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Optimization of Electro Spinning Parameters and Cytotoxicity Evaluation of Titanium Dioxide Nanofibers to be Used as a Biomaterial

Shaimaa Shaban[1], Aziza A. Elsaeid[1], Basma Ekram[2], Areg E. Omar[1] and Bothaina Abd El-Hady[2]

[1]Physics Department (Biophysics Branch), Faculty of Science, Al-Azhar (Girls' Branch), Nasr City, Cairo, Egypt.
[2]Polymer and Pigments Department, Chemical Industries Inst. National Research Centre, Dokki, Cairo, Egypt.

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ABSTRACT

The TiO_2 nano fibers were prepared using the conventional sol gel method and the electro spinning technique. Also Polyvinyl pyrrolidone (PVP) polymer using a combination of sol-gel and electro spinning techniques. The effects of sol-gel and electro spinning on TiO_2/PVP nanofibers diameter, in addition to polyvinyl pyrrolidone (PVP) concentration, needle Tip-to-collector distance and applied voltage were evaluated. The optimum combination was determined to be a 10% PVP concentration, with the needle tip-to-collector distance 10 cm, and the applied voltage at 20 kV. The cytotoxicity of the prepared fibers was evaluated using fibroblasts and the results showed that, the fibers increased the cell viability of human normal fibroblasts (BJ1 cells), which indicates that, it can be safely used as a biomaterial.

Keywords: Polyvinyl pyrrolidone, titanium dioxide nanofibers, electro spinning, cytotoxicity

1. Introduction

Titanium dioxide (TiO_2) has attracted the interests and considerations of the researchers because of its low cost, environmental friendly and incomparable chemical stability with unusual optical and electronic properties (Tang *et al.,* 2016). It is a transition metal oxide which can be found naturally in four polymorphs (Gupta and Tripathi 2011). Titania nanofibers have a remarkably large active surface area and good pore size distribution. It has been used in many applications such as chemical sensors (Bai and Zhou 2014; Bertuna *et al.,* 2016), catalytic filters (Dai *et al.,* 2016), battery materials (Lee *et al.,* 2017), super capacitors (Da Silva *et al.,* 2019), solar cells (Mustafa *et al.,* 2019), environmental usages (Khosravi *et al.,* 2020), and as a biocompatible material (Jafari *et al.,* 2020).

TiO_2 is considered as a non-toxic substance which has been approved by the American Food and Drug Administration (FDA) to be used in food and drug products (Bonetta *et al.,* 2013) (Mccullagh *et al.,* 2007). Nanostructured TiO_2 has been broadly used in biological applications due to its good biocompatibility, low toxicity and versatile fabrication techniques. Titanium nanofibers were produced by various ways including electro spinning, hydrothermal and template methods (Altaf *et al.,* 2020). Electro spinning is known as the most versatile technique to fabricate nanofibers such as metal oxide nanofibers (Tang *et al.,* 2016). A typical electro spinning process includes a high voltage source connected to a needle and a metallic collector where the fibers are deposited (Caratão *et al.,* 2014). High voltage is applied between the metallic needle and the collector. In this way, the electric field stretches the drop that forms on the needle tip, deformed into a conical shape (Taylor cone). By increasing the electric force, the Taylor cone will overcome the surface tension and then undergoes a continuous elongation forming the electro spun fibers on the collector (Pascariu *et al.,* 2019). The produced fibers have a high surface area-to-volume ratio, the fibrous mats are highly porous and exhibit excellent mechanical properties (Khan, 2012).

Corresponding Author: Shaimaa Shaban, Physics Department (Biophysics Branch), Faculty of Science, Al-Azhar (Girls' Branch), Nasr City, Cairo, Egypt.

In general, electro spinning parameters affect nanofiber diameters. These parameters include polymer concentration, solvent used, tip to collector distance, electrical conductivity, viscosity and applied voltage (Ekram *et al.,* 2017).

