Effects of Electrospinning Setup and Process Parameters on Nanofiber Morphology Intended for the Modification of Quartz Crystal Microbalance Surfaces

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ABSTRACT
To improve the performance of mass sensitive biosensors, the surface of a piezoelectric quartz crystal transducer, is expanded by employing electrospun nanofibers to its surface. This work describes the effect of vertical - horizontal electrospinning setups and electrospinning parameters on fiber morphology. The research objective was to obtain finer and non-beaded fiber morphologies, via controllable and repeatable process parameters, for further applications of QCM surfaces in high performance DNA-, Aptamer-, Immuno-sensor applications.

INTRODUCTION
Quartz crystal microbalances (QCMs) are one of a broad class of acoustic wave (AW) techniques. QCM is highly sensitive to mass changes in the presence of a coating that interacts with the target when used as mass sensitive biosensors [1-3]. Biosensor performance such as sensitivity, selectivity, and response time is largely influenced by the properties of the sensing films [4,5].

Electrospun nanofibers which have controllable membrane thickness, fine structure and large surface area to volume ratio, are expected to be an ideal candidate as the structure of sensing materials [6]. Polyvinyl alcohol (PVA) which is an important polymer that is widely used in electrospun coatings due to its excellent chemical and physical properties such as non toxicity, process ability, good chemical resistance, good film formation capacity, complete biodegradability and high crystal modulus, is used to modify QCM surfaces [7,8].

Even though electrospinning is a novel method for sensor surface modification, it's an old technique that has been the basis for theoretical studies and new applications for more than a hundred years. The Rayleigh limit, Taylor cone, perturbations, and instabilities are the theoretical backgrounds of the electrospinning process [9,10,12]. Many researchers are investigating novel apparatus to overcome instabilities and chaotic behavior of nanofibers for the purpose of obtaining aligned and reproducible nanofibers [11,12].

Considering polymer solution parameters such as molecular weight, solution viscosity, dielectric effect of the solvent; processing conditions such as voltage, flow rate, temperature, effect of collector, distance between tip and collector; and ambient parameters such as temperature, humidity, and pressure; many experiments have been done to determine the effects of these parameters on fiber morphology [8,11,12].

Following the modification of QCM surfaces by the electrospinning method, it is predicted that the low pressure plasma polymerization technique, which is an elegant method for generating functional polymer surfaces, will be used for creating specific groups on those surfaces for the purpose of novel biosensor applications [2,3].

In this paper, for the modifications on QCM surfaces, vertical and horizontal electrospinning setups are observed and effects of the process parameters on polyvinyl alcohol nanofiber morphology are reported. The appropriate parameters for QCM surface modifications are chosen by the interpretations of SEM images.

EXPERIMENTAL DETAILS
Materials
For the electrospinning process, aqueous solutions of polyvinyl alcohol (10 - 15 wt %) (*Mw*=70000–100000 (LALLS), SIGMA, Germany) were prepared at 80^{o}C in an ultrasonic water bath (Ultrasons Medi II - J.P. SELECTA).

As a mass sensitive transducer, quartz crystals (5MHz, with Ti/Au electrode) were supplied from

MAXTEK Inc. (USA). The preliminary studies, prior to original quartz crystal usage were performed on gold (Au) coated glass surfaces (NANOVAK, Turkey) and the basic characterizations such as fourier transform infrared spectroscopy- attenuated total reflectance (FTIR-ATR), atomic force microscopy (AFM), scanning electron microscopy (SEM) spectrums and images were obtained.

Methods

In the horizontal electrospinning setup, the syringe pump is placed parallel to the floor and the collector is placed perpendicular to the floor, across the needle of syringe. Because of the resultant electrical field vector is parallel to the floor this setup is called horizontal and when the resultant electrical field vector is perpendicular to the floor it is called vertical electrospinning setup.

