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A novel biodegradable micro-nano tubular knitted structure of PGA braided yarns and PCL nanofibres applicable as esophagus prosthesis

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A novel multi-layered knitted structure with the coating of the nanofibrous web has been developed for using as a esophageal prosthesis. Poly glycolic acid (PGA) braided yarn has been knitted into tubular form and polycaprolactane (PCL) nanofibres are coated on the surface of PGA knitted fabrics with the electrospinning process. Finally, a gelatin coating has been applied on the surface of the developed tubular fabric. The effect of the stitch density parameters on the mechanical properties of the multi-layer knitted fabric has been investigated and the optimum process conditions for electrospinning of the PCL nanofibres are obtained by studying the uniformity in the diameter distribution of the fibres. The surface roughness of the gelatin coated fabric is calculated with image processing method before and after the coating process. Degradation behavior of the three-layer esophageal prosthetic is also investigated. Results show that the knitted tubular fabrics has mechanical properties similar to real esophageal tissue. Coating of gelatin increases the surface roughness of the PCL nanofibrous web and also retains the porosity of the final structure. Degradation test also shows that the PGA knitted fabric coated with the PCL nanofibres and gelatin is degraded in a controlled manner. It can be said that this tubular knitted structure can be considered as a substitute for prosthetic application in different esophageal diseases after biological investigations.

Keywords: Esophageal prosthesis, Knitted fabric, Nanofibre, Nylon 6, Polycaprolactane, Poly glycolic acid, Tubular knitted fabric

1 Introduction

Weft knitting is a historical textile process for making the fabric, by forming a unique yarn in to loop structure across the fabric width with a set of needles. Today, the weft knitted fabrics have found a wide range of applications due to their excellent extensible properties. In fact, the interlooping yarns in the fabric allow the knitted structures to have greater circumferential properties than in longitudinal direction. In addition, these structures have a porous structure that makes them useful for medical and regenerative treatments.

Tubular knitted fabrics have been used as a basic structure for making the vascular grafts. Wang *et al.*[1] embedded the polyester single jersey tubular knitted fabric into polycaprolactane (PCL) composite structure to make a vascular graft. This results showed that this vascular prototype has good mechanical properties due to the knitted structure. In another study, Yang *et al.*[2] used the polyester/spandex tubular weft knitted fabric to reinforce a three layer vascular graft. They evaluated the radial mechanical property of the developed multi-layer structure and reported that the radial tensile property of the vascular grafts has improved with the use of knitted fabric. These studies approve that the use of weft knitted fabrics can enhance the mechanical properties of the tubular prosthesis and vascular prototypes.

Esophagus is a tubular shaped muscular/mucosal tissue that connects the mouth and throat to the stomach. The researches have shown that there is a nonlinear and exponential relationship between force and elongation of the esophagus tissue[3]. The mechanical properties of the native esophagus tissue, including the axial and circumferential strengths and strains have been measured as 2.19 MPa, 1.41 MPa, 70%, and 82.5% respectively[4].

Some researchers have focused on providing the self-expanding metallic and plastic stents, however the application of metallic stents in benign esophageal strictures is limited due to high risks and problems, such as stent displacement, forming the new

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strictures, fistula, and hyper-plastic reaction of the tissue in the long-term[5,6]. Knitted textile structures are used as biodegradable stents for treatment of the esophageal disorders. Saito *et al*.[7] developed a novel biodegradable knitted poly l-lactic acid (PLLA) stent for use in the benign esophageal strictures. This is a novel method for developing the textile based biodegradable stents. However, the mechanical properties of this stent haven't been measured for long-term applications. This stent also has a significantly open structure that makes it impossible for use as a tissue engineering scaffold considering the proper conditions for cell growth and proliferation. Hoogenkamp *et al*.[8] have developed a hybrid seamless tubular structure as esophageal prosthesis. They have knitted the PCL yarns into plain sheet structures and then coated the knitted structure by collagen polymer. However, they used the silk knots for making the tubular shape of PCL knitted sheets by surrounding knitted fabrics around a mandrel. These sutured points may be torn due to mechanical loadings during its application and may create leakage sites. Muste *et al*.[9] also used successfully the polyester knitted prosthesis for treatment of the esophageal disorder in a dog.

