A preliminary study of greyscale intensity and deposited electrospun fibres using image analysis technique

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ABSTRACT – The amount of deposited electrospun fibres is difficult to measure due to their extremely small size and mass. In this study, a new method using image analysis technique to predict the amount of deposited fibres is proposed. It is hypothesized that the amount of deposited fibres can be predicted by measuring the greyscale intensity of a sample. Samples were produced and scanned to 8-bit greyscale images and ImageJ software was used to analyze the samples. Preliminary results showed that the greyscale intensities has the potential to be used for measuring the amount of deposited electrospun fibres.

1. INTRODUCTION

Electrospinning is a simple method of producing polymeric nanofibres from polymer solutions using electric charge [1][2]. When an adequate electric potential is applied to the polymer solution (Figure 1) a straight jet of polymer is ejected from the pipette tip before undergoing a spiral looping motion known as the whipping instability [3]. As the jet moves towards the collector, solvent evaporates and fibre stretching continues before landing on the collector as randomly oriented fibres.

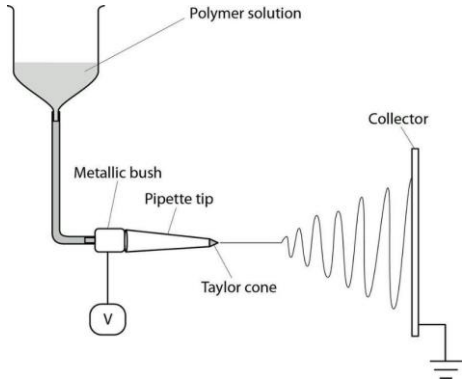

Figure 1 Electrospinning process**.**

Due to the extremely small fibre size and mass, electrospun fibre exhibit unique advantages such as high surface area to volume ratio, light weight, small pore size and high permeability. Electrospun fibres have been proposed in numerous applications such as filtration, tissue engineering scaffolds, drug delivery system and sensors [1]. However, measurements of the deposited fibre amount by means of membrane's physical thickness or mass are difficult to obtain especially at short deposition times. Previously, measurements were made using scanning electron microscope images, digital micrometer and light profilometry. These methods are difficult to manage and could damage the structure of the samples [4]. To overcome the problems, a non-destructive method using image analysis technique to predict the amount of deposited electrospun fibres is proposed.

2. METHODOLOGY

Electrospinning process was carried out using poly (vinyl alcohol) (PVA) with an average molecular weight of 124,000-130,000 g/mol. The PVA was dissolved in distilled water to a final concentration of 8 wt.%. The solution was stirred for approximately 2 hours at 60 ^{0}C using a magnetic stirrer Model C-MAG HS7 (Ika Works, Malaysia). Black A4 papers were used as substrates to aid visibility. These papers were weighed using four figure balance Model AG204 (Mettler Toledo, Switzerland) before and after samples collection. Samples were collected at different deposition times ranging from 1 to 30 minutes. The samples were left overnight to make sure the solvent has fully evaporated. Electrospinning parameters used in this study are summarized in Table 1. Samples were scanned to 8-bit greyscale images using a commercial Canon Model MG5500 scanner and analyzed using ImageJ software (National Institutes of Health, NIH). This software was used to measure the greyscale intensities of the samples. All samples were produced using Model ES1a electrospinning machine (Electrospinz Ltd., New Zealand).

Table 1 Electrospinning parameters.

Parameters	**Value**
Temperature (^{0}C)	21-24
Humidity (%)	60-75
Voltage (kV)	10
Distance from tip to collector (cm)	10

3. RESULTS AND DISCUSSION

Based on visual observation, the white intensity of the deposition spot increased with deposition time (Figure 2). Furthermore, the size of the white spot also increased as the deposition time increased. A closer examination also revealed that the intensity of the white spot was at a maximum at the centre. These results suggest that the amount of deposited fibres was proportional with deposition time and the densest area was located at the centre of the deposition spot.

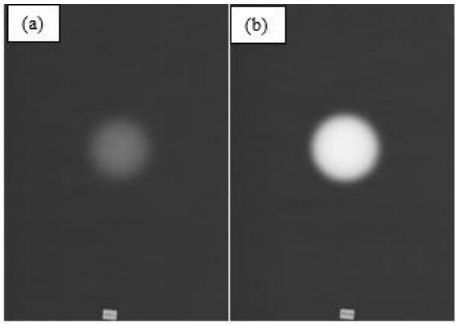

Figure 2 The deposition spot of samples collected at different deposition times (a) 1 minute (b) 15 minutes.

Plots of greyscale intensities from image analysis confirmed the visual findings (Figure 3). The greyscale intensity curves resembling normal distribution curves with minimums at both end of the curves. The peaks of the curves at the centres reasserted the findings that the densest region was located at the centre of the deposition spots. In addition, the peak values of the greyscale intensity curves seem to be proportional with deposition times.

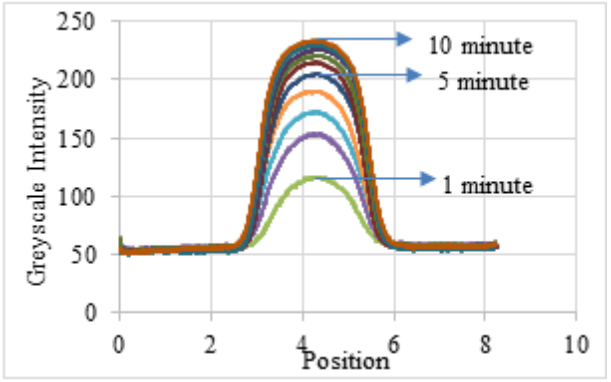

Figure 3 Greyscale intensity curves profiles as a function of position for samples collected at different deposition times.

From Figure 3, the highest peak values of the curves were taken to construct a graph of greyscale intensity versus deposition time (Figure 4). The data produced a curve with a minimum value of 117 for sample of 1 minute deposition time and a maximum value of 233 for sample of 10 minutes. A best fit line was fitted to the data with an R-squared value of 0.88.

Interestingly, a stronger correlation between greyscale intensity and deposition time was found at the lower range of deposition times (inset of Figure 4). This is evidenced by the R-squared value of 0.96. The results suggest that the image analysis technique may be best used at a short deposition times. The inferior result when applying this technique for longer deposition times was most probably caused by the flattening pattern at the higher end of the curve. This was caused by limitations of the scanner in distinguishing the greyscales at the very end of an 8-bit scale.

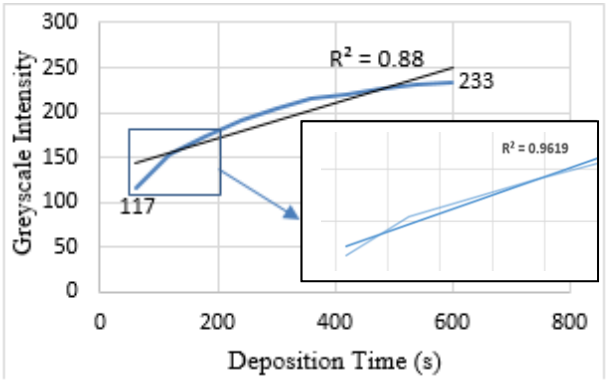

Figure 4 Greyscale intensity as a function of deposition time.

4. CONCLUSION

Visual observations on samples suggested that the amount of deposited fibres increases with time. This finding was confirmed by the greyscale curves obtained from the image analysis. Furthermore, a strong correlation between greyscale intensity and deposition time was found at shorter deposition times. These preliminary results could be an interesting start for a new technique for predicting the amount of deposited electrospun fibres.

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