

## Silk Fibroin Nano-Coated Textured Silk Yarn by Electrospinning Method for Tendon and Ligament Scaffold Application

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**Abstract.** In recent years, the use of combined nanofibers with textile structures for various applications such as tissue engineering has been highly regarded. Among the different methods, electrospinning mechanism is more important to produce polymeric nanofibers in extensive diameters that has been used to fabricate silk fibroin nanofibers in this research. On the basis of the statistical analysis and analytic hierarchy process optimization method, the optimum electrospinning parameters to produce good morphology of nanofibers and the best conditions of texturing process to fabricate suitable structure of textured silk yarn have been obtained, respectively. The following step to produce nano-coated textured yarn was defined as a nano-coating process on the surface of textured silk yarn. Finally, the morphological and mechanical properties of these samples including no-textured silk yarn, textured silk yarn and nano-coated textured silk yarn analyzed and compared. Based on the finding of this study, the nano-coated textured silk yarn was found to be a promising construct for engineered scaffolds in tendon and ligament tissue engineering.

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## Introduction

The importance of designing the suitable scaffold is one of the significant aspects in tendon and ligament tissue engineering [1]. During the healing process, there are a lot of considerations that should be taken into account when engineered tendon or ligament wants to be designed or fabricated [2]. A successful tissue engineered scaffold must be biocompatible, display similar mechanical properties that are similar to or greater than the regenerated native tissue and degrade at a fitting rate that does not cause rupture or stress shielding of the new tissue [3]. Among effective parameters, the role of mechanical properties of scaffold is being increasingly recognized to provide temporary mechanical support, to maintain space for matrix formation following cell growth and rapidly to restore tissue biomechanical function [4]. Recently, a lot of different scaffolds made of textile structures including nonwovens, woven and especially knitted fabrics have been introduced in tissue engineering because of their mechanical properties similar to body tissues [5].

The major aim of this study is to combine two previously introduced tendon and ligament scaffold design methods, fiber texturing and nanofiber coating. The textured silk scaffolds are constructed from silk fibers at the best texturing process conditions that were defined by Multiple Criteria Decision Making (MCDM) method optimization to achieve suitable mechanical properties close to tendon and ligament tissues in case anterior cruciate ligament (ACL) tissue. Also, Sahoo *et al.* developed a hybrid nano/micro scaffold system made of a silk based knitted scaffold coated with electrospun PLGA nanofibers that expected to have biomechanical and degradation properties suited for ligament and tendon tissue engineering [6]. In this study, it was hypothesized that combining textured silk yarn with silk fibroin coating in one scaffold would cause better morphological and mechanical properties when compared to textured silk yarn and silk yarn without both coating and texturing process according to mechanical properties and surface roughness analysis [21].

Air-jet texturing process is an attractive method to produce spun yarn like appearance permanently by modifying the uniform arrangement of the continuous multi-filament yarns. The basis of this method is that yarn is overfed into the compressed air-jet stream, so that loops are forced

out of the yarn [7]. On the other hand, due to texturing process, the fibers curled back on themselves creating a coiled structure and increasing the volume of yarn within a given length of unloaded yarn. In addition, texturing resulted in an increase in the stiffness yarn from that of non-textured yarns due to the compromised mechanical integrity of the yarn. That is why textured silk yarn is a beneficial option to be chosen as a scaffold in tissue engineering according to Altman's research [8]. In addition to acceptable mechanical properties that can be improved by texturing process, the surface topography is a crucial factor for cell survival in synthetic extra cellular matrix (ECM) which affects cell behavior including cell attachment, cell proliferation, as well as cell differentiation [9]. So, surface roughness is one of the most significant parameters to consider by researchers that can be progressed by coating process.

