Fabrication and characterization of low-cost, bead-free, durable and hydrophobic electrospun membrane for 3D cell culture

Hajar Moghadas[1],
Mohammad Said Saidi[1*] Email: mssaidi@sharif.edu,
Navid Kashaninejad[1,2],
Amir Kiyoumarsi Oskouei[1],
Nam-Trung Nguyen[2*] Email: nam-trung.nguyen@griffith.edu.au

[1]School of Mechanical Engineering
Sharif University of Technology
Tehran, Iran

[2]Queensland Micro- and Nanotechnology Centre, Nathan Campus
Griffith University
170 Kessels Road, Brisbane, QLD 4111, Australia

Abstract

This paper reports the fabrication of electrospun polydimethylsiloxane (PDMS) membranes/scaffolds that are suitable for three-dimensional (3D) cell culture. Through modification the ratio between PDMS and polymethylmethacrylate (PMMA) as carrier polymer, we report the possibility of increasing PDMS weight ratio of up to 6 for electrospinning. Increasing the PDMS content increases the fiber diameter, the pore size, and the hydrophobicity. To our best knowledge, this is the first report describing beads-free, durable and portable electrospun membrane with maximum content of PDMS suitable for cell culture applications. To show the proof-of-concept, we successfully cultured epithelial lung cancer cells on these membranes in a static well plate without surface modification. Surprisingly, due to three-dimensional (3D) and hydrophobic nature of the electrospun fibers, cells aggregated into 3D multicellular spheroids. These easily detachable and cost-effective scaffolds with controllable thicknesses and high tensile strength are good candidates for cell-stretching devices, organ-on-a-chip devices, tissue engineering and studies of non-adherent mammalian cancer stem cells.

Keywords

PDMS membrane
Electrospinning cell culture
Hydrophobic nanofibers
Spheroid formation
Beads-free scaffolds

1. Introduction

Advances in cell culture, tissue engineering and dynamic microfluidic devices (Kashaninejad et al. 2016; Nguyen et al. 2017) has been leading to an increasing demand for efficient cell culture platforms. A membrane as scaffold for cell culture is an essential component for most bioreactors. Polydimethylsiloxane (PDMS) is a good material choice for this membrane. The transparent, flexible and non-toxic polymer is permeable to oxygen and eliminates the need for an external oxygen generator in bioreactors for cell culture (Firpo et al. 2015). However, progress has been limited towards the fabrication of efficient PDMS membranes suitable for cell culture. To date, the fabrication techniques mainly rely on either direct microfabrication of PDMS (Chen et al. 2012) or soft-lithography from a master mould (Huh et al. 2013). However, these fabrication techniques are expensive, time-consuming and mostly require cleanroom facility and equipment. Most importantly, even in a more sophisticated cell-stretching platform for organ-on-a-chip study (Huh et al. 2010), cells assembled in a two-dimensional (2D) monolayer on the integrated PDMS membrane.

Electrospinning is considered as an alternative method to fabricate simple and cost-effective three-dimensional (3D) scaffolds for cell culture application. Because of the large surface area to volume ratio as well as their controllable physical and chemical properties, electrospun scaffolds are good candidates for many applications such as biomedical research (Stamatialis et al. 2008; Haerst et al. 2014), tissue engineering (Xue et al. 2014), environmental

remediation (Homaeigohar and Elbahri 2014), enzyme and catalyst support (Chen et al. 2013), filtration media (Kaur et al. 2014; Matulevicius et al. 2014), military protective clothing (Raza et al. 2014; Serbezeanu et al. 2015), electronic and chemical sensors (Fang et al. 2014; Kim et al. 2014), reinforced nanocomposites (Carrizales et al. 2008), and protein microarrays (Yang et al. 2009). During the electrospinning process, a polymer solution is discharged from the tip of a needle, which is connected to a syringe pump. Due to the surface tension, a pendant droplet is formed. At the same time, a high voltage power supply induces a strong electric field. The electrostatic force overcomes the surface tension and eventually causes the polymer solution to erupt from the surface. Following the evaporation of the solvent, the electrospun fibers deposit and remain on the collector surface. Depending on the polymer and the adjustable operation parameters, the scaffolds with a wide range of mechanical properties can be produced (Pham et al. 2006; Sill and von Recum 2008).

