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Chloroform-Formic Acid Solvent Systems for Nanofibrous Polycaprolactone Webs

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***Abstract*—**In this study, polycaprolactone (PCL) was dissolved in chloroform:ethanol solvent system at a concentration of 18 w/v %. 1, 2, 4, and 6 droplets of formic acid were added to the prepared 10ml PCL-chloroform:ethanol solutions separately. Fibrous webs were produced by electrospinning technique based on the horizontal working principle. Morphology of the webs was investigated by using scanning electron microscopy (SEM) whereas fiber diameters were measured by Image J Software System. The effect of formic acid addition to the mostly used chloroform solvent on fiber morphology was examined.

Results indicate that there is a distinct fall in fiber diameter with the addition of formic acid drops. The average fiber diameter was measured as 2.22μm in PCL /chloroform:ethanol solution system. On the other hand, 328nm and 256 nm average fiber diameters were measured for the samples of 4 drops and 6 drops formic acid added. This study offers alternative solvent systems to produce nanoscaled, nontoxic PCL fibrous webs by electrospinning technique.

***Keywords*—**Chloroform, electrospinning, formic acid polycaprolactone.

I. Introduction

BASED on the dimensional similarity of natural extracellular matrix, nanofibrous scaffolds are inevitable environments which promote cell attachment and proliferation [1]-[3]. Producing nanofibers by using electrospinning technique, has potential in developing ideal scaffold designs [4]. Vascular grafts are the scaffolds where electropun fibrous webs are nominated as candidates for single layer or multilayer designs.

Polycaprolactone is a semi crystalline linear hydrophobic polymer which has a wide application area in biomedical applications [5], [6]. Due to the fact that PCL has five $–CH_2$ moieties in its repeating units, it has the lowest degradation rate among other biodegradable polyesters such like poly (glycolic acid), poly (lactic acid), poly(lactic-co-glycolic) acid. This lower degradation property makes PCL preferable for the applications that require the use of synthetic polymers [7], [8]. Biocompatability and long term biostability after implantation are the preference reasons of polycaprolactone to be used in electrospun vascular grafts [9].

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Since there are many toxic solvents for polycaprolactone (such like; dimethylformamide, tetraflouraethylene, methylene chloride, dichloroethane, prydine [10]), chloroform is the one that is mostly preferred based on its less hazardous properties [11], [12]. However, it is known that, only PCL microfibers around 2–5 μm diameters [10]-[13] can be achieved from chloroform solvent. Therefore, researchers are studying on different solvent systems for PCL in order to produce nanofibers without losing biocompatibility of the fibrous webs for scaffolds.

In this study, PCL was dissolved in chloroform:ethanol solvent system and 1, 2, 4 and 6 droplets of formic acid were added to the prepared 10 ml solution in order to investigate its effect on fiber morphology. PCL/chloroform:ethanol solutions were prepared at a concentration of 18% w/v. Standard electrospinning technique was used to produce fibrous webs. Fiber morphology was investigated based on SEM analysis and fiber diameter measurements.

II. Materials And Methods

Poly-ε-caprolactone (Mn 45000, Sigma–Aldrich) was dissolved in chloroform:ethanol (9:1, v:v; Sigma–Aldrich) at a concentration of 18 w/v % solution. 1, 2, 4 and 6 drops of formic acid were added seperately to the choloroform:ethanol solvent system. Lastly pure formic acid was used as a solvent. Solutions with sample codes and details were listed in Table I.

TABLE I
Solutions Prepared to Be Electrospun

Sample Codes	ChEth	FA1	FA2	FA4	FA6	FA
18%PCL dissolved in FA drops	Ch:Eth (9:1)					FA
	-	+1	+2	+4	+6	-

The addition of ethanol is required to lower the fast evaporation rate of chloroform. It improves the spinnability of polymer in chloroform solution. For homogenity, prepared solutions were mixed at magnetic stirrer for around 10 hours.

The basic electrospinning apparatus (Inovenso, Turkey, Fig. 1) was used in horizantal feeding direction. Since this study is for optimization of fiber morphology, flat collector was used. Production steps can be seen in Fig. 1.

Distance between needle tip and collector was fixed to 20 cm while needle diameter was chosen as 0.8mm. Voltage, flow rate and spinning time were varied from 12kV to 16kV, 0.4ml/h to 1ml/h, and 10min to 15min (for pure acid 30min.) respectively based on used solvent systems. Spinning time was adjusted in order to get adequate wall thickness. Detailed

information about variable production parameters are given in Table II.

Fig. 1 Electrospinning unit

TABLE II
PRODUCTION PARAMETERS VARIED

Sample Codes	Production time [min]	Voltage [kV]	Feed rate [ml/h]
ChEth	10	14.3	1
FA1	15	12.3	1
FA2	15	12.9	0.4
FA4	15	14.8	0.4
FA6	15	16.1	0.5
FA	30	15	0.5

Morphology of the fibrous scaffolds were examined by using scanning electron microscopy and fiber diameters were measured from at least 100 fibers for each sample by Image J Software System (version 1.48).