As stated above, most of the previous works are focused on the use of potential of weft knitted fabrics for making the biodegradable stents and prosthesis. However, these structures have mechanical and morphological limitations. In this research, we have developed a novel biodegradable tubular hybrid structure which can provide the mechanical properties of the native esophageal tissue and also introduce a good surface morphology for further cell growth and proliferation during the implementation period. This multi-layer structure is consisted of the PGA knitted tubular fabric, nanofibrous coating of the PCL polymer and an additional layer of gelatin coating. The biodegradable nature of the applied polymers and yarns eliminates the need of further surgeries for removing the prosthesis and also will expose the different functional groups (such as carboxyl groups) to cells. In fact, the use of PGA and PCL as knitted structure and nanofibrous coating leads to a balance in degradation during the implantation in the body. This issue is overcome with hydrophilic property of PGA against low contact surface of filament yarn in comparison with hydrophobic property of PCL against high contact surface of nanofibres.

2 Materials and Methods

2.1 Materials

Poly glycolic acid (PGA) and Nylon 6 yarns with the yarn count of 62.27 tex were used for knitting of the tubular fabrics. PGA braided yarn was supplied by Supabon Co., Iran. Polycaprolactane (PCL) biodegradable polymer (M_w=80000 g/mol) and gelatin (bovine skin, type B) were purchased from Sigma Aldrich. Methylene Chloride (CH_2Cl_2) and dimethylformamid (DMF) solvents and ethanol 70% (v/v) were also supplied by Merck. Phosphate buffer solution (PBS, pH 7.4) was purchased from Cyto Matin Gene Co., Iran.

Zwick universal testing machine 1446-60 (USA) was used to measure the mechanical properties of the tubular knitted structures. Innova-4080 incubator (Eppendorf Co., Germany) was used for conditioning the test samples. SEM images were captured by Seron AIS-2100 (Korea) scanning electron microscope.

2.2 Methods

2.2.1 Knitting of Tubular Fabrics

Double jersey V-bed weft knitting machine (Santagostino, 12 gauge) was used to produce the PGA tubular fabrics of plain structures. In order to investigate the effect of stitch density on the mechanical properties, three samples of nylon fabrics were also knitted with different stitch densities. The lengths of all fabrics were set at 60 mm to simulate the characteristics of the actual esophagus tissue. Structural properties of the knitted fabrics are listed in Table 1.

2.2.2 Preconditioning of Tubular Knitted Fabrics

Tubular knitted fabrics should be washed prior to electrospinning process to remove the chemical spin finish agents and pollutions made by a mechanical process. For this purpose, PGA fabric was washed with ethanol 70 % (v/v) for 30 min at 35°C. After that, the tubular fabric was rinsed by distilled water to remove the residual ethanol. Finally, the washed PGA fabric was dried at the room temperature (20±2 ^{0}C) for 24h.

Table 1—Properties of weft knitted tubular structures

Sample code	Courses/ cm	Wales/ cm	Width mm	Stitch density cm^{-2}	Thickness mm
Nylon 1	7.1	7.1	32	50.41	0.8
Nylon 2	7.9	7.9	29	62.41	0.8
Nylon 3	9.5	10.2	25	96.9	0.9
PGA	7.1	6.3	34	44.73	0.8

2.2.3 Electrospinning of PCL Nanofibres on PGA Knitted Structure

In order to provide a uniform and complete covering of PCL nanofibres on the PGA knitted tubular fabrics, an aluminum drum with the length of 20 cm and a diameter of 22 mm was developed. Figure 1 shows the schematic diagram of the electrospinning process of the PCL nanofibres on the PGA tubular knitted fabrics.

