# Electrospinning of aligned medical grade polyurethane nanofibres and evaluation of cell–scaffold interaction using SHED stem cells

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Polyurethane (PU) nanofibres are widely used in tissue engineering of elastic tissues such as tendon and ligament, vascular and arterial grafts etc. Alignment of the PU nanofibres is also a desirable property of some tissues to mimic the morphology of the extracellular matrix. Elasthane™ 55D nanofibres were produced using a common electrospinning method and a rotating drum to collect the aligned fibres. Four flow rates and five collector speeds were investigated for their effects on nanofibres alignment and diameter. Field emission scanning electron microscopy (FESEM) images of nanofibrous webs were captured, and the diameter and alignment of the nanofibres were measured. Finally, stem cells from human exfoliated deciduous tooth (SHED) were seeded on the surface of the PU nanofibres to investigate the cell–scaffold interaction. Statistical results showed that the flow rate had a significant effect on the fibres diameter. Enhancing the flow rate led to increasing the diameter of the PU nanofibres. The results showed that the fibres alignment index was promoted by increasing the rotating speed of the collector. In addition, the morphological observation of SEM images indicated that cells grew in the alignment direction of the nanofibres. The results of this study can be applicable to produce and control the aligned PU nanofibres for specific applications such as vascular grafts, esophagus prosthesis, arterial grafts and tissue engineering of tendon and ligament.

**1. Introduction:** Nanofibrous structures are widely used in tissue engineering applications due to their unique properties, such as high surface area and porosity that can mimic the extracellular matrix of the human body [1]. Electrospinning is a common and efficient method used to fabricate the nanofibrous structures from a broad range of polymer solutions [2]. In electrospinning, an electrostatic force produced by a high-voltage supply is used to drive the spinning process. This field is applied to droplets of polymer solution (or a melt) passed from the tip of a fine orifice. The equipment needed for electrospinning on a laboratory scale is composed of three main components: the high-voltage power supply, a syringe pump and a collector, which can be a sheet of aluminium foil [3]. The variable parameters of the electrospinning process can be classified in three categories including the solution parameters (such as viscosity and concentration), process parameters (such as voltage, electrospinning distance and flow rate) and ambient condition (such as temperature and humidity). These parameters, individually or in combination, may influence the nanofibre features such as fibre diameter, surface morphology, web alignment.

Biomaterials must be elastomeric in some special tissue engineering applications such as vascular grafts, oesophagus prosthesis and arterial grafts [4]. Polyurethanes (PUs), which play an important role in medicine, are used at all scales, from the construction materials used to build the bedding materials to surgical instruments, medical implants and microscale encapsulation devices; this is due to their superior mechanical, thermal and chemical properties, and excellent elasticity [5].

Many studies have been focused on investigating the effective parameters in the electrospinning of different PU grades. Zhou *et al.* [6] investigated the effects of process parameters (applied voltage, flow rate) and solution concentration on the morphology of shape memory polyurethane (SMPU). They concluded that flow rate and solution concentration directly affected the diameter and morphology of the SMPU nanofibres. Yanilmaz *et al.* [7] also produced the PU nanofibres for using in tissue engineering applications. They studied the effect of process parameters (applied voltage and tip to collector distance) on the diameter and morphology of the PU nanofibres. Statistical results showed that both parameters had significant effects on the diameter and uniformity of the electrospun fibres. These attempts have been made to produce the PU nanofibres with random patterns. However, alignment of the PU nanofibres is also important in some specific applications. Jia *et al.* [8] found that aligned PU nanofibres represented anisotropic and proper mechanical properties for the regeneration of the artery. They concluded that the alignment of the PU nanofibres promoted smooth muscle cell alignment through contact guide. Mi *et al.* [9] also developed different blends of unidirectional and orthogonal thermoplastic polyurethane (TPU) nanofibres to assess the culture condition of fibroblast cells on them. Their findings showed that aligned fibres exhibited a more pronounced differentiation and cell migration behaviour, in comparison to the random webs. However, they applied static copper plates in two unidirectional and orthogonal patterns to collect the nanofibres. Such a method cannot be useful to produce aligned nanofibres for specific applications involving cylindrical tissues (artery, oesophagus, vascular grafts etc.). Sheikh *et al.* [10] worked on the electrospinning of the composite PU nanofibres containing the multi-walled carbon nanotubes to evaluate the fibroblast cells culture for the tissue engineering of the tendon and ligament. Their results showed that alignment of nanofibres led to increasing the mechanical properties, which could be a desirable feature for such applications.