Sol gel combined with electro spinning is the most versatile method to produce titanium nanofibers, it includes titanium precursor and biodegradable polymer like polyvinyl pyrrolidone (PVP) (Albetran, Dong, and Low 2015a; Nuansing *et al.,* 2006; Jae Young Park and Kim 2009; Tang *et al.,* 2016). PVP was selected as the base polymer due to, its good solubility in alcohol, acetic acid and its compatibility with some titania precursors (Ju Young Park and Lee 2010).

Many studies have been focused on the fabrication of TiO_2 nanofibers by electro spinning, but there are no sufficient studies on either the optimization of the electro spinning parameters or on the effect of the TiO_2 electro spun fibers on cytotoxicity.

The aim of the present work is optimization of the electro spinning TiO_2/PVP using a suitable solvent, demonstrating the feasibility of producing bead-free nanofibers with the use of such benign solvents. Also, the effect on the cytotoxicity was evaluated to study TiO_2 safety to be used as a biomaterial.

2. Materials and Methods

2.1. Materials

Titanium (IV) isopropoxide ($C_{12}H_{28}O_4Ti$, Mr 284.26, Fluka, Sigma-Aldrich), Polyvinyl pyrrolidone (PVP, Mw 360, 000, Sigma-Aldrich), (Acetic acid 96% and Ethanol 99.8%, Edwic, Egypt) were used as purchased without any purification.

2.2. Preparation of the electrospinning solution

Polyvinylpyrrolidone (PVP) solutions were prepared by dissolving a calculated amount of PVP in ethanol. In parallel a calculated amount of Titanium (IV) isopropoxide was dissolvent in acetic acid and ethanol 1:1. Both solutions were left under magnetic stirring up to 3h for complete dissolution.

2.3. Electrospinning of TiO_2/PVP nanofibers

The electro spinning equipment was operated in a vertical mode and consisted of a syringe pump, rotating wheel collector and high voltage power supply. The polymer solutions were pumped from 0.5ml syringe in to a 10 cm long needle with the constant flow rate of 1 ml/h. The voltage was adjusted using a Glassman High Voltage, INC, high bridge, NJ08829 source (voltage range 0-20 kV). The positive voltage was connected to stainless steel needle and the negative one to the rotating collector. Electro spinning was carried out at room temperature.

The effects of most important process parameters, such as solvent ratio, PVP concentration, voltage difference, and finally tip to collector distance on the TiO_2 electro spun.

2.4. Electro-spinning parameters study:

2.4.1. Different PVP concentrations study

In order to study the optimum PVP concentration, all the other electro spinning parameters such as applied voltage and tip to collector distance were kept constant. The concentration of PVP was variable set at 8, 10 and 12 %.

2.4.2. Applied voltage study

To study the optimum applied voltage, all the other electro spinning parameters, such as concentration and tip to collector distance were kept constant. The voltage was set at three different values (10, 15 and 20 kV).

2.4.3. Tip to collector distance study

To study the optimum tip to collector distance, all the other parameters, such as concentration and applied voltage were kept constant. The distance was set at three different values (8, 10 and 12 cm).

2.5. Characterizations

2.5.1. Fiber morphology

Fiber morphology was studied under field emission scanning electron microscope (FESEM), Jeol JXA 840. The diameter of the electro spun fibers was determined by measuring about 50 individual fibers with the image analysis software (image J. 1.429 software (NIH, Betheseda, Maryland, USA).

2.5.2. Rheological measurements

Viscosity measurement was examined at 22˚C as a function of time on the polymer solution system using Brookfield viscometer (model DV-III ultra). The polymeric solution was put in the built-in stainless steel container attached with a temperature controller.
Measurements were done using S21 spindle at 50 RPM under uniform temperature.

2.5.3. Conductivity measurements

The change in conductivity by varying the polymer concentration was done at 25˚C three times and the average value was taken, using conductivity meter (HC 3010, Trans-instruments).

2.6. Cell culture

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan (Mosmann,1983).
Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Human normal fibroblasts cell line (BJ1) was used. The cells were suspended in DMEM-F12 medium with1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO_2.