Coating a functional layer to add certain properties such as stability, abrasion or chemical resistance have been extensively used to functionalize filaments in the textile industry for instance, coated filaments were woven into fabrics instead of coating the already woven textiles is more attractive. Coating techniques such as drop coating, melt coating, chemical vapour deposition coating and dip-coating have been used to produce applicable coatings on filaments, but the electrospinning coating offers a flexible technique enabling coating of nanofibres onto textile fabrics in various applications like implant stents, filters, protective cloths and scaffolds [10]. According to Zhou *et al.* investigations, by choosing suitable biomaterials, nano-coated yarns comprised of coated nanofibers on to the surface of twisted yarns have a good potential to develop tendon and ligament scaffolds [11].

To fabricate coated nanofibers, electrospinning is a well-established process capable by electrically charging a suspended droplet of polymer solution or melt [12]. In order to form nano-scale fibers with this technique, electrospinning is to use an electric field to draw polymer solution from a needle to a collector and high voltages are used to generate sufficient surface charge to overcome the surface tension in a pendant drop of the polymer fluid [13]. These kinds of nanofibers

are more appropriate for various applications due to useful properties such as high specific surface area and high porosity [14].

Silk as a major material that have been used in this work is produced by a variety of insects including silkworm that is a typical fibrous protein comprised of two types of proteins, fibroin and sericin. Sericin layers should be removed by an especial way as a degumming process when fibroin is more important protein that forms the filaments of silk and can be regenerated in various forms depending on application, such as gels, powders, fibers, or membranes. Recently, silk fibroin is one of the attractive candidate materials for biomedical applications and has been investigated by researchers [15]. The main reasons to use silk fibroin in biomedical application are distinctive biological properties including good biocompatibility, slow biodegradability and perfect mechanical properties [16].

According to previous mention, we expect to improve the properties of a tendon and ligament scaffold by using both texturing and coating method comprised of coated silk fibroin nanofibers onto the surface of textured silk yarn in best structure according to MCDM optimization. In comparison, nano-coated textured silk yarn were constructed and compared to textured silk yarn without coating process and no-textured silk yarns without texturing and coating process. Mechanical and morphological behavior of yarns as scaffolds was analyzed by mechanical tester and SEM images and therefore surface characteristics have been evaluated by digital signal processing.