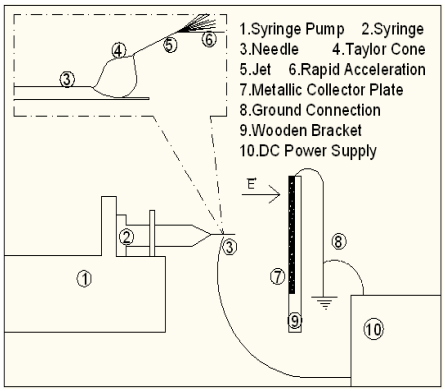

FIGURE 1. Diagram of horizontal electrospinning setup and the droplet shape in detail.

In the vertical electrospinning setup, the collector is placed on the insulating floor and the syringe pump is settled above the collector. Both in horizontal and vertical electrospinning setups, the collector is chosen as an iron plate which has a 15x15 cm^2 surface area. To start the electrospinning process at the center of the collector plate, the syringe pump is placed across its center.

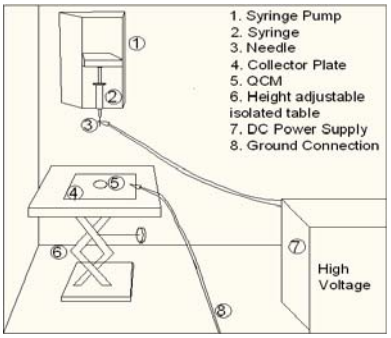

FIGURE 2. Vertical electrospinning setup.

The temperature of PVA solution is varied in the range of 25-80 °C prior to electrospinning process. Electrospinning is carried out by charging the PVA solutions at 10-25 kV with a direct current (DC) high voltage power supply (Gamma High Voltage Research Inc., USA). The fibers are collected on a grounded collector that is placed 10 cm away from the capillary tip. Flow rate is adjusted by the syringe pump around 0.5-10 ml/h. Experiments are performed under atmospheric pressure at 25°C room conditions.

After electrospun PVA nanofiber (nanoPVA) modification, total mass uptake is calculated with the frequency shifts, which are obtained by means of research quartz crystal microbalance (RQCM, MAXTEK Inc.) measurements. Modified surfaces are characterized by SEM, AFM, FTIR-ATR and contact angle measurements.

Sessile drop contact angle measurements were carried out by pipetting a 5 µl drop of distilled water onto the sample surface and using a computer microscope (Intel® Play™ Q*3™**)** to capture images of the drop. The inner contact angles between the curvature surface of water drop and the solid surface were measured.

RESULTS AND DISCUSSIONS

Since the electrical field vector is parallel to the floor in the horizontal electrospinning setup, polymer droplets are inclined to do projectile motion with increased voltage. When electrospinning begins, even if the Taylor cone consists upwards, it is observed that fibers are collected downwards of collector plate still as doing a projectile motion.

It was predicted that electrospinning could begin in the center of the collector plate in the vertical electrospinning setup. But it was observed that beginning point of the collecting fibers is randomly located on the plate. This behavior shows the chaotic case of electrospinning via bending instability. Also little defects on the needle tip change the roughness of the needle and the shape of the droplets. Therefore it is nearly impossible to determine the beginning point on the collector plate.

Due to this fact, to prepare a totally repeatable and controllable surface with the same thickness, homogeneous fiber dispersion and the exact fiber morphology instabilities should be obstructed. With these instabilities, electrospinning can't be repeated even though the same parameters are applied again.

Surface Characterization via SEM and AFM

In our preliminary studies, we modified gold surfaces by nanoPVA by employing various process parameters. The appropriate parameters for the finer fiber structures were selected for the modification on QCM surfaces.

The effect of process parameters on nanofiber morphology was investigated in detail and some SEM images are given in the following tables with the correlations and interpretations.