Cells were batch cultured for 5 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated alone (negative control). After 48 h of incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) was added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC.

The absorbance was measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program (Bassyouni *et al.,* 2014).

2.7. Statistical analysis

The values reported are the average ± standard deviations. Statistical analysis was performed with the one-way ANOVA test, attached by excel software Values of $p< 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. Different PVP concentrations study

3.1.1. Rheological and conductivity measurements

The viscosity of different PVP concentrations in ethanol results were presented in Fig. 1. As noticed from the figure, that the viscosity increased as the concentration of the PVP increased and there was no noticeable change in stability with time (Watthanaarun, Pavarajarn, and Supaphol 2005).
In Table (1), it was recognized that by increasing the concentrations of PVP, the conductivity values were increased due to the increase of the PVP content, where the polymer solution conductivity is mainly dependent on the intrinsic polymer properties. An increase in electric conductivity tends to decrease the fiber diameter. However, above a critical point it may prevent the formation of Talyor cone and fiber formation (Soares and Borges 2021).

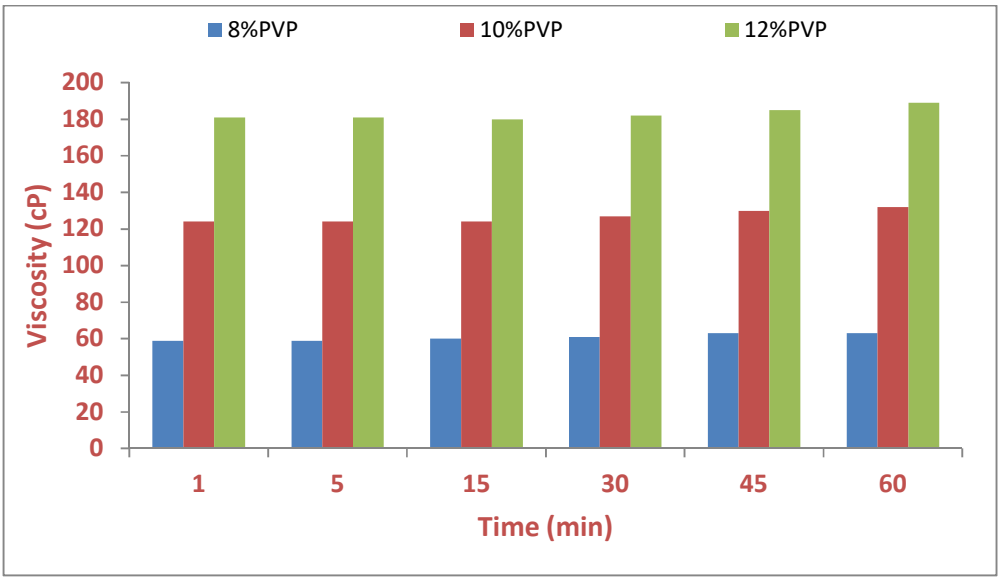

Fig. 1: Viscosity with time for 8, 10 & 12% PVP concentrations

Table 1: Variations in conductivity versus different polymer concentrations

	PVP concentrations (wt%)		
Parameter	8	10	12
Conductivity (µs)	4.0±0.2	4.71±0.4	4.83±0.3

TiO_2/PVP fibers were produced successfully by unscathed mixture as acetic acid/ethanol for TiO_2 and ethanol for PVP, mixing of both solutions resulting of formation of emulsion, which was used for further electrospun.

