FACTA UNIVERSITATIS
Series: **Mechanical Engineering** Vol. 1, N^{o} 10, 2003, pp. 1 - 2

SURFACE TREATED AND FIBRIN COATED ELECTROSPUN POLYACRYLONITRILE FIBER FOR ENDOTHELIAL CELL GROWTH AND PROLIFERATION

Nur Syazana Rashidi[1], Irza Sukmana[2,*], Agung Mataram[3], Noor Jasmawati[1], Mohd Ramdan Mohd Rofi[1], Mohammed Rafiq Abdul Kadir[1]

[1]Medical Devices and Technology Group (MEDITEG), Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia
[2]Department of Mechanical Engineering, Faculty of Engineering, University of Lampung, Bandar Lampung 35143, Indonesia
[3]Department of Mechanical Engineering, Faculty of Engineering, University of Sriwijaya, Ogan Ilir, South Sumatera, Indonesia

Abstract. *Polyacrylonitrile (PAN) is a synthetic biocompatible polymer that has been utilized as filtering membrane in hemodialysis and enzyme immobilization purposes. However, its potential usage in other medical applications is limited due to its poor hydrophilicity. To overcome this problem, two groups of electrospun PAN fibers were treated with sodium carbonate (Na_2CO_3) and sodium hydroxide (NaOH) respectively at 100^{o}C for 5 minutes. Fibrin gel was then coated to the treated samples before seeding with human umbilical vein endothelial cells (HUVECs) for 1 and 3 days. X-ray diffraction results showed increased crystallinity of PAN fibers when treated with Na_2CO_3 and NaOH. Contact angle measurements showed that the hydrophilicity of Na_2CO_3 treated and NaOH treated samples improved from 115° to 88° and 64° respectively. Fourier transform infrared spectroscopy confirmed their hydrophilicity was due to the existence of carboxyl and hydroxyl groups. However, tensile strength of PAN fibers reduced by 34% when treated with Na_2CO_3 and 42% when treated with NaOH. Cytotoxicity tests showed increased absorbance in day 3 for both treated samples. The absorbance value for NaOH treated PAN fibers maintained until day 7 while Na_2CO_3 treated PAN fibers showed a slight increase in absorbance on day 7. In vitro tests showed increased cell adhesion and proliferation after 3 days of culture. PAN treated fibers coated with fibrin are therefore proven to attract HUVEC cells and promote endothelialization.*

Key words: *Polyacrylonitrile fibre, surface treatment, scaffold, endothelial cells*

[*]**Received:**
Corresponding author: Irza Sukmana
Affiliation: Department of Mechanical Engineering, Faculty of Engineering, University of Lampung, Jl. Prof. Soemantri Brojonegoro No. 1, Bandar Lampung 35143, Indonesia
E-mail: irza.sukmana@eng.unila.ac.id

1. INTRODUCTION

Due to its high biomechanical strength, thermal stability, tolerance to solvents as well as being resistant to bacteria and photo irradiation effects, Polyacrylonitrile (PAN) has been used extensively to produce various manufactured materials [1]. These include products such as commercial carbon fibers, filtration [2], adsorption [3], as well as weaved products such as blankets, carpets and clothes [4]. PAN is also proven as a biocompatible material and it is suitable for biomedical purposes such as tissue engineered bioartificial skin [5], hemodialysis [6], and biocatalysts immobilization [7].

However, due to its poor hydrophilicity, the use of PAN without surface treatment appears limited. Surface treatments to overcome this issue have been previously described. These include methods such as plama treatment [8-10], plasma initiated graft polymerization [11,12], and photoinduced grafting [13]. These methods nevertheless have inherent limitations. Compounded by the high cost involved in the treatment process, many of these methods have been deemed impractical for large scale manufacturing purposes [14]. It has been suggested that the use of Na_2CO_3 and NaOH solutions in Millipore-purified water can be used to provide similar effects without involving exorbitant cost [15,16].