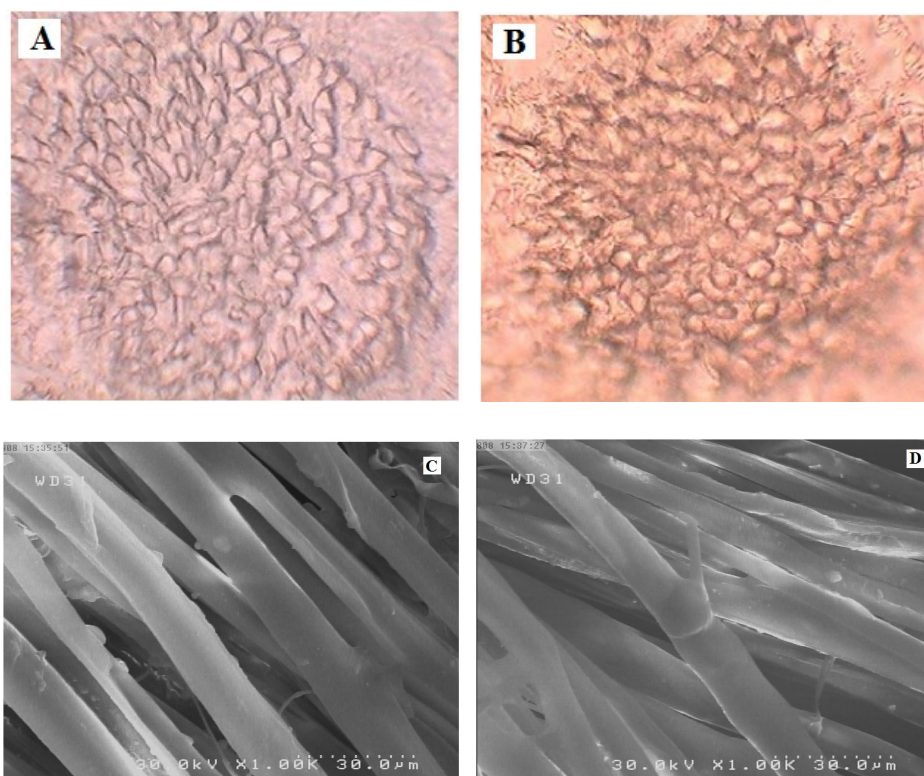
## Experimental

**Preparation of Textured Silk Yarn.** Silk that was used in this research was composed of 4-twisted multifilament, each multifilament comprised of 64 monofilaments by 300 twist per meter. Thereafter, a degumming process was necessary to remove sericin layer and to increase biocompatibility of silk yarns. A degumming solution of 0.25%  $\text{Na}_2\text{CO}_3$  was prepared and raw silk yarns were immersed in solution with temperature between 95 and 98 °C for 30 min. The process

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was repeated in fresh solution for 60 min until removing the majority of sericin. The yarn account of silk yarn was measured 204.4 Denier after degumming process.

The following process was defined air-jet texturing to convert common silk yarns to textured silk yarns. Adjusting the variable parameters of texturing process that can affect final properties like the speed of process and air pressure are important because of their significant influence on the architectural characteristic in final structure. By using MCDM approach and Analytic Hierarchy Process (AHP) optimization method, the optimized parameters of texturing process were obtained 80 rpm and 40 Psi for speed and air pressure, respectively. The AHP method is one of the well-known methods of multiple criteria decision making method that is based on decomposing a complex MCDM problem into a system of hierarchies [17]. In this research, the ranges of speed and air pressure in texturing process as variable parameters were defined according to texturing machine limitations. Then, 21 samples in different situations of speed and air pressure designed and produced, the former adjusted from 20 to 80 rpm and the latter changed from 20 to 40 Psi. Finally, textured silk yarns as an initial material in this research were prepared according to obtained conditions (air pressure: 40 Psi and speed: 80 rpm) as a result of AHP optimization method. The images of cross section and longitude view of textured silk yarn and no-textured silk yarn that have been observed using microtome method, optical microscope and FE-SEM have been illustrated in Fig. 1.

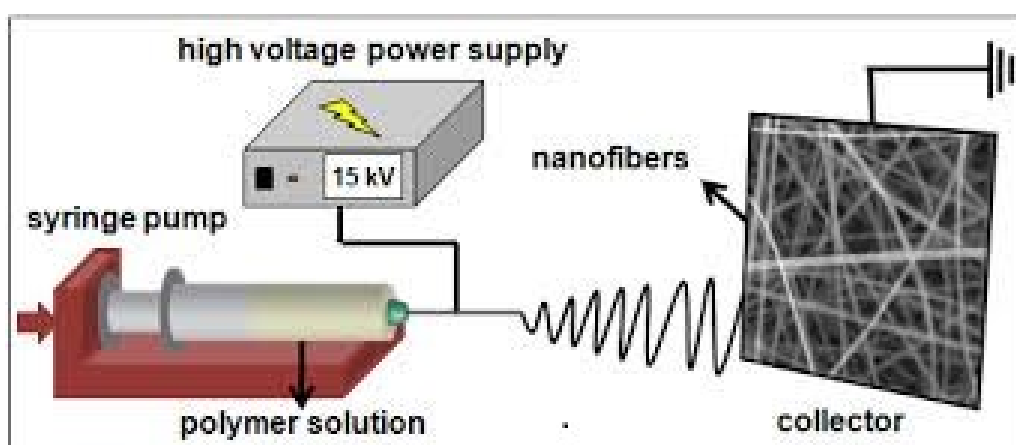


**Fig. 1.** The images of cross section of A) no-textured silk yarn, B) textured silk yarn by optical microscope (OM) and longitude view of C) no-textured silk yarn and D) textured silk yarn by FE-SEM.