3.1.2. Fiber morphology

The scanning electron microscopy images of the produced fibers of 8, 10 and 12% PVP were represented in Fig.2 and Table 2. They show the variations of morphology and distribution of the diameters for the obtained fibers according to the change in concentration of PVP. At 8% PVP, the mean fiber diameter was found to be 259.1 nm. This may be due to the low polymer concentration produce low viscosity and high surface tension of the solution and also of the instability of the jet under electric field (Ju Young Park and Lee 2010). At 10% PVP, the mean fiber diameter was found to be 226.3 nm; the fibers became smoother, since uniform beadles Nano fibers can be achieved at a specific solution concentration. As the concentration gets closer to this critical value beads, the shape was changed from a round to an ellipse to smooth fibers (Caglayan and Basal 2020). Thus viscosity increases due to higher polymer concentration which leads to an increase in chain entanglement among the polymer chains. These entanglements aid in overcoming surface tension, thus producing uniform beadles fibers (Soares and Borges 2021). The mean fiber diameter at 12% PVP was found to be 256.5 nm. Hence, increasing PVP concentration led to an increase in the fibers diameters with broader distribution. Thus increasing in the fiber diameters with increasing PVP concentration is a result of the increase in the viscosity of the spinning solutions. However, the increase in the viscosity of the spinning solutions could lead to non-uniform ejection of the jet when the viscosity becomes too great (Watthanaarun, Pavarajarn, and Supaphol 2005).

As 10% PVP concentration leads to better distribution in diameters among formed fibers and adequate fiber diameter, this is why that value was selected for the present study.

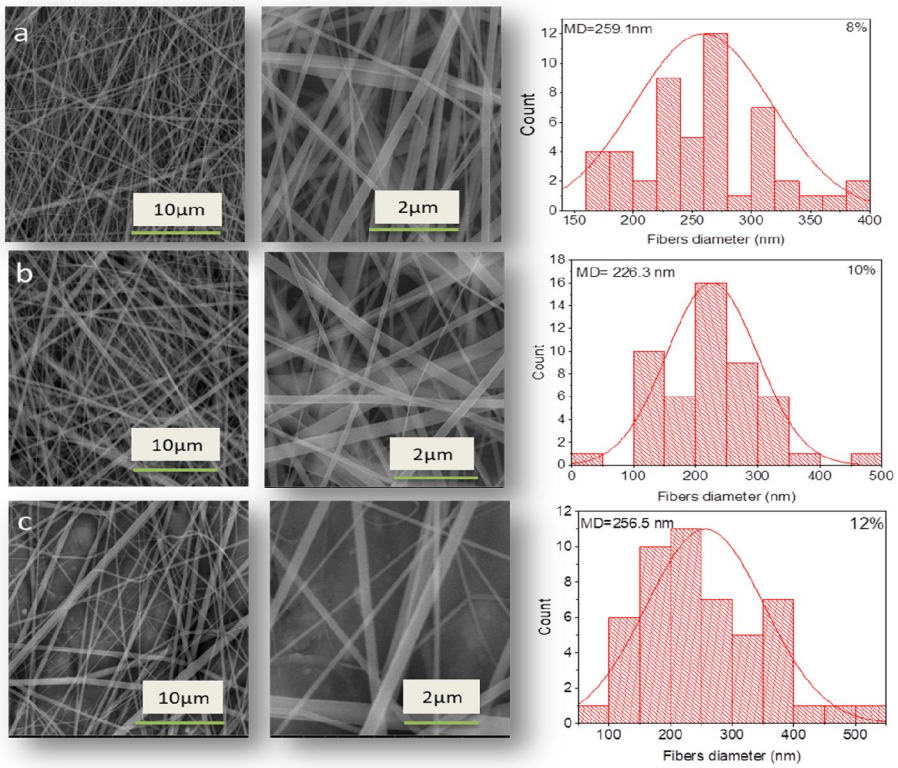

Fig. 2: SEM images of the TiO2 with PVP fibers produced for a) 8%, b) 10% and c) 12% PVP concentration at two magnifications and their distribution charts.

Table 2: Different concentrations of PVP solutions used for fabrication with obtained fiber morphology and average fiber diameters.