The Effect of Flow Rate on Nanofiber Diameter and Beaded Structure

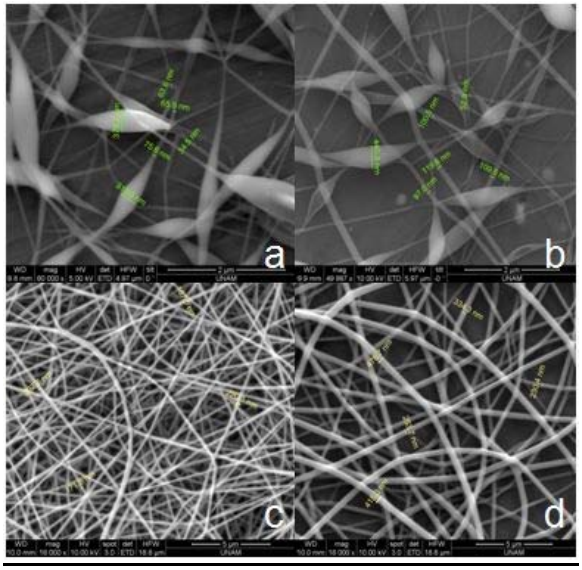

FIGURE 3. SEM images of (10 cm, 12 kV, horizontal setup) electrospun nanofibers a) flow rate of 10 ml/h, 30-60 nm fiber diameter, 300 nm bead diameter, b) flow rate of 6ml/h, 50-100 nm fiber diameter, 440 nm bead diameter, c) flow rate of 1.6 ml/h, 170-220 nm fiber diameter, non-beaded structure d) flow rate of 1.1 ml/h, 230-476 nm fiber diameter, non-beaded structure.

As can be seen from *Figure 3a* and *Figure 3b*, when the flow rate is decreased with other parameters kept constant, it is observed that there is a decrease in the bead size whereas there is an increase in nanofiber diameter. When comparing the two images, *Figure 4a - Figure 4b,* it is inferred that with the decrease in flow rate, bead size could get smaller until the non-beaded structure is obtained. Further decrease in flow rate urges the necessity of an increase in voltage in order for the electrospinning to take place. When a non-beaded structure is obtained as in *Figure 3c*, still the decrease of the flow rate increased the fiber diameter *Figure 3d*.

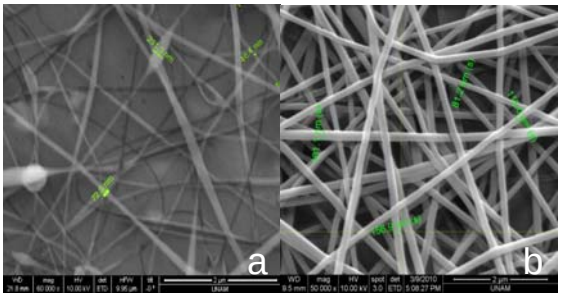

FIGURE 4. SEM images of (10 cm, 15 kV, horizontal setup) electrospun nanofibers a) flow rate of 6 ml/h , 4070 nm fiber diameter, 250 nm bead diameter b) flow rate of 1.6 ml/h, 80-150 nm fiber diameter, non-beaded structure

The Effect of Voltage on Nanofiber Diameter and Beaded Structure

When other parameters are kept constant and voltage is increased, nanofiber diameter decreases, however the bead sizes increase (*Figure 3b-Figure 4a*). The beaded structure shows that a stable Taylor cone could not be obtained. With the increase of voltage, greater amounts of charge emerge, causing the droplets and the jet to accelerate toward the collector faster, thus larger beads are formed.

It is predicted that to obtain a Taylor cone and non-beaded fiber morphology, flow rate and voltage should be changed together. If flow rate is changed voltage should be changed to a new critical voltage value. Also for a given voltage, there should be a certain flow rate to collect non-beaded nanofibers. If one of these two parameters is changed alone, beaded structure or fibers, which have larger diameters, are observed. As it is seen from *Figure 3c* and *Figure 4b*, when flow rate is increased, critical voltage value is also increased for non-beaded fiber morphology.