Despite the advantages, the main obstacle of PDMS electrospinning is its relatively low viscosity. As a result, PDMS itself cannot be spun through the conventional electrospinning process. The polymer should be spun in combination with other polymers or by other techniques such as core shell electrospinning (Xue et al. 2014). In a pioneering work, Tungrapa and coworkers blended PDMS in a mixture of poly (ethylene glycol)(PEG)-PDMS-PEG with polyethylene oxide (PEO) which resulted in relatively rough fibers (Tungprapa et al. 2006). By adopting the sol-gel process, cross-linked PDMS as non-uniform fibers with either diameters larger than 10 μm (Kim et al. 2009) or amino-functionalized PDMS with a diameter of 125 μm were formed with electrospinning (Boyacı et al. 2013). Furthermore, PDMS combination with silicone-urea copolymer through a relatively complex synthesis process resulted in fibers with porous surfaces (Yilgor et al. 2012). In another work, polyacrylonitrile-graft-poly (dimethyl siloxane) was applied to create super-porous fibers (Bayley and Mallon 2012). Recently PDMS as core and polyvinylpyrrolidone (PVP) as sheath layer were spun by modified core-shell method which resulted in rough but extraordinary elastic scaffold (Niu et al. 2014). Later, PDMS in the form of silicone-acetone diluted solution spun as very rough fibers (Haerst et al. 2014).

For cell culture application, the polymers for electrospinning should be biocompatible. Yang *et al.* showed that PMMA powder dissolved in a solution of tetrahydrofuran (THF) and dimethylformamide (DMF) can be used a carrier polymer for successful PDMS electrospinning.

However, the weight and volume ratios used in that work led to production of fragile PDMS/PMMA nanofibers and required a glass support. The material also needed additional heat treatment to cross-link the electrospun fibers. The maximum possible weight ratio of PDMS to PMMA was low. Later, Igreja *et al.* used this electrospun fibers with PDMS to PMMA weight ratio of 3 as chemical sensitive layers on transducers (Igreja et al. 2013). Adjusting the electrospinning parameters such as voltage and injection rate can form an efficient membrane for water desalination (Ren et al. 2017).

In all reported works, additional procedures such as heat treatment (Niu et al. 2014) and vacuum pump (Yang et al. 2009; Igreja et al. 2013) were needed to separate the scaffold from the collector and to remove the excess solvent. Moreover, most of the PDMS electrospun fibers were either non-uniform beaded fibers, and usually have non-homogenous porous surfaces. A PDMS: PMMA ratio of 3:1 was the highest amount of PDMS ever used for relatively smooth and beads-free electrospun fibers (Yang et al. 2009). Therefore, there is a need to fabricate a cost-effective, biocompatible, beads-free, robust electrospun membrane, specifically suitable for cell culture application.

In this paper, the scaffolds are fabricated from a variety of PDMS to PMMA weight ratio from 1:1 to 6:1. PMMA is mainly used as a polymer carrier. As a major novelty, the fabricated scaffolds peeled off very easily from the collector. Hence, additional procedures such as heat treatment or vacuum pump are not required. The significant characteristics of the fabricated scaffold such as hydrophobicity, strength, flexibility, integrity, scaffold width and mobility were examined. Moreover, the relationship between the PDMS content and the diameter of the fiber as well as its wetting properties were evaluated. The main purpose of the paper is fabricating a scaffold with high PDMS content to benefit from the advantageous properties of PDMS such as flexibility, strength, transparency and mobility. We also investigated the effect of the increasing PDMS content on the hydrophobicity of the scaffold and on the subsequent cell culture application.

2. Materials and methods

2.1. Materials

PDMS (Sylgard 184 silicone elastomer kit) and PMMA (M_W = 350,000) were purchased from Sigma-Aldrich, USA. Tetrahydrofuran (THF) and dimethylformamide (DMF) were obtained from Merck KGaA, 64,271 Darmstadt, Germany.

