest average fiber diameter is found within this range and is further affected by change in flowrate, where 0.5 mL/h significantly produces larger fiber diameters than 0.9 mL/h. Fiber diameter is more greatly affected by flowrate in both voltages of 20kV and 25 kV, showing that larger fiber diameters are achieved at a flowrate of 0.5 mL/h at a spinning distance of 100mm. Fiber diameter will consistently be at its finest with increase in spinning distance.

### 3.2 Evaluation of the tensile property of the PVDF-based scaffolds

It was observed that the samples would tear and stretch rather than break when compared to the experiment done on porcine kidney as reference. The electrospun scaffolds can sustain a significant amount of tensile force even exceeding the breaking point of the reference porcine kidney sample. This shows the advantage of PVDF-based scaffolds in terms of mechanical integrity.
3.3 Comparison of properties with porcine kidney
Based on the evaluation of fiber diameter and pore size of the PVDF-based scaffolds, the electrospun scaffolds appear to have a thinner average fiber diameter and smaller average pore size as compared with that of the porcine kidneys which are 25.28μm and 11.80μm, respectively and as seen in Table 6. This difference might be because the porcine kidney was decellularized prior to analysis. The process when cells were removed caused the thickening of the fibers and affected the pore sizes. Another difference would be the tensile nature of the scaffolds in comparison the native decellularized kidney ECMs, wherein the fibrous nature of the electrospun PVDF-scaffolds allowed it stretch and tear. The kidney ECM when subjected to tensile stress would eventually break when applied with more than 1.75N of force as seen in Table 6.

| Table 6. Porcine kidney structural and tensile properties |
|--------------------------------------------------------|
| Average Fiber Diameter (μm) | 25.28 |
| Average Pore Size (μm) | 11.80 |
| Force (N) | 1.75 |
| Displacement (mm) | 15.5 |
| Breakage | Yes |

4. Conclusion
This research work demonstrated the possibility of creating scaffolds from synthetic materials capable of mimicking the native tissue by manipulating the parameters of electrospinning. The applied voltage, spinning distance, and flowrate have strong influence on the fiber diameters and pore sizes of the electrospun scaffolds. Lower spinning distance and lower applied voltage resulted in the production of fibers with higher fiber diameters, which led to larger pore sizes. Fibers with smaller fiber diameters and smaller pore sizes resulted to the faster stretching and tearing of the scaffolds when applied with tensile stresses. Meanwhile, increased flowrate resulted to higher fiber diameters and occasional overflowing of the polymeric solution from the electrospinning nozzle. Nonetheless, the PVDF-based scaffolds appear to be superior in terms of mechanical integrity. It can be concluded that PVDF-based scaffolds could be used as promising materials intended renal bioengineering. The optimal values for the parameters in fabricating a PVDF electrospun scaffold would be a voltage of 20kV, a spinning distance of 100mm and a flowrate of 0.5ml/h. This is because of the larger pore size given. Larger pores may allow better cell movement within the scaffolds itself, seeing as to how compared to the native kidney ECM. To maintain constant uniformity of the fibers, the applied voltage must not be at 35kV or higher, any less than 20kV may not result in the formation of fibers as it is not strong enough to cause jetting to the solution.

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