The promotion of endothelial adhesion and vascularization to prevent thrombosis as well as intimal hyperlasia of small diameter vascular graft has been tested to the PAN fibres. Previous studies showed that without the fibrin coating process, cell differentiation onto the material surfaces would be non-existence thus rendering the material less useful for vascularization of an engineered tissue substitute [17]. The role of fibrin actively directs cellular responses through specific receptor-mediated interactions with endothelial cells of the vessel wall [18]. Therefore, the use of fibrin as a coating active matrix onto a bioactive scaffold to support endothelialization is of interest. However there is no study yet showing electrospun PAN fibers with Na_2CO_3 and NaOH surface treatment followed by fibrin coating can form endothelial cells monolayer for blood vessel.

Surface treatment using NaOH nevertheless is already established for PAN materials by authors mostly in ultrafitration and haemodylisis applications [19,20]. However, the effects of sodium carbonate treatment on the PAN mechanical and physical properties have not been intensively studied compared with NaOH surface treatment. Research on Na_2CO_3 surface treatment onto PAN only covered hydrolysis part while biocompatibility was not addressed before [15,20]. The present study aims to analyze hydrophilicity of treated PAN fibers with Na_2CO_3 and NaOH. PAN treated surfaces then were coated with fibrin gel to provide covalently attached bioactive compound. Therefore, the present study needs more cell attachment in order for future tissue engineering and we have managed to develop a new technique utilizing fibrin gel coating on treated PAN fiber. This study also determines change in biophysical surface appearance, surface chemistry and biomechanical properties, as well as bioactivity and biocompatibility of PAN fibres before and after undergoing surface treatment followed by fibrin coating.

2. MATERIALS AND METHODS

2.1 Materials

Polyacrylonitrile (PAN; Mw=150,000; 53.06 g/mol), N,N-dimethylformamide (DMF; 73.10 g/mol) and acrylamide (AM; 71.08g/mol) were bought from Aldrich Chemical and were used without further purification. Other materials are Sodium hydroxide (NaOH; Sigma Aldrich, USA), Sodium bicarbonate (Na_2CO_3; Sigma Aldrich; USA), Fibrinogen and Thrombin (Sigma Aldrich; USA).

2.2 PAN fiber preparation

The random PAN fibers were developed through electrospinning under optimum conditions. Briefly, 14% (w/v) of PAN was dissolved in a DMF solution. This solution was loaded into a 5 mL glass syringe and the flow rate of 2 $mL.h^{-1}$ was controlled by syringe pump. A high voltage (21 kV) was applied using a high voltage regulated DC power supply (Model ES 30P-5W, Gamma High Voltage Research, Ormond Beach, FL). A piece of aluminum foil was placed towards the tip at a distance of 12 cm as grounded collector. Afterwards, the electrospun fibers were peeled off from the aluminum foil and the residual solvent was released in an oven at 40^{o}C. The dried electrospun fibers were then stored in desiccator prior to further processes.

Electrospun PAN fibers mat was cut by 2 cm x 2 cm. 1% w/v aqueous solution of Na_2CO_3 was prepared. PAN fiber was dropped into boiling Na_2CO_3 at temperature 100^{o}C for 5 minutes and next was rinsed with plenty of water until pH~7. The substrate then was dried at ambient temperature (T~25^{o}C) for 24 hours. This step was repeated for NaOH surface treatment. PAN fiber was sterilized by immersing the fiber in 10% penicillin-streptomycin under ultraviolet light (UV) for 15 minutes. Next, the substrate was dried under UV for 1 hour.

In 24 well plates, fibrin gels were prepared using 500 µl/well of fibrinogen solution (2.0 $mg.mL^{-1}$) made in phosphate buffered saline (PBS). This solution was directly mixed with 1 mg / 500 µl (100 $U.mL^{-1}$ in PBS) for polymerization process of fibrinogen into fibrin. PAN fiber after treated with Na_2CO_3 and NaOH was transferred and deposited in the prepared fibrin gel. Fibrin-coated PAN fiber was then stored in incubator at 4^{o}C overnight. The substrate then was dried on filter paper under room temperature in sterile condition.

Human umbilical vein endothelial cells (HUVECs) were cultured in endothelial cell growth (Promo Cell) supplemented by 1% antibiotic penicillin-streptomycin (Gibco, USA). The cells were replaced twice a week and cultures were maintained at 37^{o}C in incubator containing 5% CO_2. HUVEC between passages 3 and 5 were used in all experiments. After coating process, sterile fibrin-coated PAN fibers treated with Na_2CO_3 and NaOH were seeded with HUVECs at a density of $3x10^4$ cells/cm^2 and observed for day 1 and day 3.