The Relation Between the Concentration of Solution and the Critical Voltage

Fiber diameter is increased with increased polymer concentration. Also the critical voltage value for electrospinning is increased when the concentration of the solution is increased. With the SEM images of nanofibers, which are obtained from 15 % wt PVA solution, it can be observed that in some sections, nanofibers are stuck to each other and spoiled. The reason why they are stuck is that the solvent could not fully evaporate before reaching the collector *Figure 5.*

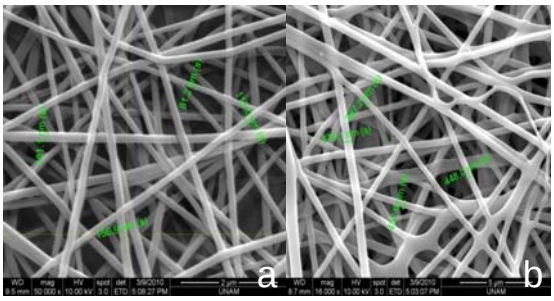

FIGURE 5. SEM images of (horizontal setup, 10 cm, flow rate of 1.6 ml/h) electrospun nanofibers a) 15 kV, 10 %wt PVA, 80-150 nm fiber diameter, 50000X b) 17 kV, 15 %wt PVA, 70-220 nm fiber diameter, 16000X.

The Relationship of Horizontal and Vertical Setups with Spinning Parameters

Gravitational force affecting the polymer is negligible with respect to the electric field forces causing the polymer to spin while electrospinning. However, gravity has an effect on the shape of the polymer droplet and the Taylor cone. This causes a difference in electrospinning parameters observed in horizontal and vertical systems *Figure 6.* In the vertical system, the shape of the droplet forming on the needle tip depends on the flatness of the needle tip. This is why the needle tip is straightened.

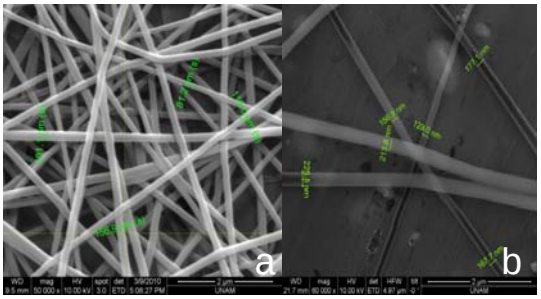

FIGURE 6. SEM image of (10 cm, 11 kV) electrospun nanofibers a) Horizontal system, Syringe with regular needle, 15 kV, Flow rate of 1.6 ml/h, 80 - 150 nm fiber diameter, 50000X b) Vertical system, Syringe with straightened needle tip, 11 kV, 4 ml/h, 70 - 220 nm fiber diameter, 60000X.

SEM results show that with the use of a 10 %wt PVA solution and a 10 cm distance, horizontal electrospinning parameters are flow rate of 1.6 ml/h and critical voltage of 15 kV, vertical electrospinning parameters are flow rate of 4 ml/h and critical voltage of 11 kV *Figure 6.* It is predicted that with the control of ambient parameters, these process parameters are suitable for finer and non-beaded fiber morphology.

The Effect of Temperature on Nanofiber Morphology

In the preliminary studies, the morphology of the fibers were found to be "beaded" for the low temperature (<40°C) solutions, but at higher (>60°C) temperatures of polymer solution, the fibers became flat. The bead formation was eliminated at low temperature polymer solutions by changing the other electrospinning process parameters. The results obtained at low temperature of polymer solutions were more predictable due to the controllable vapor pressure of solution.

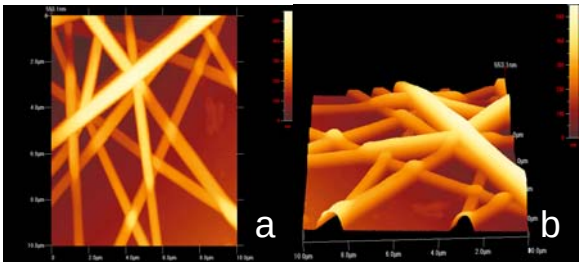

FIGURE 7. AFM images of PVA nanofiber modified gold surface, 10 cm distance between tip and collector, needle diameter of 0.80 mm, 10 %wt PVA, 80ºC, 13kV, 0.5 ml/h, a) 2D view b) 3D view.