III. RESULTS AND DISCUSSION

A. Morphological Analysis

SEM analysis results are given below.

In the first sample, ChEth, smooth microfibers were observed in SEM image (Fig. 2). Web area that was accumulated on flat collector was narrow and it was easy to remove the fibrous web from the collector.

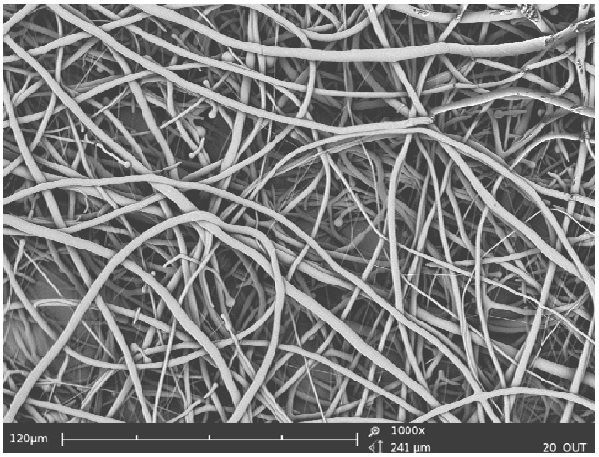
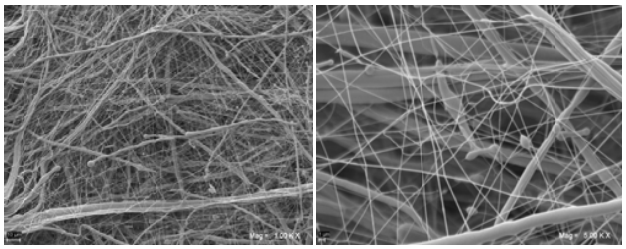


Fig. 2 SEM image of sample ChEth. Magnification is 1000x

On the other hand, both microfibers and nanofibers were observed in the SEM images of FA1. Microfibers in the structure are discontinuous (Fig. 3). It is most probably resulted from the poor effect of 1 drop formic acid addition.

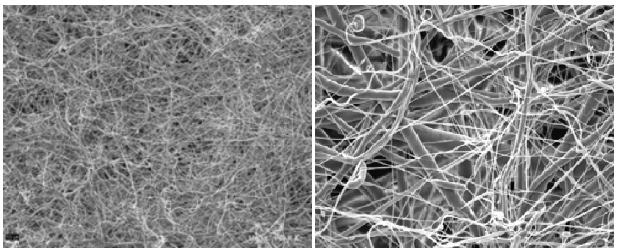


(a) (b)

Fig. 3 SEM images of sample FA1. Magnifications: (a) 1000x, (b) 5000x

Similarly, both microfibers and nanofibers were observed in SEM images of FA2 but structure includes more nanofibers than the structure of sample FA1. Some beads were observed in nanofibers rarely. Most fibers are in curled form while some fibers are in ribbon like form (Fig. 4).

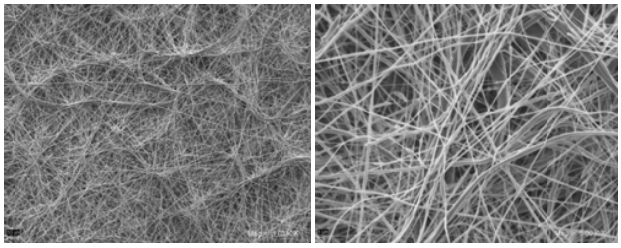
It can be said that, the increase in nanofibers are the results of effectiveness of increased formic acid drops. However, it is not possible to say this web is a nanofibrous webs.



(a) (b)

Fig. 4 SEM images of sample FA2. Magnifications: (a) 1000x, (b) 5000x

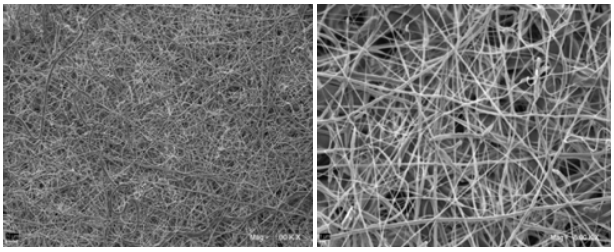
Although there are some thick fibers in the fibrous structure, nanofibers were observed dominantly in the SEM images of FA4 (Fig. 5). Too wide collecting area was observed during production. Smoother and more homogeneous nanofibrous webs are achieved from sample FA4.



(a) (b)

Fig. 5 SEM images of sample FA4. Magnifications: (a) 1000x, (b) 5000x

In a similar manner, although there are some thick fibers in the fibrous structure, nanofibers were observed dominantly in the SEM images of FA6 (Fig. 6). It can be said that both in sample FA4 and FA6, nanofibrous webs were produced.