Human lung cancer epithelial cell line (A549) was obtained from Sharif Biotechnology Laboratory. Dulbecco's Modified Eagle's medium (DMEM)/ F-12 (Cat. No. 11320033) and fetal bovine serum (FBS) (Gibco, 10,270–106) were purchased from Thermo Fisher Scientific, USA. Penicillin-Streptomycin (P4333–100 ml), phosphate buffered saline (PBS), glutaraldehyde and 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) were purchased from Sigma-Aldrich, USA.

2.2. Preparation of carrier polymer

PMMA was chosen as the carrier polymer, due to its biocompatibility for cell culture application. Dry PMMA powder has to be dissolved first in a solvent. We first chose THF as the solvent for PMMA. THF is an organic and volatile liquid, which can dissolve most plastic solvents. The volatile property of THF is highly desirable, as this solvent evaporates quickly after electrospinning and does not affect the chemical composition of PDMS/PMMA membrane for later cell culture. We dissolved the PMMA powder in THF with different concentrations ranging from 4 to 20% by weight then stirred gently for 24 h at room temperature of 25 °C. As discussed in Section 3, PMMA: THF electrospun scaffolds are not suitable for cell culture. The best possible result was obtained at a concentration of 6%. We subsequently added another organic liquid, DMF, as a co-solvent while PMMA concentration was fixed at 6% in the solution. A high concentration of DMF in culture media had been proven to be cytotoxic (Jamalzadeh et al. 2016). Therefore, we did not increase the volume concentration of DMF, but increased the volume concentration of THF. Accordingly, THF: DMF volume ratios of 1:1 and 2:1 were examined. The most satisfying result was obtained with 6% PMMA in 2:1 of THF: DMF. Finally, this optimized combination was chosen as a carrier polymer for electrospinning of PDMS.

2.3. Electrospinning procedure

We successfully conducted the electrospinning procedure for a variety of concentrations of both carrier polymer (either PMMA dissolved in THF or PMMA dissolved in THF:DMF mixture in concentrations explained in previous part) and PDMS:PMMA:THF:DMF solutions. In each experiment, all the parameters related to electrospinning process were fixed. These parameters include solution volume, flow rate, voltage and working distance. Fig. 1 shows the setup with 5 ml polymer solution was loaded in a syringe pump. The flow rate generated by the syringe pump was adjusted to 1 ml/h. A high DC voltage generator was used to provide 18 KV. To this aim, an alligator clip was used to connect the metal tip of the syringe needle (the anode) to an aluminum foil mounted on the ground (the cathode). The distance between the needle tip and the aluminum foil was fixed to 15 cm. The aluminum foil was covered on the cylindrical collector with a diameter of 10 cm.

Fig. 1 The electrospinning system used in our experiments. A volume of 5 ml polymer solution (PMMA:THF, PMMA:THF:DMF or PDMS:PMMA:THF:DMF) was loaded in a syringe connected to a syringe pump (flow rate fixed at 1 ml/h). A high DC voltage generator imposed 18 KV between the syringe needle tip and the aluminum foil connected to the ground. The first inset shows how easily the fibers can detach from the collector. The second inset shows the SEM image of bead-free electrospun PDMS: PMMA membrane

2.4. Surface characterization

Preliminary observation of the fibers was performed by an optical microscope (Optika - B350). The scaffold was evaluated via scanning electron microscopy (SEM, Leica 440) at a working distance of 39 mm and an accelerating voltage of 25 kV. The average fiber diameter and pore size were calculated using ImageJ software by measuring at least 100 objects that were selected randomly.

2.5. Porosity measurement

Porosity (ε) is defined as the ratio of pores volume (V_p) to the total electrospun membrane (EM) volume (V_t). With a known membrane volume and fiber mass), porosity can be simply calculated as $\varepsilon = 1 - m_f/\rho_f V_t$ where ρ is the density of the EM fibers. The 1-mm thick EM was cut into 10 × 10 mm pieces. The mass (m and the volume (V_t) of each EM peace were then measured. The difference between the density of PDMS and PMMA is less than 2%. So the fibers density was estimated as the average of two polymers densities.

2.6. Tensile test

The mechanical tensile strength of the $1 \times 5 \times 20 -$ mm EM specimen was evaluated by Hounsfield H10KS tensile testing machine (Test Resources Company). The EM thickness was measured by a micrometer screw gauge.