Literature review shows that some studies have been performed to evaluate the effective parameters on the morphology aspects of random PU nanofibres. Some *in vitro* assessments also indicate that alignment of the PU nanofibres and its blends with other polymers can significantly affect the cells behaviour after seeding, as well as their mechanical properties. It has also been found that there is no study regarding the effects of electrospinning parameters




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on the alignment and diameter of the orientated PU nanofibres in the case of using the cylindrical collectors. In this study, the process parameters including the flow rate and the rotational speed of the collector were evaluated for their effects on medical grade biodegradable PU (Elasthane™ 55D) nanofibres. Statistical analysis was also performed to express the results in a clear manner. In addition, the porosity of different layers of the electrospun scaffold was measured based on an image processing method. The effect of fibres alignment on the morphology of cell growth was investigated by seeding the nanofibres surface with the teeth-derived stem cells. The results of this study can be useful for the engineering of specific tissues that need the aligned morphology of the culture substrate.

**2. Materials and methods:** Elasthane™ 55D biomedical grade PU was supplied by DSM Co. (the Netherland). Dimethylformamide (DMF) solvent was supplied by Merck Co. (Germany). Field emission scanning electron microscopy was used to capture the images of the PU nanofibres (S4160, Hitachi Co., Japan).

Electrospinning of the PU nanofibres was performed using an aluminium drum with the length of 15 cm and the diameter of 25 mm. The optimum conditions of the electrospinning process were determined via several trials. The aluminium collector was placed 15 cm from the needle tip of a syringe with the diameter of 0.6 mm. Then, a positive voltage of 13 kV was applied between the needle tip and the aluminium collector. The PU/DMF solution of 10% (w/w) concentration was prepared. The flow rate of the polymer solution and the rotational speed of the aluminium collector were changed to investigate the effects of process parameters on the diameter and alignment of the PU nanofibres. Four flow rates (0.15, 0.20, 0.25 and 0.3 ml/h) and five collector speeds (100, 200, 300, 400 and 500 rounds per minute, r.p.m.) were used for the electrospinning of the PU nanofibres. Needle carrier was also arranged for a reciprocating motion of 15 cm distance, so that a uniform coating of the PU nanofibres would be formed on the whole area of the aluminium drum. The electrospinning setup has been shown in Fig. 1.

Mean angle of nanofibre orientation, relative to the direction of the collector rotation ($\mu$) and the average angular standard deviation ($\sigma$) of the PU nanofibre was measured to calculate the alignment index of the different webs. The angular standard deviation index proposed by Fisher was calculated as follows [11]

$$\mu = \tan^{-1}\left(\frac{\sum_{i=1}^{n}\sin 2\theta_i}{\sum_{i=1}^{n}\cos 2\theta_i}\right) \quad (1)$$

$$\rho = \frac{\sqrt{\sum_{i=1}^{n}\sin 2\theta_i + \sum_{i=1}^{n}\cos 2\theta_i}}{n} \quad (2)$$

$$\sigma = \frac{1}{2}\sqrt{-2\ \ln \rho}, \quad (3)$$

where $n$ is the number of investigated nanofibres in each sample (here $n = 100$), and $\rho$ is the mean resultant length of nanofibres.