Different concentrations of PVP (wt. %)	Fiber morphology obtained	Average fiber diameters (nm)
8	Bead-free fibers with larger diameter	259.1±57.9
10	Bead-free uniform fiber	226.3±73.5
12	Bead-free non uniform fibers	256.5±95.6

3.2. Fiber morphology of applying voltage study

The scanning electron microscopy images of the produced fibers using the applied voltage of 10, 15, 20 kV were represented in Fig.3 and Table 3. At 10 kV, the mean fiber diameter was found to be 264.4 nm and the reproducibility of the fibers was the lowest. Therefore at lower voltage, the instability resulting from the electric field was small and the charge stress contributing to the elongation of the fibers was small (Kim *et al.,* 2021). By increasing the applied voltage (i.e., strong electrical repulsive forces), the fiber diameter reduction occurs resulting in highly stretched and elongated fibers (Albetran, Dong, and Low 2015b). At 15 kV, the fibers seemed to be tangled and with a higher reproducibility even though with a higher fiber 257.7 nm. The mean fiber diameter for 20 kV was found to be 196.5 nm. However, by increasing the applied voltage the reproducibility increases. Therefore increasing the applied voltage not only decreased the average nanofibers diameters but also changed the morphology of the beaded nanofibers to smooth ones with lowest average nanofibers diameters (196.5±67.5 nm) (Mirmohammad Sadeghi *et al.,* 2018). Since fibers electro spun at 20 kV own better distribution, good homogeneity and lowest fibers diameter; this value was selected for further investigation.