2.7. Contact angle measurement

A customized set up was designed to measure the values of the contact angle (CA) and the wettability of the EM. To minimize the effect of gravity on the droplet shape, the volume of the droplets was fixed at 3 μL, where the corresponding Bond number (Bo = 0.2) is less than unity. A micropipette was used to generate the desired volume from deionized (DI) water. The DI water droplet was placed very gently on the surface of the EM to minimize the impact. Once the droplet stabilized, the side view of the droplet was captured with a high-resolution camera (USB 8LED 50-500X 2MP Digital Microscope Endoscope Magnifier Video). Subsequently, the captured images were analyzed using ImageJ software. To ensure the measurements repeatability, the left and right CAs of 5 droplets were measured and the averaged value was evaluated.

2.8. Cell culture

To prepare the culture medium, DMEM was supplemented with 10% FBS to provide the necessary proteins and growth factor for the cells. To avoid bacterial contamination, it was further mixed with 100 unit/ml penicillin and 100 µg/ml streptomycin. Several portions of the EM with dimensions of 0.5 × 0.5 cm were sterilized by pure isopropyl alcohol and then washed with PBS for three times. The culture remained below a biological hood for about 2 h to become completely dry. Then, they were placed on 24-well plate and, 20 µl of the cell suspension with a density of 5×10^5 cells/ml was added to each portion of the EM. After 1 h, 48 µl of the culture medium was very gently added to the cultured cells on the EM. Due to non-adhesive nature of the EM, any flow of the medium could disturb cell configurations on the substrate. A 24-well plate was incubated and the medium was changed every 24 h. To conduct the cell imaging, each EM portion was immersed in 4% glutaraldehyde for 4 h then washed with PBS. The light turned off and, DAPI (10 µg/ml in PBS) was added to the EM. After one minute, the EM was again carefully washed and observed under a fluorescence microscope.

3. Results and discussions

3.1. Characterizations of electrospun membrane

For all different concentrations of PMMA dissolved in THF from 4 to 20%, the SEM images confirmed that the fibers contained several beads. The beads grow as the concentration increased. At concentrations lower than 4%, snow-like particles erupted from the needle (Fig. **2**a) whereas at concentrations higher than 20%, a few micrometers fibers were formed (Fig. **2**b). The lowest bead density corresponded to the case of 6% (Fig. **2**c). Magnified image in Fig. **2**d, demonstrates that beads with 10-µm diameter are relatively large compared with 1-µm fibers. That is not suitable for cell culture application because the beads are in order of the cell diameter and even higher which can disturb cell analyzing.

Fig. 2 The Morphology of the electrospun fibers at different PMMA: DHF concentrations. a) Snow-like particle at a concentration of 3%; b) Separated micrometer fibers at a concentration of 20%; c) The lowest possible beaded fibers obtained at 6%; d) An enlarged view of the beads with 10-μm diameters.

Accordingly, we added DMF solvent while kept the PMMA concentration constant at 6%. Finally, beads-free electrospun fibers were produced when 6% PMMA was dissolved in 2:1 (THF: DMF) as shown in Fig. 3a.

Fig. 3 SEM images of PDMS electrospun membrane with different PMMA: PDMS weight ratios as indicated in each image. a) 1:0; b) 1:1; c) 1:2; d) 1:3; e) 1:4; f) 1:5 and g) 1:6. In all the experiments, PMMA as a carrier polymer was dissolved in 2:1 (THF: DMF) in a concentration of 6%.

We also tried 10% PMMA concentration in 2:1 (THF: DMF). However, not only the fibers contained some beads, but heat treatment was also required to cross-link and cure the fibers after the electrospinning process. This was consistent with the work reported by Yang *et al.* where they dissolved 10% PMMA in 1:1 (THF: DMF) and then dried the fibers at 70 °C for 2 h (Yang et al. 2009). All results reported in this paper are obtained at room temperature without any heat treatment.

After obtaining the desired carrier polymer, we added PDMS into the PMMA: THF: DMF solution. Fig. 3b-g shows the SEM images of such EM with different PDMS weight ratios ranging from 1 to 6. This figure illustrates the beads-free, smooth and homogenous fibers without any cracks or small holes. By increasing the amount of PDMS, the morphology of the fibers changes from relatively straight and flat structures to curved and curly ones. However, increasing the amount of PDMS itself did not generate any beads.