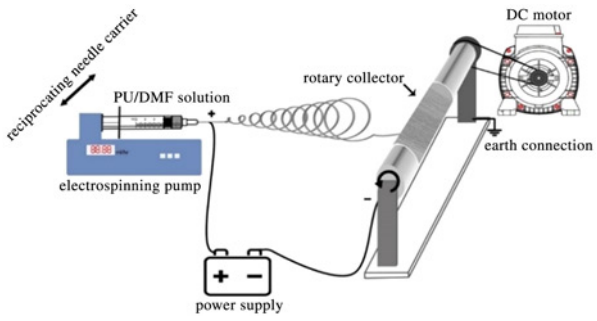


**Fig. 1** *Schematic setup for the electrospinning of PU nanofibres*

$\theta_i$ also represents the orientation angle of an individual nanofibre. The lower value of ($\sigma$) represents a more aligned nanofibrous web.

Porosity of various layers of the electrospun nanofibres (surface layer, middle layer and visible lower layer) was also measured using an image processing method proposed by Mobarakeh *et al.* [12]. At first, the SEM images were converted to binary images. Then, the porosity values were calculated using different thresholds based on the mean and standard deviation of the image matrix [12].

The aligned electrospun web was also investigated for the effects of fibres alignment on the morphology of the stem cells seeded on the surface of the scaffold. The nanofibrous scaffold was exposed to UV radiation for 2 h, washed three times with Phosphate buffered saline (PBS), and incubated with DMEM (Gibco, 12800) supplemented with 15% ES-FCS medium for 24 h before cell seeding. The stem cells from human exfoliated deciduous tooth (SHED) were further seeded on the PU nanofibrous web placed in a 24-well plate and tissue culture plate (TCP) control at a density of $5 \times 10^4$ cells/well; they were then grown in culture media and incubated at 37°C, 5% $CO_2$ incubator with 95% humidity.

The morphology of cells on the surface of the aligned PU nanofibres was investigated by SEM images. After 7 days of cells seeding, samples were fixed with 3% glutaraldehyde for 2 h. Then, the specimens were rinsed in water and dehydrated with the graded concentrations (50, 70, 90, 100%, v/v) of ethanol. Finally, the samples were sputtered with gold to observe the cell morphology.

**3. Results and discussion:** Table 1 shows the mean diameter of the different PU nanofibre samples. The diameter of the PU nanofibres was in the range of 520–725 nm. Angular standard deviation values were calculated using (1)–(3), as presented in Table 1. Figs. 2–5 show FESEM images of the PU nanofibrous structures in flow rates of 0.15, 0.20, 0.25 and 0.30 (ml/h), respectively.

As expected, the diameter of the PU nanofibres was increased with enhancing the flow rate of the polymer solution in a specific speed of the collector. The lower values of the flow rate made smaller polymeric jets, resulting in the electrospinning of more fine fibres. The average diameter of the electrospun fibres in the flow rates of 0.15, 0.20, 0.25 and 0.30 (ml/h) (for different collector speeds) was 577, 630, 635 and 700 nm, respectively.

**Table 1** Morphological properties of the PU nanofibres under different electrospinning process conditions

| Flow rate, ml/h | Collector speed, r.p.m. | Mean diameter, nm | Angular standard deviation, ° |
|---|---|---|---|
| 0.15 | 100 | 521 ± 59 | 0.74 |
| | 200 | 541 ± 51 | 0.62 |
| | 300 | 567 ± 43 | 0.55 |
| | 400 | 628 ± 100 | 0.47 |
| | 500 | 630 ± 56 | 0.34 |
| 0.20 | 100 | 605 ± 70 | 0.76 |
| | 200 | 616 ± 96 | 0.68 |
| | 300 | 621 ± 64 | 0.61 |
| | 400 | 649 ± 70 | 0.54 |
| | 500 | 660 ± 62 | 0.42 |
| 0.25 | 100 | 608 ± 73 | 0.61 |
| | 200 | 620 ± 77 | 0.56 |
| | 300 | 628 ± 80 | 0.50 |
| | 400 | 650 ± 78 | 0.42 |
| | 500 | 675 ± 58 | 0.34 |
| 0.30 | 100 | 660 ± 130 | 0.55 |
| | 200 | 675 ± 99 | 0.42 |
| | 300 | 718 ± 122 | 0.35 |
| | 400 | 721 ± 139 | 0.31 |
| | 500 | 723 ± 97 | 0.26 |