The optimum conditions of the electrospinning process were determined via several trials. The aluminum collector was placed 12 cm from the needle tip of a syringe with the diameter of 0.6 mm. Cylindrical drum was covered by tubular fabric and a positive voltage of 14KV was applied between needle tip and aluminum collector. Finally, electrospinning of the PCL solution was performed with the feed rate of 1 mL/h on the tubular fabric which had been covered on the rotating drum. Electrospinning apparatus was modified to provide a complete and uniform coating of PCL on whole external area of fabrics. For this purpose, the syringe carrier was arranged for a reciprocating motion with the distance range of 2 cm and 20 traverse/min. Electrospinning process was completed after 50 min, so that a uniform coating of PCL was formed on the whole area of the tubular fabrics.

2.2.4 Coating the Gelatin on PGA Tubular Fabrics Covered by PCL Nanofibres

PGA fabrics that are covered by PCL nanofibres should be preconditioned prior to gelatin coating process. PGA tubular fabric was soaked in ethanol 70 % (v/v) at room temperature for 30 min and was dried for 24h at ambient temperature. In the next step, the gelatin solution (0.1%, w/v) was prepared by dissolving the gelatin in deionized water at 40°C temperature for 30 min. Coating process was performed as reported by Chen *et al.*[10]. PCL coated fabric was soaked in the gelatin solution for 2 min. Gelatin coated fabric was incubated for 5 min at 37°C. This procedure was done 3 times to obtain a uniform coating of gelatin on the composite structure of the PGA tubular fabric/PCL nanofibres.

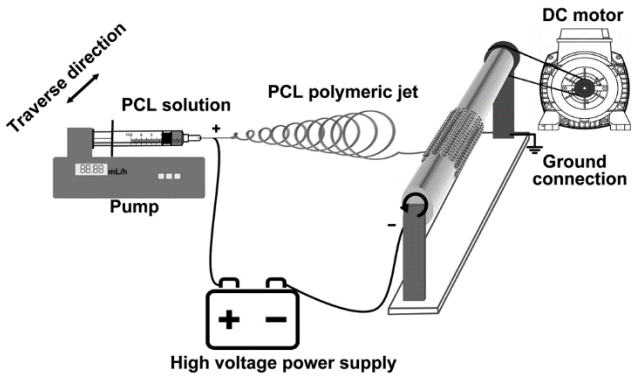

Fig. 1 — Schematic diagram of electrospinning process of PCL nanofibres on PGA tubular knitted fabric

2.3 Mechanical Tests

2.3.1 Axial Test

Five tests were performed for each sample, and circumferential & axial tenacity and strain values were averaged. All results were obtained before the jamming condition of the fabrics. Jamming phenomena occur when the loops of the knitted structure attain their maximum tensile strength. In fact, after this point the applied force will be applied on the yarns. The strain rate for axial tests was set at 30mm/min and specimen length was 30 mm.

2.3.2 Circumferential Test

Two fixtures were designed to calculate the circumferential strength of the tubular fabrics. The circumferential stress was calculated by the following equation:

$$\sigma_t = \frac{F}{2Lt} \text{ (MPa)} \qquad ...(1)$$

where F is the maximum force (N); and L and t, the specimen length (mm) and thickness (mm) respectively.

2.4 Measuring Surface Roughness of Gelatin Coated Fabrics

Since the surface roughness of the prosthesis is a critical property that affect the cell attachment and proliferation, this property was measured before and after the gelatin coating. The surface roughness of tubular fabric was measured according to developed procedure by Semnani *et al.*[11]. In this method, the surface roughness criterion (R_s) was calculated based on image processing technique. The roughness level is average of the five different roughness features which represent the surface relief properties. Matlab software was used for image analysis of the surface of the gelatin coated structure. The roughness criterion (R_s) was defined in the range of 0 and 1 to represent the smooth and rough surfaces respectively.