In addition, as shown in Fig. 4a-b, increasing the PDMS content led to more transparent electrospun fibers. Furthermore, the PMMA fibers are fragile and damaged during detachment from the collector. Whereas adding PDMS led to the production of flexible fibers which were easily detached from the aluminum foil as shown in Fig. 4c.

Fig. 4 By increasing the amount of PDMS, fibers diameters become both larger and more transparent. a) Electrospun fibers from pure PMMA are thin and opaque; b) Electrospun fibers with a ratio of 1:6 of PMMA: PDMS are thicker and more transparent; c) Durable electrospun membranes are easily detached from the aluminum foil

Table 1 summarizes the characteristics of the evaluated EMs. The fiber average diameter and the pore size increase significantly as the PDMS content increases, while the porosity increased slightly. The thickness of the fabricated EM varied from 30 to 100 µm, which can be adjusted by changing the collector area and/or time of the electrospinning process.

Table 1 Electrospun membrane characterization

PMMA:PDMS ratio (*w*/w)	Diameter of fiber (µm)	Average pore size (µm)	Max pore size (µm)	Porosity (%)
1:0	1.15	3.9	11.2	90.7
1:1	1.1	4.8	13.3	91.0
1:2	1.6	6.4	13.6	91.0
1:3	1.8	7.1	14.4	91.4
1:4	2.1	7.9	15.4	91.5
1:5	2.8	8.9	17.2	91.6
1:6	3.6	9.6	18.7	91.7

Figure 5a shows the typical tensile curve of an EM. The curve has many fluctuations due to the rupture of the fibers while the force was applied. A comprehensive investigation of such

phenomenon was reported previously by Andersson *et al.* (Andersson et al. 2014). To compare the data of different EMs, the corresponding fitting curves of the stress-strain characteristics are plotted in Fig. 5b. The maximum strain of pure PMMA fabrics is almost 20% whereas the elasticity of the polymer mixture is much higher (its strain is up to 40%). There is no clear correlation between the polymers ratio and the ultimate tensile stress. That is because not only the polymers ratio but also the structures and orientations of the fibers affect the mechanical properties of an electrospun membrane.

Fig. **5** (a) Force-extension curve of an EM with 1:2 (PMMA: PDMS) weight ratio; (b) Stress–strain curves for EMs at different PMMA: PDMS weight ratios

The dispensed water droplets on the EMs show remarkable hydrophobicity. A sheet of PMMA is moderately hydrophilic (with water CA of 68°), while PDMS is slightly hydrophobic (with water CA of 106°). However, their electorspun copolymer scaffold showed higher water-repellency as shown in Fig. 6. Interestingly, water CA of pure PMMA electrospun scaffold is 124.5° which is 83% larger than its solid surface. The average increase of CA on the EM with 1:1 (PMMA: PDMS) is 4.5° compared to the EM with 1:0 (PMMA: PDMS). Adding PDMS tends to increase the hydrophobicity of the electrospun scaffold. The figure also indicates that CA increases with increasing PDMS concentrations until the ratio of 1:4 (PMMA: PDMS) and then decreases. This pattern reveals that both the PDMS content and the surface morphology contribute to the surface hydrophobicity. At a PDMS content higher than 1:4, the diameter of the fibers increases and the solid fraction of the surface in contact with water increases. According to Cassie-Baxter equation, when droplet is bounced on a surface with air cavity, increasing the effective area of the solid in contact with droplet (decreasing void fraction) leads to a higher wettability (Cassie and Baxter 1944). Nonetheless, the diameter of the electrospun fibers is also a function of electrospinning parameters such as voltage difference, flow rate and needle tip-to-collector distance. Here, we systematically varied the polymer concentrations while other electrospinning parameters were fixed.