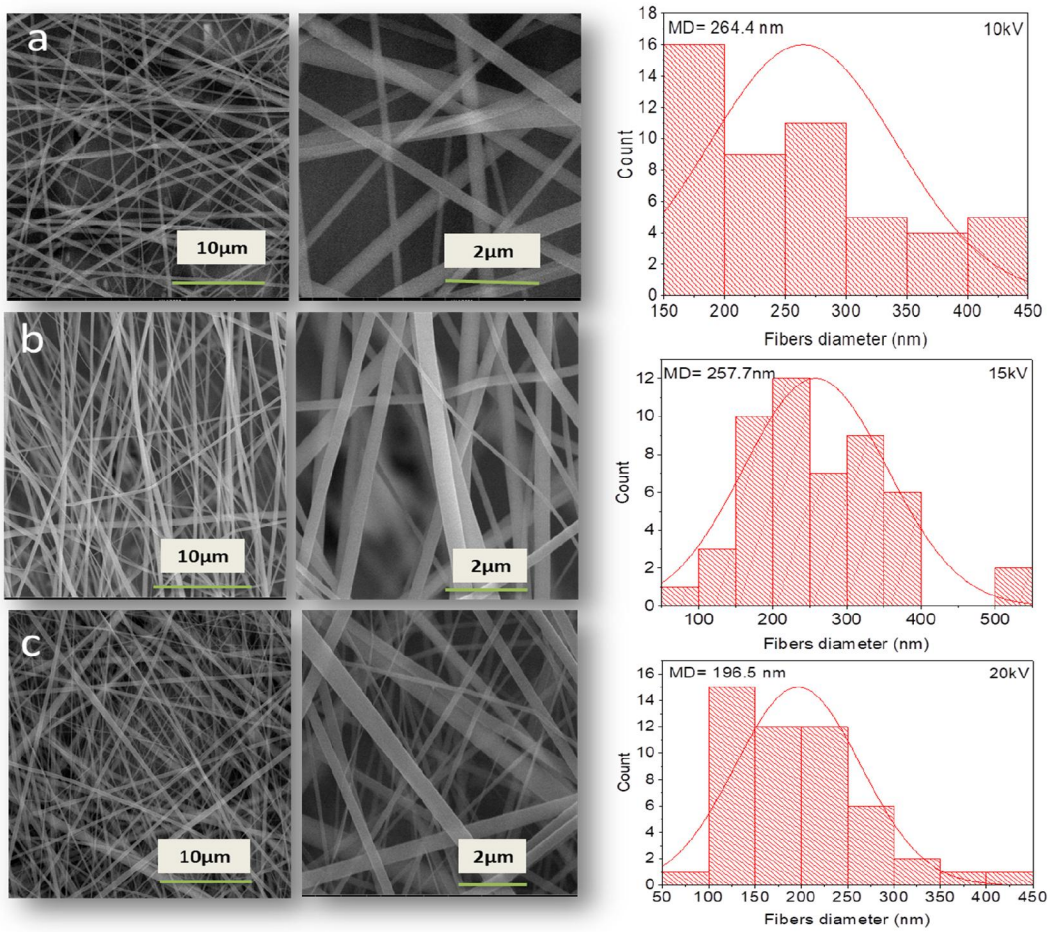

Fig. 3: SEM images of the electrospun TiO_2 fibers produced from a) 10 kV, b) 15 kV and c) 20 kV applied voltage at two different magnifications and distribution charts.

Table 3: Different applied voltage on TiO_2/PVP solutions used for fabrication with obtained fiber morphology and average fiber diameters.

Applied voltage (kV)	Fiber morphology obtained	Average fiber diameters (nm)
10	Bead-free fiber with larger diameter	264.4±77.09
15	Bead-free non uniform fiber	257.7± 96.63
20	Bead-free uniform fiber	196.5±67.5

3.3. Fiber morphology of tip to collector distance study

The scanning electron microscopy images of the produced fibers at tip to collector distances 8, 10 and 12 cm were presented in Fig.4 and Table 4. Tip to collector distance plays a direct role on jet flight time and electric field strength. The mean fiber diameter for 8cm was found to be 324.8 nm, since the flight time was shortened, also the solvent evaporation time, and the electric field strength was increased, which results in the increase of bead formation. At 10 cm, the mean fiber diameter was found to be 230.5 nm in which more time was provided for fluid jet to stretch fully and for solvent to evaporate completely and thus producing thinner fibers. At 12 cm, the mean fiber diameter was found to be 334.0 nm, since the gap was increased; so the collected fibers were partially dried before reaching the collector and fully stretched, and therefore fiber diameter was reduced; however the fibers reproducibility decreased and this is similar to what was mentioned previously (Soares and Borges 2021) . These results about the effect of spinning distance on the fibers are harmonized with the results explained by (Albetran, Dong, and Low 2015b). The optimum tip to collector distance was found to be 10 cm which leads to suitable distribution, homogeneity and fiber diameter. As a conclusion, the optimum

combination was shown to be for 10% PVP concentration, with the needle tip-to-collector distance at 10 cm, and the applied voltage at 20 kV.