2.3 Surface characterization

The morphology of the fabricated scaffolds before and after surface treatment and the coated PAN fibers were observed by a high-resolution field emission scanning electron

microscope (FESEM; *ZEISS Supra 35VP*), operated at an acceleration voltage of 10 kV. The fibers diameters were measured from the SEM (JEOL, JSM-6390LV) micrographs measured randomly within an average of 50 fibers. Composition of PAN fiber before and after surface treatment and coating was determined from Energy dispersive X-ray spectroscopy (EDS).

Fourier transformed infrared (FTIR) spectra of the fibers were collected on Perkin–Elmer Spectrum 2000 to analyze the chemical structure of electrospun PAN fiber over a range of 400-4000 cm^{-1} with typically 32 scans at a resolution of 4 cm^{-1}. Also, the crystallinity of sample was characterized by x-ray diffraction (XRD) (D5000, Siemens, Germany) in a thin film mode at 40 kV and 40 mA. The data were recorded in 2θ range using CuKα radiation of 1.5406 Å.

Water contact angle of PAN fibers before and after surface treatment were measured using VCA Optima (AST Product, Inc.) mounted with a CCD camera. The fiber was placed on the sample stage and a drop of ultrapure water (Milli-Q, 2 μL) was dropped to the surface for contact angle measurement. Five points per fiber were measured to determine the mean value of the water contact angle (n = 3).

2.4 Mechanical properties

Mechanical properties of the electrospun fibers were verified using a tensile machine (Instron 8845) with a load cell capacity of 10N. The appropriate specimen gripping is required to prevent the fibers from breaking or slipping at the grips. According to ASTM D882, samples were mounted and had a 25 mm gage length. Samples were prepared for testing at a crosshead speed of 5 mm/min at ambient conditions. The initial modulus, ultimate strength and elongation at ultimate strength were measured.

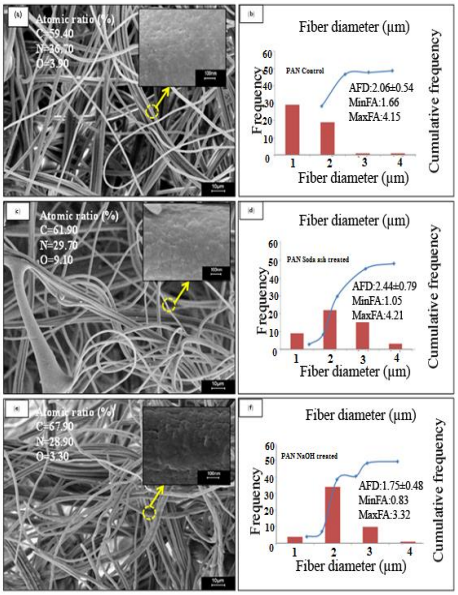
2.5 Endothelial cell responses

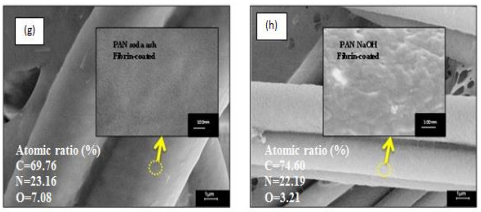
Cell morphology was studied using Axio Vert A1 inverted microscope and SEM (Carl Zeiss). The substrates were fixed in 2% glutaraldehyde (0.1 M phosphate buffered) for 2 h then washed with ethanol series before observed under SEM. For staining procedure, HUVEC-seeded on fibers were gently washed with PBS (3 times) and fixed with formaldehyde solution (3.75% wt/v) in PBS for 20 min. Sample was then washed three times with PBS and then permeabilized with a Triton X-100 solution (0.5% v/v in PBS) for 15 minutes. The sample was finally rinsed three times in PBS and stained with Hoechst 33258 (1:10,000 dilution, catalog #B2883; Sigma Aldrich) for 1 hour at room temperature and in dark. To assess the intracellular of HUVECs, sample then was washed with PBS three times before stained with Alexa Fluor 488, Life technologies for 1 hour. Finally, after washing with PBS, sample was conserved with 3 mL per well of PBS and observed under microscope. The MTT assay was used as a measure of relative cell viability. After the HUVECs were cultured for 1, 3 and 7 days in 96-well plate, the cell viability was evaluated using the MTT assay (MTT; Sigma), in which 100 ml of MTT (5mg/ml) was added to each well and incubated at 37^{o}C for 4 h. At the end of the assay, the blue formazan reaction product was dissolved by adding DMSO. The absorbance was measured at 570 nm using a microplate reader (Thermo scientific; US).