**Preparation of Regenerated Silk Fibroin Solution.** To improve the biocompatibility properties of silk, sericin layer of silk must be removed and fibroin extracted before using in engineered scaffolds and fabricating silk fibroin nanofibers. Removing sericin and extracting silk fibroin has several steps including crushing, cleaning and boiling in the Polymat machine for 1 h at 120 °C when L: R was defined 1:30. In the next step to take out fibroin, mixed materials of previous step was filtered and washed for several times and then dried. Therefore, the triple solution made of calcium chloride / ethanol / water with molar ratio (8:2:1) used for 7 h at 70-80 °C to dissolve completely. The achieved solution was purified by dialysis cellulose membrane of solution and extract silk fibroin sponge [22].

**Preparation of Silk Fibroin Nanofibers and Fabrication of Nano-Coated Silk Textured Yarn.**

Obtained silk fibroin sponge of previous step was set under an electric heater placed as a solution of 15% concentration and 98% formic acid at 50 °C for 3 h. In order to produce nano-coated textured yarn, stretched textured silk yarn was fixed in front of spinneret, very close to collector via horizontal electrospinning apparatus. Time coating as a variable parameter that can effect on the final properties of nano-coated textured yarn was changed for 5, 10 and 15 minutes. In Fig. 2, a schematic view of horizontal electrospinning has been shown.



**Fig. 2.** Schematic view of horizontal electrospinning.

In order to find the best conditions to fabricate silk fibroin nanofibers via electrospinning, different values of variable parameters including voltage, tip to collector distance, concentration and feed rate were altered. The details of different conditions are summarized in Table 1.

**Table 1.** Conditions to produce silk fibroin nanofibers by altering variable parameters.

Sample number	Feed rate (ml/h)	Concentration (w/t) %	Tip to collector distance (cm)	Voltage (kv)
Sample 1	1.5	6.5	15	14
Sample 2	1.5	15	15	15
Sample 3	1.5	17	15	15
Sample 4	1.5	17	15	14

**OM, SEM and FE-SEM Analysis.** Scanning electron microscopy (SEM) analyses were performed to study the morphology of silk fibroin nanofiber, the structure of textured silk yarn and the effect of silk fibroin nano-coating on the surface of silk textured yarn. In addition, SEM images were used to surface roughness analysis by digital signal processing. The type of SEM that used was a Phenom G2 proX model. Also, optical microscope Flexicam-sairan-IFC-100 and FE-SEM Hitachi-s-4160 model used to analyze the image of cross section and longitude view.

**Nanofibers Diameter Analysis.** The SEM images of silk fibroin nanofiber samples were analyzed by choosing 150 random fibers in order to calculate the average diameter and other aspects related to nanofiber diameter. SPSS 20 software was used and statistical analyses were done to find the best sample.

**Mechanical Properties Analysis.** The tensile behavior of constructs including no-textured silk yarn, textured silk yarn and nano-coated textured silk yarn were characterized using a Zwick Universal Testing Machine(1446-60, Germany) at a rate of 200 mm/min and gauge length 50 mm. The test of each sample was repeated 5 times.

**Surface Roughness Analysis.** In the present study, a system based on digital signal analysis was used to determine the surface roughness of triple yarn scaffolds including no-textured silk yarn, textured silk yarn and nano-coated textured silk yarn according to previous researches [18]. In this method, surface profile of SEM image of the mentioned yarns was converted into a 2-dimensional signal and surface properties were measured in terms of image intensity differences of the surface microscopic image. Surfaces can be analyzed by roughness indexes including below [19, 20]:



1-Roughness average ( $S_a$ ): is a dispersion parameter obtained as the mean of the absolute values of the surface departure above and below the mean line that is calculated according to Eq. 2:

$$\bar{Z} = \frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} Z(x_k, y_l) \quad (1)$$

$$S_a = \frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} |Z(x_k, y_l) - \bar{Z}| \quad (2)$$

Where,  $Z_{kl}$  is the position of pixel  $k$  and  $l$  in SEM image with  $M*N$  dimensions. 2- Root mean square roughness ( $S_q$ ), is one of the dispersion parameters for characterizing the surface roughness and can be gained by squaring each height value in the dataset, then taking the square root of the mean according to Eq. 3:

$$S_q = \sqrt{\frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} [Z(x_k, y_l) - \bar{Z}]^2} \quad (3)$$