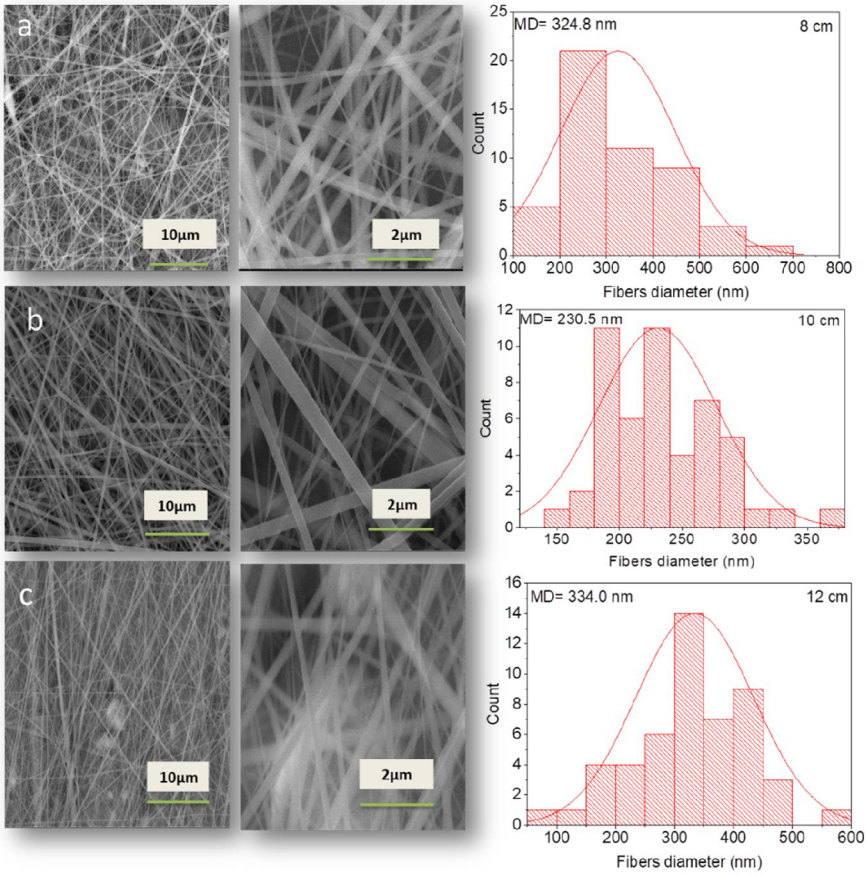

Fig. 4: SEM images of the electrospun TiO_2 with PVP fibers produced from a) 8 cm, b) 10 cm and c) 12 cm tip to collector distances at two different magnifications and their distribution charts, respectively.

Table 4: Different tip-to-collector distances of TiO_2 /PVP solutions used for the fabrication with the obtained fiber morphology and average fiber diameters.

Tip-to-collector distance(cm)	Fiber morphology obtained	Average fiber diameters (nm)
8	Fibers with lesser amount of beads	324.8±122.5
10	Bead-free uniform fibers	230.5±47.1
12	Bead-free fiber with larger diameter	334.0±99.5

3.2. Cell Viability

The cell viability after 48 h of TiO_2 nanofibers compared to the positive control was shown in Figure 5. It was shown that the TiO_2 nanofibers as prepared have positive impact on the cell growth, it enhanced the cell growth by 56% in comparison to the control. This is due to its increased conductivity which favors cells growth and propagation.

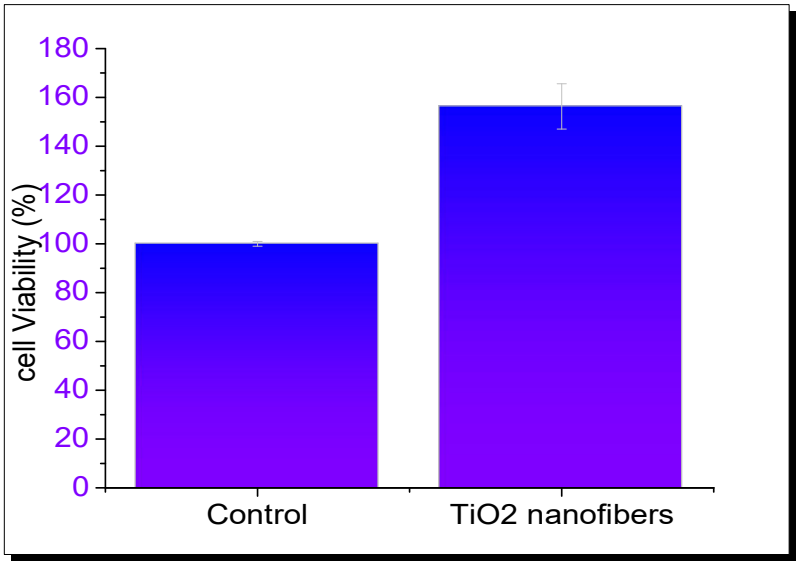

Fig. 5: Cell viability of control cells and TiO_2 nanofibers with cells after 48 h.

Conclusions

TiO_2 Nano fibers were prepared via the electro spinning technique. The optimum parameters for preparing the Nano fibers were examined and it was found that it is by 10% PVP concentration, with the needle tip-to-collector distance at 10 cm, and the applied voltage at 20kV. The prepared fibers were evaluated by fibroblasts viability *in vitro* and it was shown that it enhanced the cell growth by 56% in comparison with the control. From the previous results, it can mentioned that prepared TiO_2 Nano fibers are recommended for further tissue engineering studies.

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