2.5 In-vitro Degradation Test

The final three-layer structure, including the PGA tubular knitted fabric covered by PCL nanofibres and

coated by gelatin polymer, was placed in a closed bottle containing PBS (pH 7.4) and then was incubated at 37°C and at time intervals of 1, 3, 7 and 14 days. After each degradation interval, the specimen was washed with distilled water and then dried in vacuum oven for 24h. The remained weight percentage of the degraded structure was calculated as follows:

$$\text{Remained weight}\,(\%) = \frac{W_d}{W_0} \times 100 \qquad \ldots(2)$$

where W_d and W_0 are the specimens weight after and before the degradation respectively.

3 Results and Discussion

3.1 Mechanical Tests

3.1.1 Mechanical Properties in Axial Direction

Five tests have been performed for each sample in axial direction and results are averaged. Mechanical results for tubular multi-layer structures in the axial direction are shown in Fig. 2(a). Results show that the increase in stitch density of the nylon fabrics causes increase in strength of fabrics prior to jamming point (108, 137.84 and 185.41 N for Nylon1, Nylon2 and Nylon3 fabrics respectively). It is also observed that the tensile stress of samples in axial direction increases with the increase in stitch density parameter (2.09, 2.94 and 4.04 MPa for Nylon1, Nylon2 and Nylon3 fabrics respectively). Thus, it can be said that a dense fabric will attain its jamming point later and vice versa. Increasing the stitch density leads to increase in knitted loops in a given area, and thus more loops will contribute against the deformation caused by equal force values. PGA tubular fabric also indicates a similar trend to Nylon1 fabric. This can be explained by considering the approximately same values of the stitch densities for both fabrics.

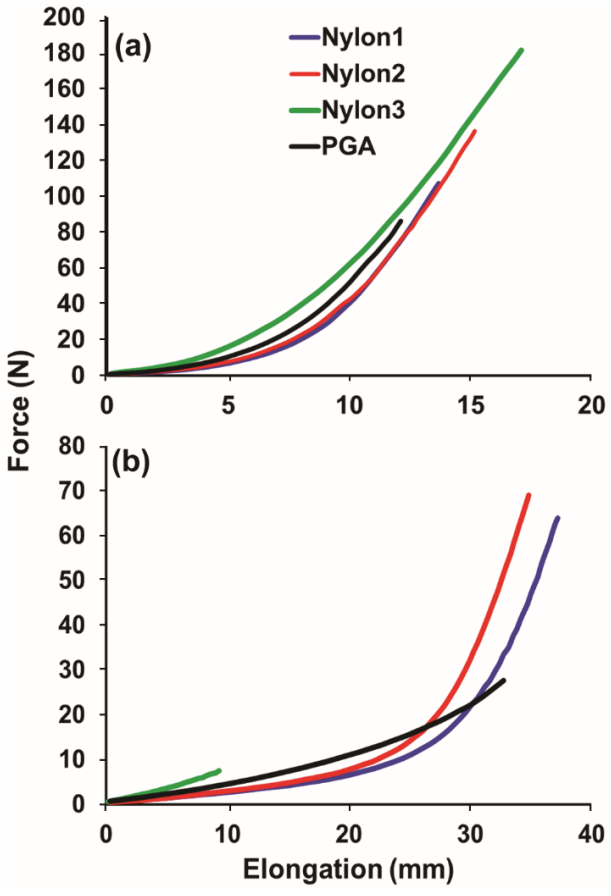

Fig. 2 — Force-elongation curves of tubular fabrics (a) axial direction and (b) circumferential direction

3.1.2 Circumferential Stress Measurement

The results of mechanical tests in the lateral direction are shown in Fig. 2(b). Results show that there is an optimum point for stitch density level of the fabrics. Increasing of the stitch density from Nylon1 to Nylon2 samples (increased by 23%) leads to increase in strength of tubular fabrics and also decrease in elongation values. With the increase in stitch density in Nylon3 fabrics (increased by 55% than Nylon2 and 92% than Nylon1 fabrics), strength and elongation values of the tubular structures are dramatically dropped. This behavior indicates that an optimal point must be selected for the stitch density level to achieve an appropriate values of the strength and elongation.