3- Surface skewness ( $S_{sk}$ ), is an asymmetry value of the surface deviation about the mean line. For a symmetric shape of surface height distribution like Gaussian surface, the skewness is zero. Negative skew shows a predominance of valleys, while positive skew is a surface with peaks. This parameter can be effective to describe the shape of the topography distribution according to Eq. 4:

$$S_{sk} = \frac{1}{MNS_q^3} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} [Z(x_k, y_l) - \bar{Z}]^3 \quad (4)$$

4- Surface kurtosis ( $S_{ku}$ ), is a value of the peaks or sharpness of the surface height distribution and its spread on the surface. A Gaussian surface has a surface kurtosis value equal to 3. A centrally distributed surface has a kurtosis value more than 3 while the kurtosis of a well spread distribution is less than 3 that can be calculated by Eq. 5:

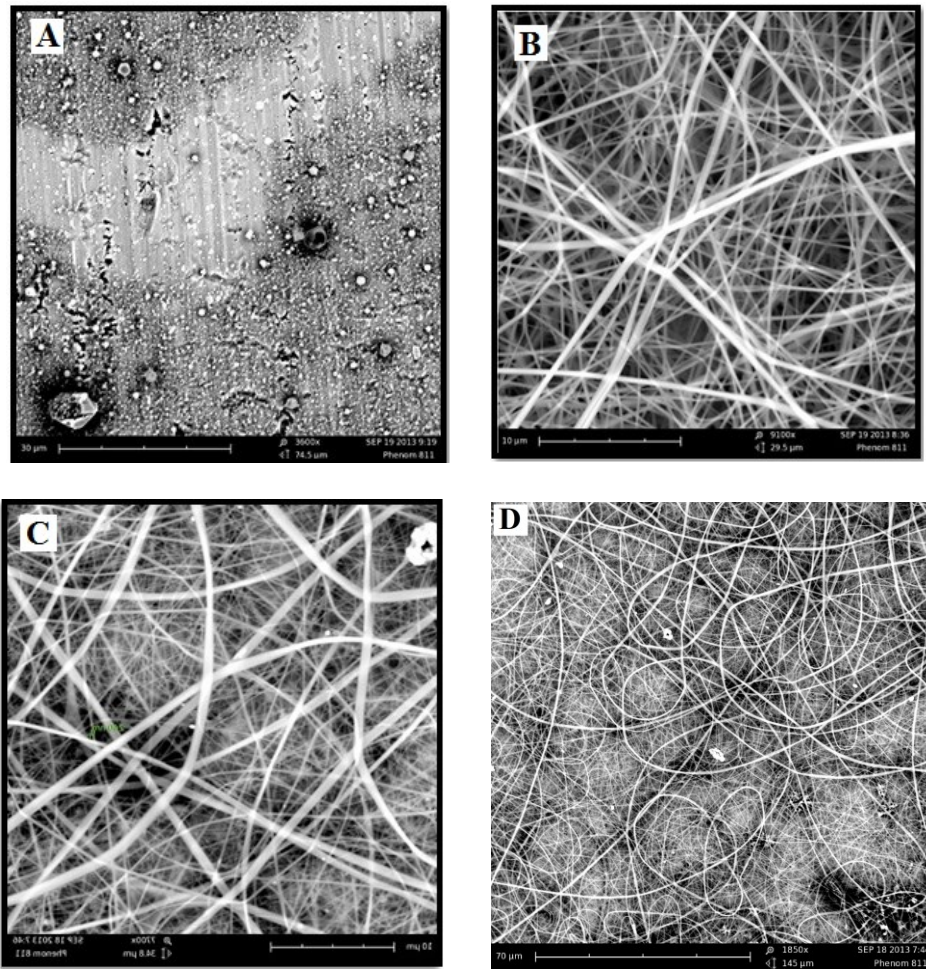
$$S_{ku} = \frac{1}{MNS_q^4} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} [Z(x_k, y_l) - \bar{Z}]^4 \quad (5)$$

Roughness indexes were calculated based on available equations by MATLAB software R2011A and each measurement was made twice for two separate samples cut for each sample, so finally the six resulting values were averaged.