The atomic force microscope was used to examine the morphology of nanofibers and the thickness of the nanoPVA film. The thickness of the film had a range from 0 to 560nm. It is very well known that, by increasing temperature, viscosity is decreased and the mobility of polymer molecules is increased, therefore the critical voltage required for the electrospinning is decreased by using the higher temperature of polymer solution. Regarding this fact, the AFM images of nanofiber obtained via the 80ºC polymer solution, vertical electrospinning setup parameters of 13 kV and 10 cm, flow rate of 0.5 ml/h are given in *Figure 7*.

Flat fiber morphology is observed by 3D views of AFM, *Figure 7b*. It is seen that nanofibers have 157±22 nm of height and 611±113 nm of width. By the assumption of elliptical cross-section, it is predicted that, before the wet fibers touch on the collector plate, they are approximately 154±22 nm in diameter. This indicates that, not only the beaded structure but also the shape of the nanofibers are affected by the temperature changes. It is predicted that, increasing the flight time by increasing the distance between the tip and the collector will be sufficient to overcome this defect.

Hydrophilicity of the Modified Quartz Crystal Surfaces

Water drops on the modified and unmodified quartz crystal surface were viewed to determine the contact angles of the surfaces. The measured contact angles of the unmodified quartz crystal and modified with nanoPVA are 74°±2 and 51°±5, respectively are given in *Table I.* Electrospun nanofiber modified quartz crystal surfaces represent more hydrophilic character when compared with untreated surfaces.

TABLE I. Contact angles of nanoPVA modified and unmodified QCM surfaces.

QCM Surfaces	Contact Angle (^{0})
Unmodified	74±2
Modified with nanoPVA	51±5

Chemical Characterization by FTIR-ATR

Chemical characterization of the nanoPVA coated surfaces were examined by FTIR-ATR.

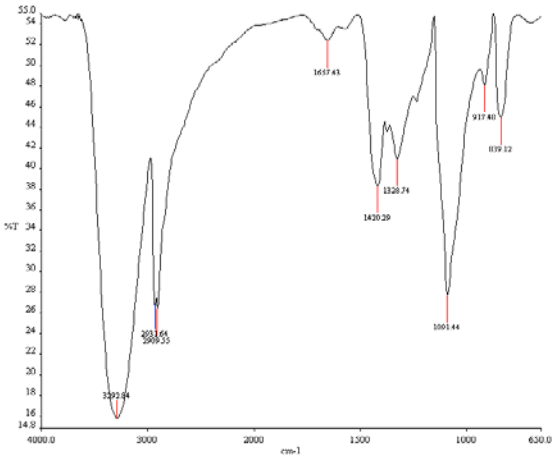

FIGURE 8. FTIR-ATR spectra of nanoPVA modified Au surface.

The FTIR-ATR spectrum presented in *Figure 8* shows the most characteristic bands of PVA and their respective assignment. The large band observed at 3292 cm^{-1} is linked to the stretching O–H from the intermolecular and intramolecular hydrogen bonds. The vibrational band observed at 2931 cm^{-1} refers to the stretching C–H from alkyl groups. All major peaks related to hydroxyl and acetate groups are observed.

Frequency Measurements

Even if all the electrospinning parameters are kept constant, the collection point of the electrospun nanofibers on the collector plate, changes. Therefore it is probably not possible to determine a constant film deposition on the quartz crystals. Hereof our results show that there is not a direct correlation between deposition time and mass deposition in the electrospinning process *Figure 9*.