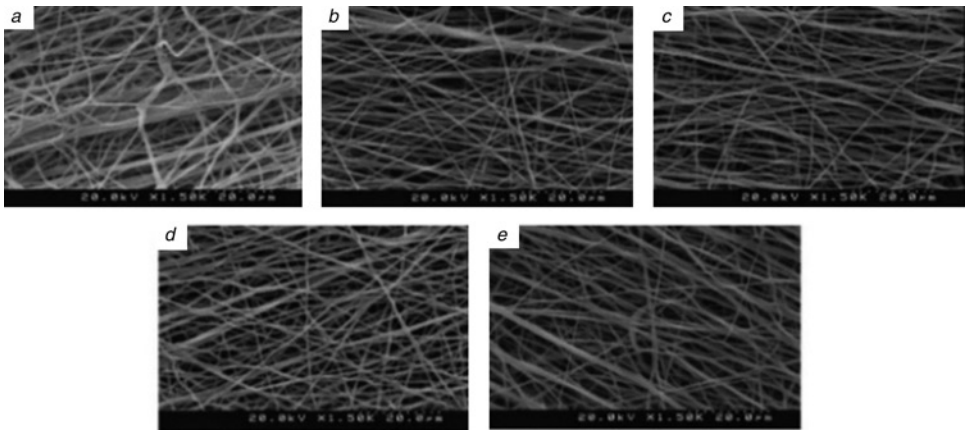


**Fig. 2** *FESEM images of PU nanofibres at the flow rate of 0.15 (ml/h) and different collector speeds*
*a* 100 r.p.m.
*b* 200 r.p.m.
*c* 300 r.p.m.
*d* 400 r.p.m.
*e* 500 r.p.m.

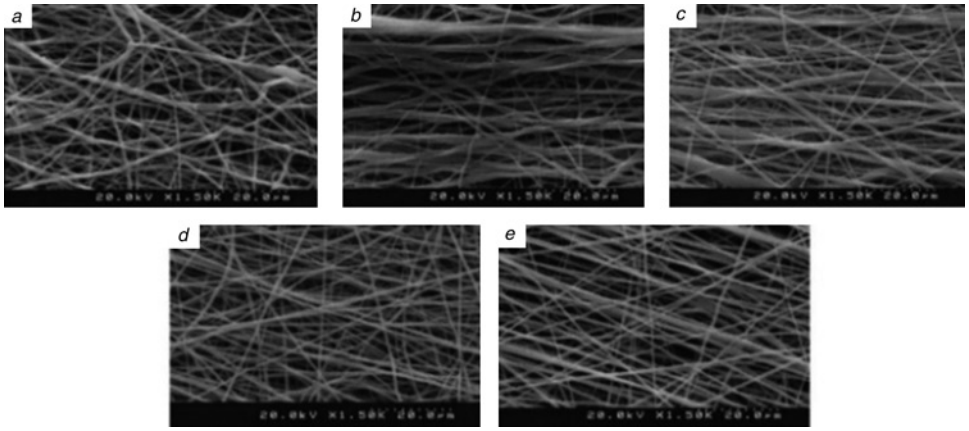


**Fig. 3** *FESEM images of PU nanofibres at the flow rate of 0.20 (ml/h) and different collector speeds*
*a* 100 r.p.m.
*b* 200 r.p.m.
*c* 300 r.p.m.
*d* 400 r.p.m.
*e* 500 r.p.m.