3. RESULTS

3.1 Morphology of untreated and treated PAN fiber

FESEM images of electrospun PAN fibers are to investigate the effect of surface treatment towards fiber morphology structure presented in Figure 1.





Mechanical Properties	PAN before surface treatment	PAN after surface treatment with soda ash	PAN after surface treatment with NaOH
Tensile modulus (GPa)	1.35±0.32	0.90±0.11	0.41±0.37
Tensile strength (MPa)	3.77±0.68	2.46 ±0.73	2.17±0.73
Failure strain (%)	35.32±16.63	31.91±13.91	23.60±9.03

Fig. 1 FESEM micrograph and distribution of untreated PAN fiber (a, b); fiber treated with soda ash (c, d) as well as with NaOH (e, f). Soda ash (f) and NaOH (g) treated fiber then coated with fibrin. Table shows mechanical properties of the electrospun PAN fibers before and after surface treatment.

Fig. 1(a) shows the micrographs of untreated PAN fibers obtained by electrospinning have bead-free and homogenous morphology. The PAN fiber surfaces also seem featureless and smooth as shown in greater detail in the figure. The morphology of PAN after surface treatment with Na_2CO_3 is shown in Fig. 1(c) has an uneven surface after treated. As indicated by the arrows, PAN fiber after NaOH as shown in Fig. 1(e) surface treatment reveals ripple and is rougher compared with Na_2CO_3 surface treatment. Surface treatment processes were proved to result PAN fibers with different diameter range before surface treatment (AFD (average fiber diameter): 2.06 ±0.54; MaxFD (maximum fiber diameter): 4.15 and MinFD (minimum fiber diameter):1.66). Next, PAN fiber after surface treatment with Na_2CO_3 become (AFD (average fiber diameter): 2.44 ± 0.79; MaxFD (maximum fiber diameter): 4.21 and MinFD (minimum fiber diameter):1.05) while PAN fiber after surface treatment with NaOH become AFD (average fiber diameter): 1.75 ± 0.48; MaxFD (maximum fiber diameter): 3.32 and MinFD (minimum fiber diameter):0.83). Measurements show that the electrospun PAN fiber diameter

increases 15% as a result of surface treatment with Na_2CO_3 and decreases 18% after treated with NaOH.

Fig. 1(g-h) showed that PAN fiber after treated by Na_2CO_3 and NaOH was fully coated with fibrin gel. EDS results reported the existence of fibrin with significant changes of nitrogen and oxygen element of PAN fiber after coating process. Increasing in carbon composition confirms the existence of fibrin. The coating is fully covering the cellular fibers due to the structure of fibrinogen transformed into fibrin gel, deposited on the surface and formed a mesh of fibrils obviously in-between of the junction fibers.

3.2 Mechanical properties of untreated and treated PAN fiber

Fig. 1(i) summarizes the tensile properties of electrospun PAN fibers before and after reaction with Na_2CO_3 and NaOH. It is noted that there are significant differences between PAN fibers before and after surface treatment with Na_2CO_3 and NaOH for tensile strength and tensile modulus. After surface treatment, overall there were decreases in tensile strength, tensile modulus and strain to failure due to loss of chain orientation. The decrease of tensile strength after Na_2CO_3 and NaOH surface treatment of PAN fiber is as much as 34% and 42%, respectively. Tensile modulus of substrate decreases from 1.35 GPa to 0.9 GPa and 0.41 GPa while strain decrease 9% and 4% after treated with Na_2CO_3 and NaOH, respectively.

3.3 Surface characterization of untreated and treated PAN fiber

Surface characterization of treated and untreated PAN fiber is presented in Fig. 2. FTIR spectra of the PAN fibers, before and after treated are included in Fig. 2(a). The control PAN molecule consists of functional groups such as methyl (CH_3) and nitrile ($C\equiv N$). It is found that surface treatment in the presence of Na_2CO_3 and NaOH includes the stage of carboxyl, amide and aldehyde.

