Program Digest, 2019 IEEE International Ultrasonics Symposium (IUS)
Glasgow, Scotland, October 6-9, 2019

Towards Chronic Wound Pads: Gradient Nanofiber Structure Generated by Ultrasound Enhanced Electrospinning (USES)

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***Abstract*—We utilized an ultrasound enhanced electrospinning (USES) technique to produce a four-layer nanofiber scaffold. In contrast to ordinary electrospinning (ES), in the USES process, acoustic radiation pressure generates a cone at the free surface of the polymer solution (4-wt% polyethylene oxide (PEO); aqueous solution), which replaces the need for the needle employed in traditional ES. The cone shape and size were modified by changing the ultrasound parameters (pulse repetition rate, cycles per pulse, and amplitude) during the electrospinning process. A four-layer sample was generated and imaged using scanning electron microscope. The produced nanofiber construct is the first demonstration of a needle-free electrospinning process featuring accurate control of fiber diameter without changes in the sample chemistry. These kinds of gradient structures could be utilized for generation of precision tailored wound pads.**

Keywords—ultrasound enhanced electrospinning, gradient structures, nanofibers, wound pad

I. INTRODUCTION

Chronic wound treatment with scaffolds demands certain properties from the scaffolds: e.g. resistance to microbes and moisture control [1]. Electrospinning (ES) can be used to produce nanofiber scaffolds. There is growing evidence that implantable nanomedical scaffolds provide an effective alternative to existing wound matrices. Nanomedical scaffolds are capable of supporting the natural wound healing process and may provide significant benefits as part of the treatment of challenging chronic wounds [2].

Tissue engineering research indicates that hierarchically designed biomaterials could be beneficial for guiding cell migration, proliferation and differentiation, as well as for tissue regeneration [3]. In addition to biochemical cues, studies have identified substrate stiffness as a significant factor that guides cell spreading, migration, proliferation, and differentiation [4].

Conventional ES does not provide rapid dynamic control of scaffold's structural properties. In conventional ES a typical way to alter fiber diameter is by changing chemistry of the spinning solution (e.g. viscosity, solvent selection) [5].

Most conventional scaffolds reported in tissue engineering feature uniform composition and pore size. They lack the structural complexity to regenerate specific tissue [6]. Gradient biomaterials are generally more difficult to fabricate than uniform or homogenous biomaterials [7].

Previously we have introduced an ultrasound enhanced electrospinning (USES) device [8] that allows us to modify the fiber diameter during the spinning process. This technology allows preparation of novel nanofibrous constructs with uniquely controlled characteristics (stiffness and porosity gradients) aimed for optimal cell proliferation and for controlling the mechanical, topological, moisture permeation, and gas exchange properties of a wound healing platform.

Since the fiber diameter can be utilized to tune stiffness of a nanofibrous structure [9] USES technology allows generation of

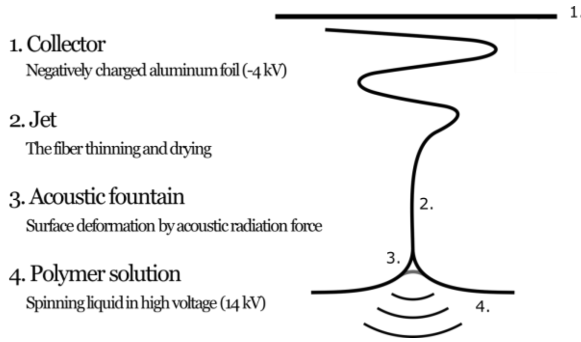

Fig 1. The USES process. Nanofiber emerges from top of the acoustic fountain. The dried fiber is collected on an aluminum foil.

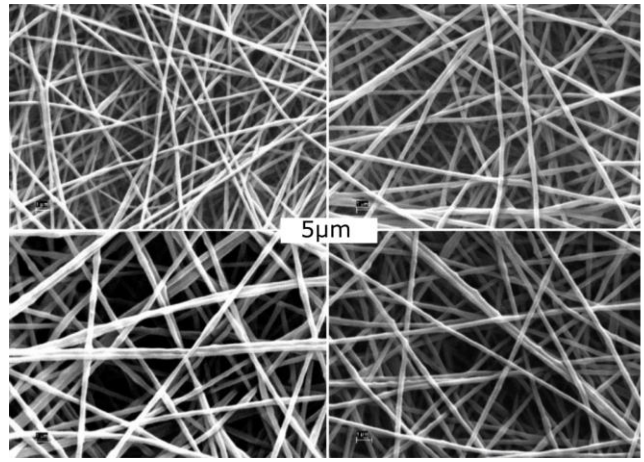

Fig 2. SEM images taken from the four layers: Layer 1 (top left), layer 2 (top right), layer 3 (bottom left) and layer 4 (bottom right)

scaffolds, potentially allowing control of structural properties and thus derivative properties such as cell migration [4].

II. Methods

The USES technology, described in [8,10] was utilized to produce a four-layer nanofiber construct. A focusing 2.16 MHz ultrasonic transducer generates an ultrasonic fountain on top of a bath of polyethylene oxide (PEO, Mw 900 kDa, 4 wt-% dissolved in ion exchanged water). This fountain acts as the base for the electric field induced Taylor cone, from where the nanofiber is spun (Fig. 1). An arbitrary waveform generator (Agilent 33120A) drove a power amplifier (Kalmus Model 121C) that transmitted the signal to the transducer. The humidity of the climate chamber, encasing the setup, was kept at 35 ± 5% with silica gel.