One-way analysis of variance (ANOVA) test was performed to draw a clear conclusion for the effects of collector drum speed and flow rate on the fibres diameter. IBM SPSS statistics 19 software (IBM Co., USA) was used to perform the ANOVA test and the 95% significance level ($\alpha = 5\%$) was chosen. ANOVA results have been summarised in Table 2.

According to Table 2, the $p$-value (0.000) of the flow rate was less than the significance level (0.05). Therefore, the flow rate had a significant effect on the nanofibres diameter. Table 2 also shows that the collector speed had no significant effect ($p$-value = 0.269 > 0.05) on the diameter of the nanofibres. In other words, increasing the collector speed led to no significant change in the diameter of the PU nanofibres. These results showed that there were at least two flow rates, such that the mean diameters of the fibres were significantly different from each other. In fact, when a significant effect is found using analysis of variance, we still do not know which means differ significantly. It is, therefore, necessary to conduct post hoc comparisons between pairs of treatments [13]. The Duncan multiple-range test was performed to compare all pairs of the flow rates and investigate the homogeneity of different subsets.

Results of the Duncan test for flow rates have been presented in Table 3. The results indicated that there was no significant difference between the effects of the flow rates of 0.20 and 0.25 (ml/h) and the diameter of the PU nanofibres. On the other hand, it was found that that there was a significant difference between the effects of the flow rates of 0.15, (0.20, 0.25) and 0.30 (ml/h) and the fibres diameter. Increasing the flow rate led to increasing the mean diameter of the PU nanofibres.

ANOVA tests were also conducted to investigate the effect of the flow rates and the rotating speed of the collector on the alignment index ($\sigma$) of the PU nanofibres. Table 4 shows that despite the flow rate, collector speed had a significant effect ($p$-value = 0.003 < 0.05) on the alignment of the nanofibres. Increasing the collector speed led to producing a more aligned nanofibrous structure (with decreasing the angular standard deviation).

Again, a Duncan test was performed to compare the means of the fibre alignment index in different collector speeds. Results of the Duncan test for the alignment of PU nanofibres have been summarised in Table 5. It could be seen that the maximum alignment (lower value of $\sigma$) was achieved in the collector speed of 500 r.p.m. The mean values listed under each subset comprised a set of means not significantly different from each other. These results indicated that the effect of the collector speed on the nanofibres alignment was significantly different with the speeds of 100, 300 and 500 r.p.m.. There




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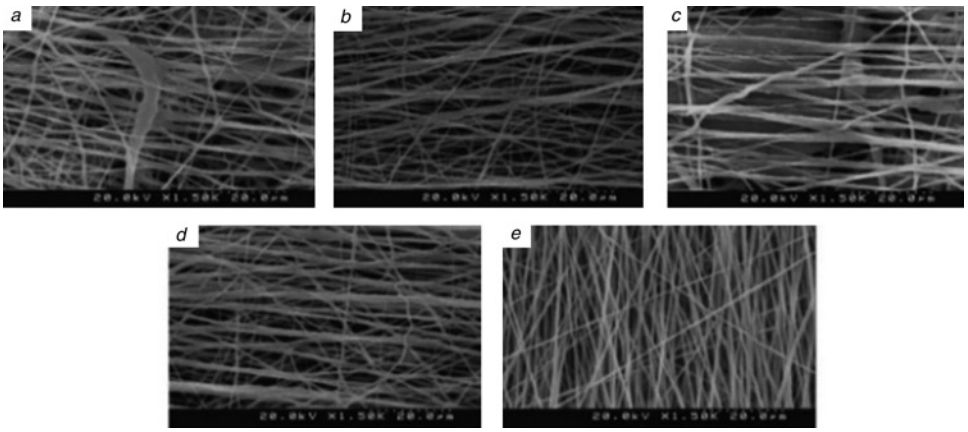


**Fig. 4** *FESEM images of PU nanofibres at the flow rate of 0.25 (ml/h) and different collector speeds*
*a* 100 r.p.m.
*b* 200 r.p.m.
*c* 300 r.p.m.
*d* 400 r.p.m.
*e* 500 r.p.m.