The figures indicate that the nylon fabrics have a low rising slope at the beginning of the experiment and then the slope of the curve increases significantly up to jamming point. However, the PGA fabric, despite the bigger loops length and a lower stitch density than nylon fabrics, reaches earlier to its jamming point. PGA fabrics are made by braided PGA yarns and this structure causes of more yarn-to-yarn friction than nylon fabrics that are made by nylon multi-filament yarns with a smoother surface. As a result, more force will be required to attain the same deformation values of the nylon tubular fabrics, and thus the force-elongation curve of PGA fabric will has a rising trend with an approximately constant slope. Table 2 shows the stress and strain values for axial and circumferential directions of the tubular fabrics. According to obtained values for PGA multilayer structure, it has mechanical properties close to the esophagus tissue

and can mimic the esophageal characteristics during substitution time.

3.2 Optimizing Parameters of Electrospinning of PCL Nanofibres

In order to obtain an optimal condition for electrospinning process of the PCL nanofibres, three voltages and three feeding rates have been applied. The applied voltages are 10, 14, and 18 kV and feed rates are set at 0.5, 1, and 1.5 mL/h. The distance between needle tip and collector drum is also set at 12 cm for all cases. Thus, nine samples have been produced and diameter distribution of the PCL nanofibres is measured randomly by Digimizer 4.1 software from scanning electron microscope (SEM) images. Several parameters have been calculated based on obtained results from diameter measurements. Mean value, standard deviation (SD), coefficient of variation (CV %), and skewness of the diameters distribution are listed in Table 3.

In order to investigate the normality of the diameter distribution, the skewness parameter is calculated for each process condition. Skewness statistic is a measure of symmetry in a distribution graph. Negative values for the skewness indicate data that are skewed left side, and positive values for the skewness indicate data that are skewed right side. A normal distribution has a skewness value of the zero. This statistic is calculated based on Pearson second skewness coefficient formulation [12], as shown below:

$$\text{Skewness} = \frac{3(\text{Mean} - \text{Median})}{\text{SD}} \quad ...(3)$$

where Median is the middle value of the diameters in a data set. As shown in Table 3, sample 5 has zero skewness value and so has a normal distribution of the diameters of nanofibres. However, sample 8 has a minimum CV% value but with a strongly negative skewness that means the distribution of diameters is not symmetric about the average diameter. Results represent that the sample 5 has a uniform structure and diameter distribution. Therefore, the process parameters for producing sample 5 are selected as optimum conditions for electrospinning of the PCL nanofibres. SEM image and diameter distribution of the sample 5 are shown in Fig. 3.

3.3 Coating Gelatin over PGA Tubular Fabric Covered by PCL Nanofibres

SEM images of the coated PCL nanofibres before and after the gelatin coating are shown in Figs 4(a) and (b). Gelatin as a natural polymer has functional groups that can facilitate the further cell attachment on the developed prosthesis. However, coating of gelatin over the PCL nanofibres reduces the pore sizes of the nanofibrous web and it will lead to an increase in diameter of the nanofibres. Thus, the time of coating process and the level of coating must be controlled. As stated above, the time of coating is too short and about 2 min. As can be seen in Fig. 4(b), there are still lot of pores on the surface of the PCL coated fabrics with the gelatin. This porous structure with the additional properties of the gelatin polymer can contribute in the proper cell attachment, growth and proliferation on the prosthetic applications.