## Result and Discussion

**Morphology of Silk Fibrion Nanofibers.** By using electrospinning technique under different experimental conditions including different conditions of voltage, tip to collector distance, concentration and feed rate, silk fibroin nanofibers were prepared and examined. SEM Images taken from various conditions according to Table 1 are shown in Fig. 3.

According to images of Fig. 3, at low concentrations for example the first sample with 6.5% concentration, there are no nanofibers virtually and to fabricate the uniform nanofibers it is necessary to use concentration more than 10%. The morphology of silk fibroin nanofibers in fourth sample is more uniform without damage visually and can be the best specimen. To recognize the best sample, the detail of statistical analysis for all samples has been mentioned in Table 2. According to One sample Kolmogorov-Smirnov test in SPSS software and related P-value, it is possible to prove that all of data for fiber diameter in 95% significant level are normal and there is a meaningful difference within data in each group.



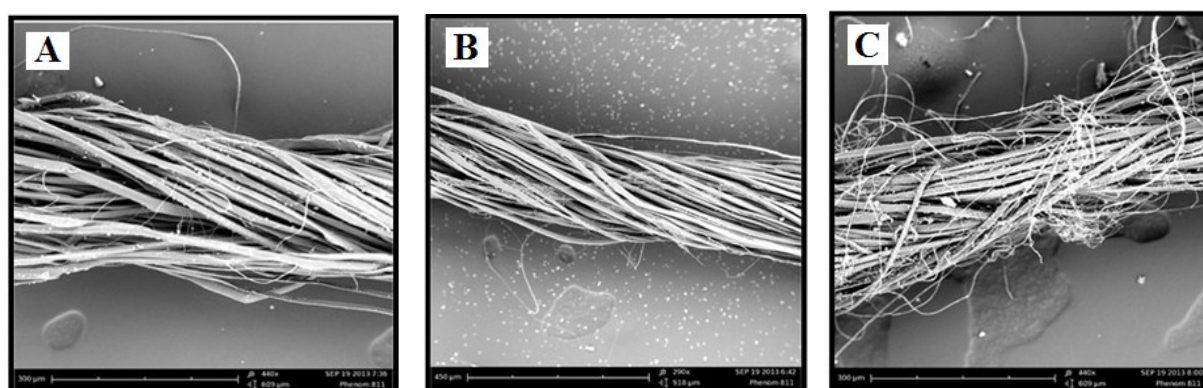
**Fig. 3.** SEM images of silk fibroin nanofibers of a) sample 1, b) sample 2, c) sample 3, d) sample 4 according to Table 1.

**Table 2.** Statistical quantities of nanofiber diameter for all silk fibroin nanofibers samples.

Sample number	Average (nm)	Median (nm)	Max (nm)	Min (nm)	Standard Deviation
Sample 1	Not spinning-able				
Sample 2	263.2	245.13	563	151	76.416
Sample 3	346.2	334.4	960	180	99.618
Sample 4	257.26	242.33	503	108	83.802

After considering the results according to Table 2, the optimum conditions were chosen. It seems the fourth sample is better than others. It is because of the value of average diameter, median, maximum and minimum of nanofibers while the lowest values are the fourth sample with an acceptable SD in comparison to others.

**Nano-Coated Silk Textured Yarn.** To design an ideal construct to use in tendon and ligament tissue engineering application, coated hybrid yarns comprised of micro and nano fibers is a new approach in recent investigations. The aim of this study is to fabricate a nano-coated textured silk yarn by electrospinning coating of silk fibroin on the surface of silk textured yarn in optimum conditions. Therefore the suitable electrospinning parameters have been chosen according to the 4<sup>th</sup> sample conditions. In order to fabricate the best construct of nano-coated yarn, different coating time including 5, 10, 15 minutes were checked. In Fig. 4, SEM micrographs of nano-coated textured silk yarn in three different coating times via electrospinning were demonstrated. According to SEM images, it is clear the more coating time has better morphology but after 15 min the structure was enough well coated, visually. Therefore, 15 minutes coating time was chosen to produce nano-coated textured silk yarn.