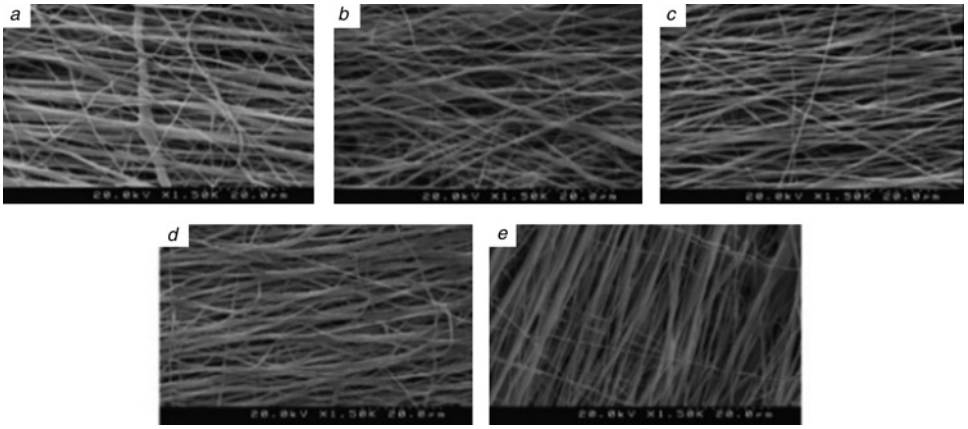


**Fig. 5** *FESEM images of PU nanofibres at the flow rate of 0.30 (ml/h) and different collector speeds*
*a* 100 r.p.m.
*b* 200 r.p.m.
*c* 300 r.p.m.
*d* 400 r.p.m.
*e* 500 r.p.m.

was also no significant effect on the fibres alignment with increasing the collector speed from 100 to 200 r.p.m., from 200 to 400 r.p.m. and from 400 to 500 r.p.m. The results showed that the collector speeds of 200 and 400 r.p.m did not significantly affect the orientation of the PU fibres. It could be concluded that the speed increment in the intervals of 200 r.p.m. could significantly change the alignment of the nanofibres.

In addition to the mechanical mechanism of the PU fibres alignment, the role of the PU microstructure should also be explained. Elasthane™ 55D is a thermoplastic polyether urethane (TPU). Fig. 6 indicates the chemistry of the polyether PUs. The thermoplastic PUs are multi-phase block copolymers composed of hard and soft segments. In these polymers, the hard segments act as physical crosslinks. At room temperature, the soft segments are above their glass transition temperature and are imparted in the rubbery behaviour of the material. In the solution state, the physical crosslinks will be broken, stretched and orientated along the tension direction exerted by the electrical field (in the electrospinning process). After the fibre formation (polymer solidifying), these

**Table 2** Results of ANOVA test for the diameter of PU nanofibres

| Source | Sum of squares | $d_f$ | Mean square | $F$ | Sig. ($p$-value) |
|---|---|---|---|---|---|
| flow rate | 37689.7 | 3 | 12563.2 | 10.95 | 0.000 |
| collector speed | 15567.8 | 4 | 3891.9 | 1.44 | 0.269 |

**Table 3** Results of Duncan test for the diameter of PU nanofibres

| Flow rate, ml/h | Replications, N | Subset for $\alpha = 0.05$ | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 0.15 | 5 | 577.4 | | |
| 0.20 | 5 | | 630.2 | |
| 0.25 | 5 | | 636.0 | |
| 0.30 | 5 | | | 699.8 |
| Sig. | | 1.000 | 0.790 | 1.000 |