Table 2 — Mechanical properties of tubular knitted structures

Sample code	Lateral direction		Axial direction	
	Circumferential strength Mpa	Lateral strain %	Axial strength Mpa	Axial Strain %
Nylon 1	1.25	116.21	2.09	45.58
Nylon 2	1.49	120.11	2.94	50.55
Nylon 3	0.17	37.22	4.04	57.04
PGA	1.16	96.28	2.03	60.63

Table 3—Diameter distribution parameters of PCL nanofibres in different conditions of electrospinning process

Sample No.	Voltage, kV	Flow rate, mL/h	Distance cm	Mean diameter nm	SD, nm	CV%	Skewness
1	10	0.5	12	266.16	120.99	45.46	-1.46
2	10	1	12	189.13	57.02	30.15	-0.57
3	10	1.5	12	183.51	44.68	24.35	-1.11
4	14	0.5	12	185.64	66.13	35.62	-2.92
5	14	1	12	200.00	47.38	23.69	0.00
6	14	1.5	12	177.27	53.78	30.34	-4.06
7	18	0.5	12	185.11	38.47	20.79	0.79
8	18	1	12	192.16	36.88	19.19	-2.67
9	18	1.5	12	186.55	64.12	34.37	-1.80

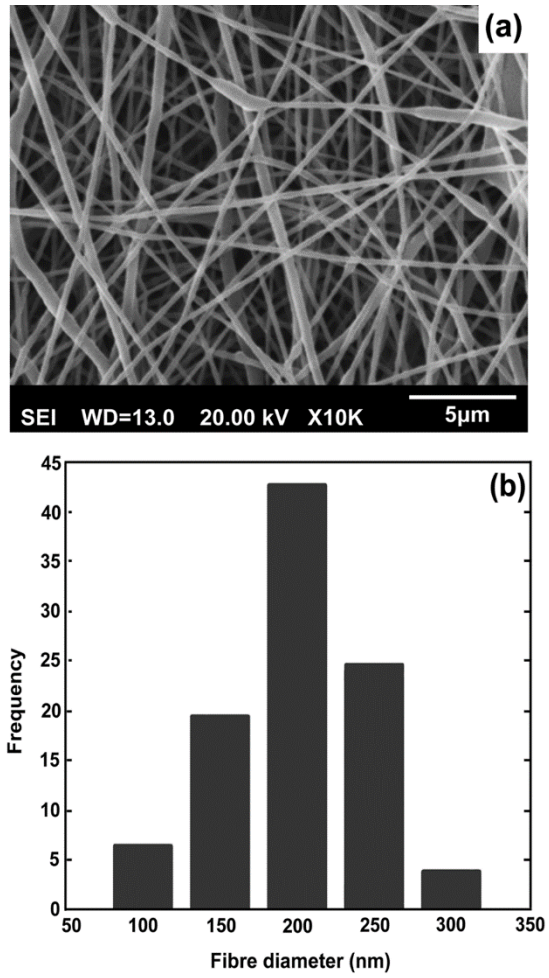

Fig. 3 — Structure of PCL nanofibrous web at the optimum condition of electrospinning process (14kV, 1 mL/h) [(a) SEM image (×10000 magnifications) and (b) diameter distribution of PCL nanofibers]

3.4 Surface Roughness Measurement after Coating of Gelatin

The surface roughness of the developed structures before and after the coating of gelatin is measured according to a previously developed method based on image analysis procedure. The surface roughness criterion is an average of the five parameters that are extracted from the surface profile of the SEM images of the PCL nanofibres. These parameters include the number of peak points in the surface profile, the variance of the distance of the peak points from the origin, the volume of the image profile, the ratio of the variance to average of the intensity values and the variance of the peak point values. SEM images with the dimension of 70×100 pixels are used for this purpose. Figures 4(c) and (d) show the 3D surface profile of the PCL nanofibres before and after the coating of gelatin.

Image analysis results show that PCL nanofibrous web prior to gelatin coating has a surface roughness criterion of the R_s=0.2434. This parameter is increased up to 0.3948 after applying the gelatin on the surface of PCL nanofibres. As expected, the surface roughness of the nanofibrous webs is increased about 15% by coating the gelatin.