**Fig. 4.** SEM micrographs of nano-coated textured yarns via electrospinning for 5, 10, and 15 minutes A, B, C, respectively. (Experimental conditions: 15.0 kV applied voltage, 15.0 cm tip to collector distance, 1.5 mL h<sup>-1</sup> flow rate and 12.0 cm coating distance).

**Mechanical Properties Behavior.** Maximum load and elongation at maximum load as mechanical properties of triple samples including no-textured silk yarn, textured silk yarn and nano-coated textured silk yarn are summarized in Table 3. The maximum load of nano-coated textured silk yarn sample is less than others but according to statistical analysis there is no significant difference among values in comparison to no-textured silk yarn and textured silk yarn in 95% significant level. There is

the same result for elongation at maximum load statistically. The maximum load of textured silk yarn is more than no-textured silk yarn and that is why this type of yarn was chosen to fabricate nano-coated textured silk yarn. In addition, the magnitude of reduction or fail mechanical properties in coated sample is suitable to use in final application in comparison by both other samples.

**Table 3.** Mechanical properties of triple samples including no-textured silk yarn, textured silk yarn and nano-coated textured silk yarn.

Yarn type	F_max (cN)	L at F_max (mm)
No-textured silk yarn	807.13	36.67
Textured silk yarn	839.7	29.92
Nano-coated textured silk yarn	785.205	31.45

**Surface Roughness Behavior.** Table 4, shows the surface roughness indexes including roughness average ( $S_a$ ), root mean square roughness ( $S_q$ ), surface skewness ( $S_{sk}$ ) and surface kurtosis ( $S_{ku}$ ) for no-textured silk yarn, textured silk yarn and nano-coated textured silk yarn.

**Table 4.** Surface roughness indexes of triple samples including no-textured silk yarn, textured silk yarn and nano-coated textured silk yarn.

Yarn type	$S_a$	$S_q$	$S_{sk}$	$S_{ku}$
No-textured silk yarn	0.4890	0.5578	0.0233	1.9260
Textured silk yarn	0.5471	0.6204	0.2388	1.8226
Nano-coated textured silk yarn	0.4466	0.5534	0.3875	2.5446

According to surface roughness indexes in Table 4, the values of  $S_a$  and  $S_q$  for nano-coated textured yarn is less than two other samples. It means surface roughness of this case is better than others and by using coating process surface properties have improved. By these results, it is expectable that texturing process leads to construct porous structure to help cells migration into inner part of scaffold and in following nanofibers coating process decreases roughness and increases uniformity of surface ( $S_a$  decreases while  $S_{sk}$  and  $S_{ku}$  increase) which are beneficial for cell attachment onto the surface.

## Conclusion

In this study, nano-coated textured silk yarn has been prepared of two main components that are micro and nano components by combining two methods including texturing mechanism and electrospinning coating process. Texturing and coating methods can be used to improve morphological and mechanical properties by changing the structure. In this reason, the optimized variable parameters in both process including air pressure and speed to produce textured silk yarn, silk fibroin electrospinning parameters to produce silk fibroin nanofibers and coating time to produce nano-coated textured silk yarn were used. No-textured silk yarn, textured silk yarn and nano-coated textured silk yarn samples analyzed and compared by SEM images and mechanical properties. In following step, digital signal processing used to analyze surface roughness. According to statistical analysis, there is no significant difference between three kinds of samples and the failure of maximum load is acceptable. The surface roughness values of textured silk yarn demonstrated more porosity while nano-coated textured silk yarn showed a surface with better uniformity that probably can be useful to cell migration and attachment. Finally, we believe this unique hybrid structure as a nano-coated textured silk yarn that was introduced have particularly promising results in artificial tendon/ ligament scaffolds to develop and design better structure.

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