The electrospinning was performed in four steps. First, a set of USES parameters (amplitude, burst rate, burst count, see Table 1) was programmed into the function generator. Subsequently, a nanofiber layer was spun for ca. 45 minutes, after which half of the sample was covered with an aluminum foil, and new parameters were set to the function generator. This was repeated three more times until the final construct was obtained. Half of the sample featured the complete four-layer construct, whereas the other half of the sample was spun on four separate aluminum foils for analysis purposes. During the spinning the polymer feeding rate was adjusted to keep the polymer surface level constant during production of the layers. The feed rate was 0.7 - 1.2 ml/h. The DC voltage for spinning solution and collector were +14 kV and -4 kV, respectively, and the distance to collector was 27 cm. These parameters were kept constant during all experiments.

TABLE I. USES Parameters for the four layers

Layer #	*Normalized Amplitude*	*Duty cycle (%)*	*Relative power*
1	1.00	1.2	1.00
2	0.55	3.0	0.76
3	0.28	7.3	0.49
4	0.17	14.6	0.33

Next, the aluminum foils were taken to a scanning electron microscope (SEM), and representative pictures (Fig. 2) were taken from each sample with 15 000 times magnification. These pictures were analyzed with ImageJ (version 1.52p) program. Manual measurement of fiber diameters (n=60) were recorded and the fiber diameter distributions calculated.

III. Results

SEM images of the different layers are shown in Fig. 2. The fiber diameter distributions of the layers are presented in Fig 3. The diameters ranged from 200 nm to 450 nm. In the first three layers the difference is visible by eye. Student's t-test values were obtained between the fiber distributions in the consecutive layers to determine statistical significance of the observed differences. The results are presented in Table 2.

TABLE II. T-test values between the layers

Layer #	*t-test p-value*
1 and 2	0
2 and 3	$2 \cdot 10^{-8}$
3 and 4	0.67

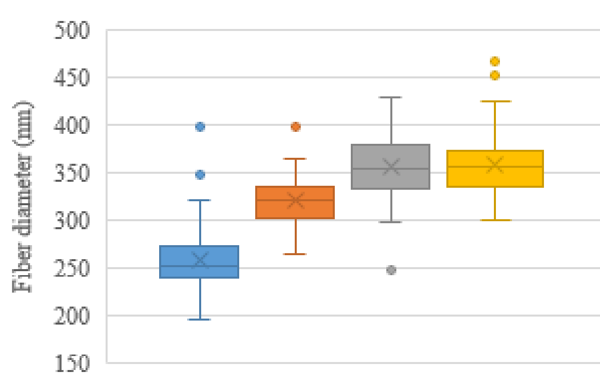

Fig 3. Fiber diameters and their variance obtained for the four layered construct (Layer 1 blue, 2 dark orange, 3 gray, 4 yellow) presented in box and whisker plot.

IV. Discussion

We generated a four-layered gradient structure, Figs. 2 and 3. The fiber diameter distribution was statistically different between the first three layers, whereas the fourth layer was not different from the third one. We suspect that this happens due to acoustic power being so small that we are out of the sensitive region of the USES process.

The construct was generated by merely varying the ultrasonic parameters during the spinning: i.e. the spinning geometry, chemistry or environmental conditions were not altered. This is a step forward in electrospinning: the presented technique allows generation of gradient structures, long sought after for biomedical engineering. The USES technology allows practical realization of gradient structures: the ultrasonic parameters can be varied rapidly (in a few seconds), and thus, precisely tailored structures modified in the depth direction can be generated. One should be able to generate a continuous gradient structure without distinctive layer boundaries.

References

[1] S. Saghazadeh, et al., “Drug delivery systems and materials for wound healing applications,” Advanced drug delivery reviews, vol. 127, pp. 138–166, 2018.

[2] M. J. Regulski and M. R. MacEwan, “Implantable nanomedical scaffold facilitates healing of chronic lower extremity wounds.,” Wounds: a compendium of clinical research and practice, vol. 30, no. 8, E77–E80, 2018.

[3] A. Di Luca, et al., “Gradients in pore size enhance the osteogenic differentiation of human mesenchymal stromal cells in three-dimensional scaffolds,” Scientific reports, vol. 6, p. 22 898, 2016.

[4] S. J. Mousavi and M. H. Doweidar, “Role of mechanical cues in cell differentiation and proliferation: A 3d numerical model,” PloS one, vol. 10, no. 5, e0124529, 2015.

[5] J. Pelipenko, P. Kocbek, and J. Kristl, “Critical attributes of nanofibers: Preparation, drug loading, and tissue regeneration,” International journal of pharmaceutics, vol. 484, no. 1-2, pp. 57–74, 2015.

[6] A. Seidi, M. Ramalingam, I. Elloumi-Hannachi, S. Ostrovidov, and A. Khademhosseini, “Gradient biomaterials for soft-to-hard interface tissue engineering,” Acta biomaterialia, vol. 7, no. 4, pp. 1441–1451, 2011.

[7] X. Miao and D. Sun, “Graded/gradient porous biomaterials,” Materials, vol. 3, no. 1, pp. 26–47, 2010.

[8] H. J. Nieminen, et al., “Ultrasound-enhanced electrospinning,” Scientific reports, vol. 8, no. 1, p. 4437, 2018.

[9] Y. Ji, B. Li, S. Ge, J. C. Sokolov, and M. H. Rafailovich, “Structure and nanomechanical characterization of electrospun ps/clay nanocomposite fibers,” Langmuir, vol. 22, no. 3, pp. 1321–1328, 2006.

[10] J. Mäkinen, et al., “FEM modeling of ultrasound enhanced electrospinning (USES),” unpublished