3.5 Biodegradability of Three-layer Tubular Structure

The degradation behavior of three layer micro-nano tubular structure is investigated using Eq. 2. PGA has a high natural hydrophilicity, while the PCL polymer has more crystalline structure and thus more hydrophobic characteristic that prevent from its hydrolysis in aqueous environments. Gelatin is also a natural polymer and has a fast degradation property in

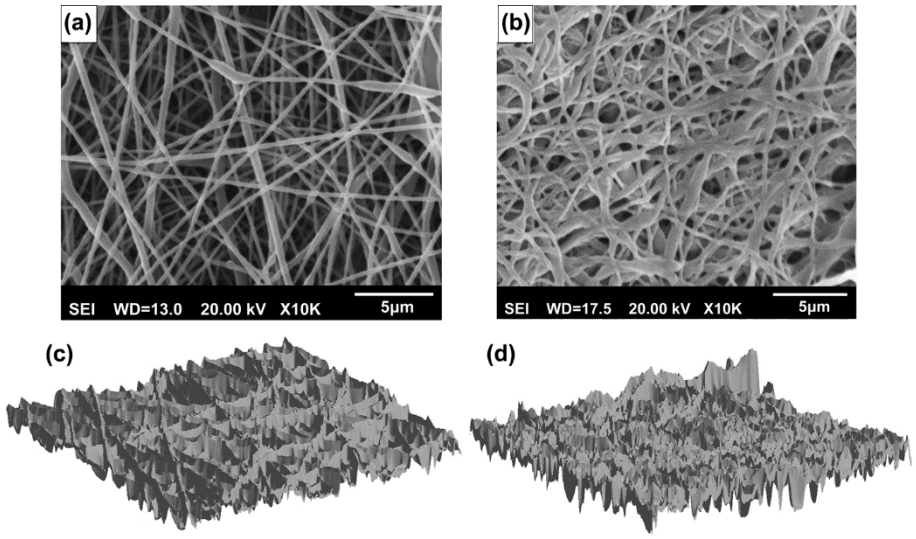

Fig. 4 — SEM images of PCL nanofibres [(a) before coating the gelatin and (b) after coating the gelatin (×10000 magnification)], and surface profile of PCL nanofibrous web [(c) before coating the gelatin and (d) after coating the gelatin]

the presence of aqueous solutions. Results show that the multi-layer structure composed of PGA knitted fabric, PCL nanofibrous layer and gelatin coating has lost about 22% of its weight after 14 days of the test. As expected, this mass loss is related to the degradation of the PGA knitted fabric and specially gelatin coating, and the whole structure of the PGA knitted fabric and PCL nanofibres layer remains as such approximately without any remarkable damage.

4 Conclusion

A multi-layer tubular structure has been developed by knitting and electrospinning of the biodegradable PGA yarn and PCL polymer for its use as a substitute for esophageal diseased tissue. Studies on mechanical properties show that the developed structure has stress and strain values close to real esophagus tissue in both directions. Investigating the effect of stitch density on the mechanical properties of the knitted structures shows that the increase in stitch density has led to increase in axial stress. However, this increase causes a decrease in circumferential stress of the tubular prosthesis from a certain point. Thus, the stitch density value must be set at an optimum value to have good mechanical properties in both directions. The surface roughness measurements for PCL nanofibres coated with gelatin shows that the coating of gelatin has led to increase in surface roughness of the esophageal prosthesis by 15% than uncovered structure. Coating the gelatin has also retained the porosity of the tubular structures. *In-vitro* tests demonstrated that three-layer micro-nano tubular structure has a controlled degradation in presence of PBS solution. Results show that the multi-layer tubular knitted prosthesis can be used as a biodegradable candidate for substituting the diseased esophageal tissues and can supply required mechanical properties, surface functionality groups, and proper surface profile for further cell growth and proliferation.

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