Fig. 6 The CAs of the fabricated scaffolds as a function of PDMS weight ratios

Fig. 7 (a) The movement of a given loose cell is marked with a pink arrow. The flow direction is shown by a purple arrow. Two fixed cells are demonstrated by a blue circle as a reference point; (b) The fluorescent images of the spheroid formation i) 24 h, ii) 72 h, iii) 96 h and, iv) 172 h after cell seeding

3.2. Spheroid formation on electrospun membranes

To validate the biocompatibility of the fabricated electrospun membranes, the epithelial lung cancer cells (A549) were cultured successfully on these surfaces. A very low density of cell suspension was seeded on these scaffolds. Due to the non-adhesive nature of these electrospun membranes (as quantified by CA), the cells adhered loosely to the surfaces. After 12 h, a relatively low flow rate could remove the cells from the substrate. The movement of a loose cell is traced in Fig. 7a. Since the electrospun membranes are hydrophobic, cells do not firmly adhere to them. Therefore, to keep the cells motionless and provide a suitable condition for proliferation, the culture medium was changed very carefully from the surfaces. The formation of the cell spheroids are shown in Fig. 7b. As the microenvironment provided by the EM is suitable for living cell, cells started division and proliferation. The hydrophobicity of the substrate restricted cell adhesion to the surface, therefore, divided cells aggregated to each other and formed a spheroid which is shown in Fig. 7b-iv. We believe that multicellular spheroid generation from a single cell is closer to *in vivo* condition. Detailed investigation of the tumor generation from a single cell would provide valuable information about the multiple aspects of the tumor microenvironment.

4. Conclusion

Previous cleanroom-based PMMA membranes led to two-dimensional (2D) cell monolayer. Though suitable for 3D protein microarrays or water desalination, reported PDMS electrospun

membrane (EM) was not suitable for cell culture. Mainly, maximum PDMS weight ratio on this EM had been low (up to 3) and was not durable enough to be used as standalone membranes. In addition, the presence of beads on this EM is detrimental for cell culture. In some cases, additional procedure such as heat treatment was necessary to separate the electrospun fibers from the collector. The current paper reports the fabrication of flexible, robust and beads-free membranes with high PDMS content. The process is based on the cost-effective electrospinning technique. These electrospun membranes can be separated from the collector easily without any heat treatment or vacuum pump. By increasing the amount of PDMS from 1 to 6, the diameter of fibers increases and their physical shape altered from straight to curved forms. The fabricated scaffold is almost superhydrophobic with a contact angle of around 138.5°. The obtained results also indicated that the contact angle could increase by increasing PDMS concentrations until a threshold (in PDMS weight ratio 4). Further increasing the PDMS ratio increases the wettability of the membrane. Therefore, both the PDMS content and the surface morphology contributed to the surface hydrophobicity. Most importantly, we showed that these electrospun membranes can provide a 3D scaffold for cell culture. In fact, the hydrophobicity of the electrospun membrane restricts cell adhesion and leads to the formation of cell spheroids from single cells. This can provide valuable insights into multiple aspects of tumorigenesis. These electrospun membranes can be an effective alternative to other costly and two-dimensional cell culture platform. In this study, we showed that the membrane can be easily incorporated into conventional static cell culture well plates. In the near future, we will investigate their potential use for dynamic cell culture such as cell-stretching devices.

Acknowledgments

We acknowledge the funds and support from Iran’s National Elite Foundation (INEF).

Author Contributions

All authors contributed to conducting the experiments and writing the manuscript.

Compliance with ethical standards

Conflicts of Interest

The authors declare no conflict of interest.

References

R. L. Andersson, V. Ström, U. W. Gedde, P. E. Mallon, M. S. Hedenqvist and R. T. Olsson, Sci. Rep. 4, 6335 (2014)

G. M. Bayley and P. E. Mallon, Polymer. 53, 5523 (2012)

E. Boyacı, N. Horzum, A. Çağır, M. M. Demir and A. E. Eroğlu, RSC Adv. 3, 22261 (2013)

C. Carrizales, S. Pelfrey, R. Rincon, T. M. Eubanks, A. Kuang, M. J. McClure, G. L. Bowlin and J. Macossay, Polymer. Adv. Tech. 19, 124 (2008)