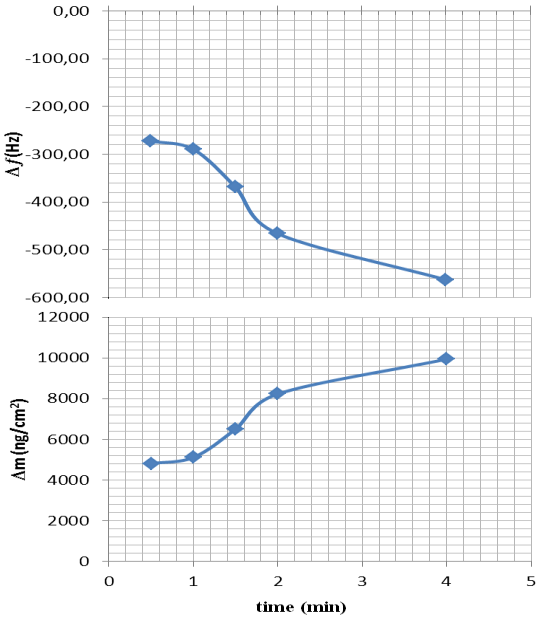

FIGURE 9. Vertical electrospinning system (15 kV- 0.5 ml/h) graphics of time versus a) frequency change (Δf-t) b) mass change (Δm-t).

CONCLUSIONS

Results of this study open a new approach to modifications on QCM surfaces via electrospun nanofibers. Use of such novel surfaces as high performance mass sensitive transducers for nanobiosensor applications are still under investigation.

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REFERENCES

[1] Karamollaoglu İ., Oktem H. A., Mutlu M., QCM-based DNA biosensor for detection of genetically modified organisms (GMOs), Biochemical Engineering Journal 44, 2009, 142–150.

[2] Mutlu S., Cokeliler D., Shard A., Goktas H., Ozansoy B., Mutlu M., Preparation and characterization of ethylenediamine and cysteamine plasma polymerized films on piezoelectric quartz crystal surfaces for a biosensor, Thin Solid Films 516, 2008, 1249–1255.

[3] Mutlu M., Biosensor in Food Processing Safety and Quality Control, Contemporary Food Engineering Series, CRS Press- Taylor Francis Group, 2011.

[4] Zhang C., Cappleman B.P., Defibaugh-Chavez M., Weinkauf D.H., Glassy polymer-sorption phenomena measured with a quartz crystal microbalance technique, J. Polym. Sci. Part B: Polym. Phys. 41, 2003, 2109–2118.

[5] Hao R., Wang D., Zhang X., Zuo G., Wei H., Yang R., Zhang Z., Cheng Z., Guo Y., Cui Z., Zhou Y., Rapid detection of Bacillus anthracis using monoclonal antibody functionalized QCM sensor, Biosensors and Bioelectronics 24, 2009, 1330-1335.

[6] Ding, B., Kim, J., Miyazaki, Y., Shiratori, S., Electrospun nanofibrous membranes coated quartz crystal microbalance as gas sensor for NH3 detection, Sens. Actuat. B-Chem, 101, 2004, 373-380.

[7] Uslu I., Daştan H., Altaş A., Yayli A., Atakol O., Aksu M. L., Preparation and Characterization of PVA/Boron Polymer Produced by an Electrospinning Technique, e-Polymers, 2007, 133.

[8] Koski A., Yim K., Shivkumar S., Effect of molecular weight on fibrous PVA produced by electrospinning, Materials Letters 58, 2004, 493– 497.

[9] Bhardwaj Nandana, Kundu Subhas C, Electrospinning: A fascinating fiber fabrication technique, Biotechnology Advances 28, 2010, 325–347.

[10] He J., Xu L., Wu Y., Liu Y., Mathematical models for continuous electrospun nanofibers and electrospun nanoporous microspheres, Polymer International 56, 2007, 1323–1329.

[11] Huang Z. M., Zhang Y. Z., Kotaki M., Ramakrishna S., A review on polymer nanofibers by electrospinning and their applications in nanocomposites, Composite Science and Tech. 63, 2003, 2223-2253.

[12] Ramakrishna S., Fujihara K., Teo W., Lim T., Ma Z., An Introduction to Electrospinning and Nanofibers,World Scientific Publishing Co. Pte. Ltd., 2005.

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