(a) (b)

Fig. 6 SEM images of sample FA6. Magnifications: (a) 1000x, (b) 5000x

No production was achieved when FA was used purely as a solvent. Since there are some studies [8], [10] in which PCL was electrospun successfully with acid solvents like glacial acetic acid, glacial formic acid, asetic acid and formic acid at lower concentrations; fibrous webs couldn't be produced in this study. It is thought that, the reason can be the molecular weight difference of used PCL. In this study Mn 45000 PCL was used which can be too low to be electrospun at a concentration of 18% w/v.

B. Image J Analysis

Fibrous webs produced from sample ChEth are composed of micro fibers. There are some nanofibers that are formed during production which can be negligible. Average fiber diameter of this production group is 2.2µm.

Image J analysis results can be seen from Fig. 7. Results indicate that, the addition of formic acid droplets causes a decrease in fiber diameter. Although 1 and 2 drops have appreciable but not so respectable effects on fiber diameter due to its high standard deviations, 4 and 6 droplets result in distinct drop of fiber diameters with low standard deviations. 328nm and 256 nm average fiber diameters were measured for sample FA4 and FA6 respectively.

These fiber diameter distributions are in consistent with the morphological analysis stated above.

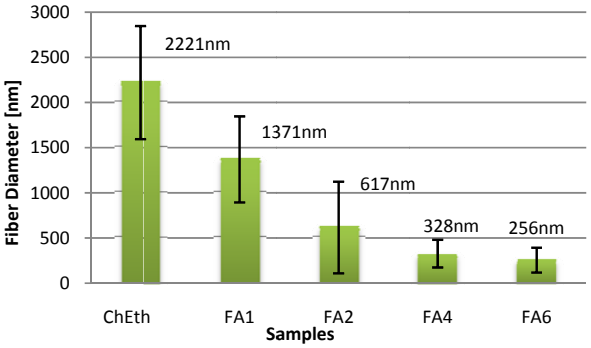
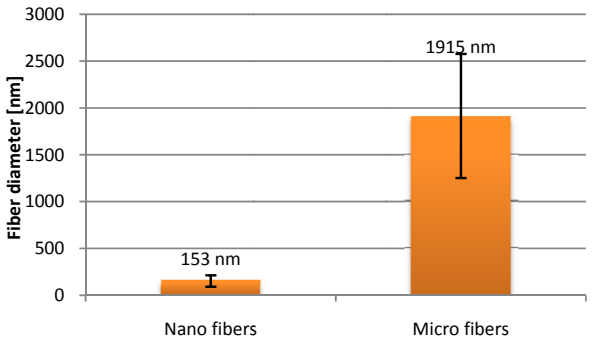


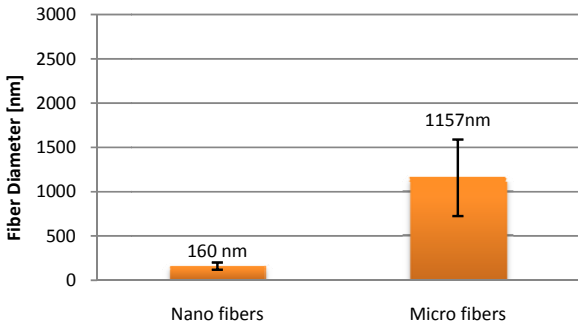
Fig. 7 Image J analysis test results

On the other hand, it can be noticed that for samples FA1 and FA2, different fiber diameter groups can be noted from SEM pictures (Figs. 3 and 4). Image J analysis results also prove these visuals. Average fiber diameters were measured as 1.37µm for sample FA1. On the other hand, it is clear from SEM pictures that these samples are composed of both nano and micro fibers which results in high deviation of average values. Therefore, while discussing the average fiber diameters, this must be taken into account.

Fig. 8 indicates that sample FA1 contents both nano and micro fibers with average of 153nm and 1.92µm diameters. Similar results were achieved for sample FA2 which is composed of 160nm and 1.16µm fiber diameters. Reason of these two grouped fiber diameter distribution can be the poor effect of formic acid solvent drops.



(a)



(b)

Fig. 8 Fiber diameter distribution for samples (a) FA1 (b) FA2

IV. Conclusion

Based on the results, it can be said that, the addition of formic acid droplets cause notable drop in fiber diameters. Especially for 4 and 6 drops additions, 326nm and 258nm fiber diameters were achieved which can be assumed that fibers are in nano scale in comparison with fibers produced from PCL: ChEth solution (2.22µm diameter).

Although chloroform has already accepted solvent for medical use of PCL with its less harmful properties, producing microfibers limits its use where mimicking ECM is a necessity. Vascular grafts are one of the potential application areas for nanofibrous PCL webs that ECM is required to be mimicked for endothelial cells' attachment and proliferation. Therefore addition of formic acid droplets to PCL:ChEth solutions can be used where nanofibrous PCL webs are required especially for medical applications due to its less hazardous properties.

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- Yalcin, I., Harakova, J., Mikes, P., Gok Sadikoglu, T., Domin, R., Lukas, D., "Design of Polycaprolactone Vascular Grafts" Journal of Industial Textiles, (online published in June 2014, Available at: http://jit.sagepub.com/content/early/2014/06/23/1528083714540701)

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