A. Cassie and S. Baxter, Trans. Faraday Soc. 40, 546 (1944)

M. Chen, C. Wang, W. Fang, J. Wang, W. Zhang, G. Jin and G. Diao, Langmuir. 29, 11858 (2013)

W. Chen, R. H. W. Lam and J. Fu, Lab Chip. 12, 391 (2012)

J. Fang, H. Shao, H. Niu and T. Lin, in *Handbook of Smart Textiles*, ed. By X. Tao (Springer, Singapore, 2014), p. 1

G. Firpo, E. Angeli, L. Repetto and U. Valbusa, J. Membr. Sci. 481, 1 (2015)

M. Haerst, V. Seitz, M. Ahrens, C. Boudot and E. Wintermante, in 6th Euro. Conf. Int. Fed. Med. & Bio. Eng. (Dubrovnik, Croatia, 2014), pp. 537–540

S. Homaeigohar and M. Elbahri, Materials. 7, 1017 (2014)

D. Huh, H. J. Kim, J. P. Fraser, D. E. Shea, M. Khan, A. Bahinski, G. A. Hamilton and D. E. Ingber, Nat. Protoc. 8, 2135 (2013)

D. Huh, B. D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Y. Hsin and D. E. Ingber, Science. 328, 1662 (2010)

R. Igreja, H. Domingos, J. P. Borges and C. Dias, Mater. Sci. Forum. 730-732, 197 (2013)

L. Jamalzadeh, H. Ghafoori, R. Sariri, H. Rabuti, J. Nasirzade, H. Hasani and M. R. Aghamaali, Avicenna J. Med. 4, 1 (2016)

N. Kashaninejad, M. R. Nikmaneshi, H. Moghadas, A. Kiyoumarsi Oskouei, M. Rismanian, M. Barisam, M. S. Saidi and B. Firoozabadi, Micromachines. 7, 130 (2016)

S. Kaur, S. Sundarrajan, D. Rana, R. Sridhar, R. Gopal, T. Matsuura and S. Ramakrishna, J. Mater. Sci. 49, 6143 (2014)

I.-D. Kim, S.-J. Choi and W.-H. Ryu, In *Handbook of Nanomaterials Properties*, ed. By B. Bhushan, D. Luo, S.R. Schricker, W. Sigmund, S. Zauscher (Springer, Berlin, 2014), p. 793

Y. B. Kim, D. Cho and W. H. Park, J. Appl. Polym. Sci. 114, 3870 (2009)

J. Matulevicius, L. Kliucininkas, D. Martuzevicius, E. Krugly, M. Tichonovas and J. Baltrusaitis, J. Nanomater. 2014, 14 (2014)

N.-T. Nguyen, M. Hejazian, C. H. Ooi and N. Kashaninejad, Micromachines. 8, 186 (2017)

H. Niu, H. Wang, H. Zhou and T. Lin, RSC Adv. 4, 11782 (2014)

Q. P. Pham, U. Sharma and A. G. Mikos, Tissue Eng. 12, 1197 (2006)

A. Raza, Y. Li, J. Sheng, J. Yu and B. Ding, in *Electrospun Nanofibers for Energy and Environmental Applications*, ed. By B. Ding, J. Yu (Springer, Berlin, 2014), p. 355

L.-F. Ren, F. Xia, J. Shao, X. Zhang and J. Li, Desalination. 404, 155 (2017)

D. Serbezeanu, A. M. Popa, T. Stelzig, I. Sava, R. M. Rossi and G. Fortunato, Text. Res. J. 85, 1763 (2015)

T. J. Sill and H. A. von Recum, Biomaterials. 29, 1989 (2008)

D. F. Stamatialis, B. J. Papenburg, M. Gironés, S. Saiful, S. N. Bettahalli, S. Schmitmeier and M. Wessling, J. Membr. Sci. 308, 1 (2008)

S. Tungprapa, I. Jangchud, P. Ngamdee, M. Rutnakornpituk and P. Supaphol, Mater. Lett. 60, 2920 (2006)

R. Xue, P. Behera, J. Xu, M. S. Viapiano and J. J. Lannutti, Sens. Actuator B-Chem. 192, 697 (2014)

D. Yang, X. Liu, Y. Jin, Y. Zhu, D. Zeng, X. Jiang and H. Ma, Biomacromolecules. 10, 3335 (2009)

E. Yilgor, O. Kaymakci, M. Isik, S. Bilgin and I. Yilgor, Appl. Surf. Sci. 258, 4246 (2012)