**Table 4** Results of ANOVA test for the angular index of PU nanofibres

| Source | Sum of squares | $d_f$ | Mean square | $F$ | Sig. ($p$-value) |
|---|---|---|---|---|---|
| flow rate | 0.14 | 3 | 0.04 | 2.85 | 0.071 |
| collector speed | 0.25 | 4 | 0.06 | 6.36 | 0.003 |

**Table 5** Results of Duncan test for the alignment of PU nanofibres

| Collector speed, r.p.m. | Replications, N | Subset for $\alpha = 0.05$ | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 500 | 4 | 0.340 | | |
| 400 | 4 | 0.435 | 0.435 | |
| 300 | 4 | | 0.502 | |
| 200 | 4 | | 0.570 | 0.570 |
| 100 | 4 | | | 0.665 |
| Sig. | | 0.193 | 0.085 | 0.193 |

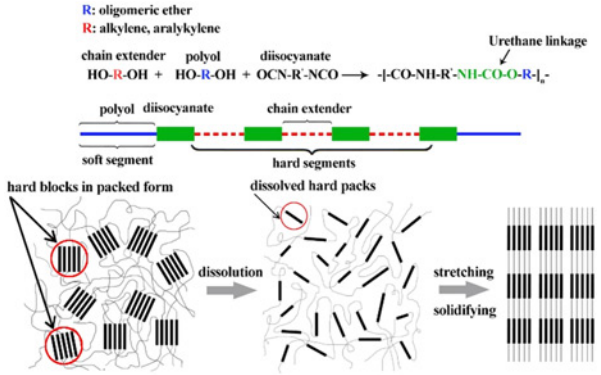


**Fig. 6** *Basic chemistry and alignment mechanism of TPU*

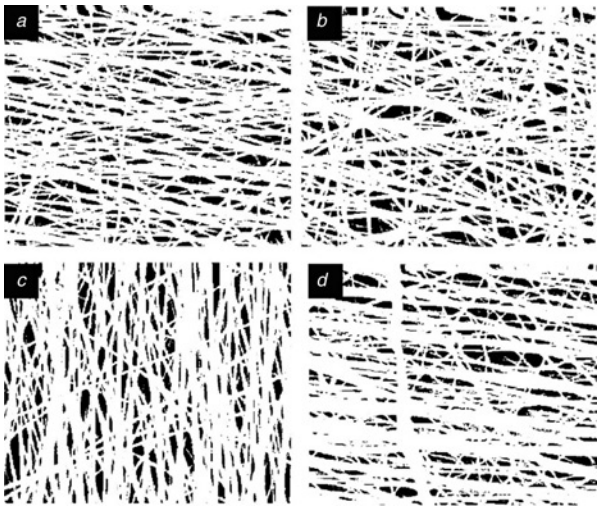


**Fig. 7** *Porosity measurement of different PU nanofibrous webs by image processing method*
*a* 0.15 (ml/h), 300 r.p.m.
*b* 0.20 (ml/h), 400 r.p.m.
*c* 0.25 (ml/h), 500 r.p.m.
*d* 0.30 (ml/h), 100 r.p.m.

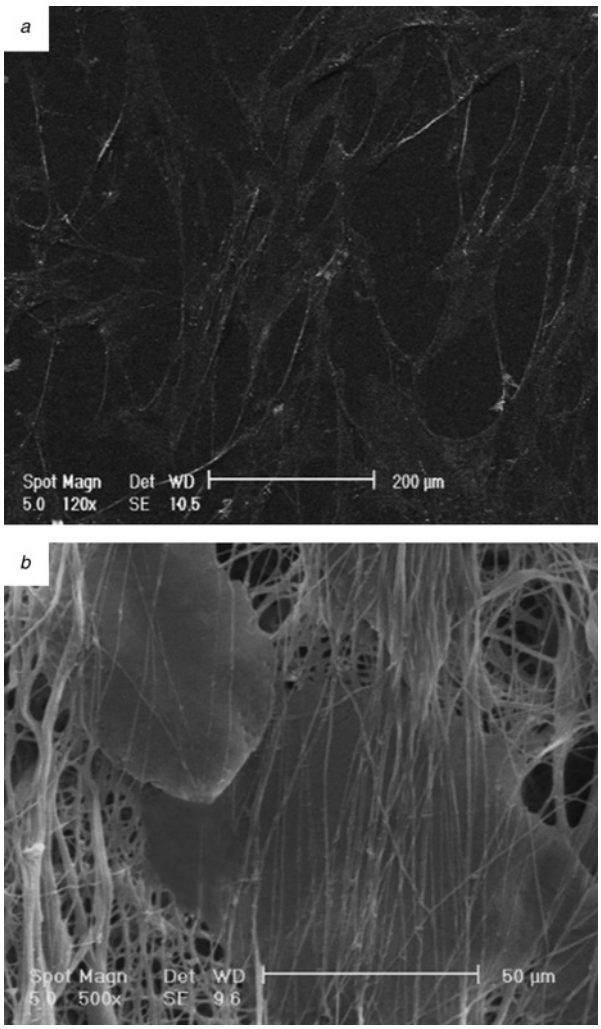


**Fig. 8** *Morphology of SHED stem cells after 7 days of seeding on*
*a* TCP
*b* Aligned PU nanofibrous web

crosslinks will repack and PU nanofibres will retain their elongated and aligned form.

Fig. 7 shows the results of the porosity measurement method applied in this study. The SEM images of the PU nanofibres were converted to the binary format and the porosity characteristic values of the different layers of the nanofibrous web were calculated. The results showed that the average porosity of 20 different scaffolds was (83 ± 1)%, (48 ± 2)% and (20 ± 1)% for the surface layer, the middle layer and the lower visible layers, respectively. The obtained values and their low standard deviation for all produced samples indicated that the electrospun nanofibres in all process conditions had approximately same porosity values. The high obtained value for surface porosity could be helpful for cell attachment and penetration into the internal layers of the scaffold to support the 3D shape production of the new generated tissue.

Fig. 8 illustrates the morphology of the stem cells on the scaffold and TCP. The SEM image shows the cell attachment and proliferation on the surface of the PU nanofibres (Fig. 8*b*). Fig. 8*b* demonstrates that the PU nanofibres could guide cell growth along their alignment direction. This feature could be useful for the regeneration of the elastic tissues, such as tendon and ligament that should support elastic properties in preferential directions.

The morphology of the SHED cells on the TCP (Fig. 8*a*) also revealed the effect of fibres alignment on the directional growth of the stem cells. It can be obviously seen that the seeded cells on TCP had no preferred growth direction.




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**4. Conclusion:** The PU nanofibres are used in specific tissue engineering applications such as tendon and ligament, vascular and artery grafts, oesophagus prosthesis etc. Alignment of the PU nanofibres is a factor of interest in some specific applications to allow the cell growth and proliferation in a directional manner. In this study, the effects of the electrospinning parameters, including the rotational speed of the collector and the flow rate of the PU/DMF solution, were on fibres diameter and alignments were investigated. Statistical analysis showed that the flow rate had a direct effect on the diameter of the PU nanofibres. Increasing the flow rate led to an increase in the fibres diameter. It was also found that increasing the collector speed in the intervals of 200 (r.p.m.) from 100 to 500 (r.p.m.) significantly affected the alignment of the nanofibres. The porosity measurements indicated that all samples had approximately same and high surface porosities (~83%) that could be helpful for cells penetration within the thickness direction of the nanofibrous scaffold. The interaction between cells and aligned PU nanofibres was also investigated by seeding the teeth-derived stem cells (SHED cells) on the surface of the scaffold. It was observed that the alignment of the nanofibres had an obvious effect on cells growth in the preferential direction (parallel to the alignment direction of the fibres). This could be useful for specific tissue engineering applications such as tendon and ligaments that require alignment of the seeded substrate.

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