PAN fiber shows a characteristic peak at 2260-2210 cm^{-1} before and after treating with NaOH and Na_2CO_3 indicating the likely and expected presence of acrylonitrile due to nitrile stretch. The alkali reaction of Na_2CO_3 and NaOH will attack chain of nitrile to become carboxyl group. The interface between these reactions involves amide formation, as can be seen in the spectra of 3400-3250 cm^{-1} for 1^o and 2^o amide then proceeds through a carbonyl group which can be proven by conversion of -CN to $-COO^-$ groups.

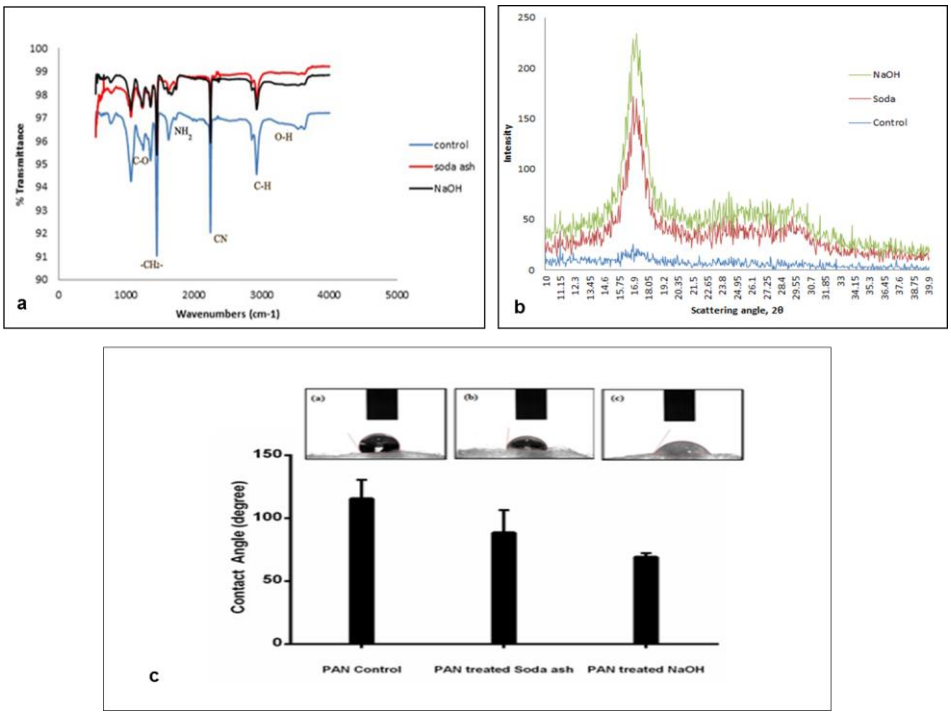


Fig. 2 (a) FTIR, (b) XRD, and (c) Contact angle results of PAN fiber, PAN treated with soda ash and PAN treated with NaOH.

Briefly, the peak at 1645 cm^{-1} is assigned to carbonyl stretching, but the peak broadened after treated into 1652 cm^{-1} for NaOH and 1649 cm^{-1} for Na_2CO_3 surface treatment. In conjunction with the peaks at 1320 cm^{-1} and 1000 cm^{-1} range (due in part to C-O stretch), 1760-1665 cm^{-1} (due to C-O-C bend), and broad absorption around 3300 cm^{-1} and 2500 cm^{-1} (due to -OH stretch) the carbonyl peak seems to confirm the presence of acrylate (H C=C(CH)CO R) as a co-monomer for NaOH surface treatment. This carboxylic acid formation will lead to the formation of secondary amines in which hydroxyl group has been replaced by amine. However, in the presence of Na_2CO_3 surface treatment, it does not include the stage of amide formation for second degree and does not result in the complete exhaustion of nitrile groups in a PAN considering that Na_2CO_3 is not really strong reducing agent like NaOH and will not fully attack and hence will not harm the substrate.

The obtained XRD patterns were shown in Fig. 2(b). A number of crystalline peaks were observed for pure PAN, which can be attributed to the semi- crystalline structure of this polymer. The polymer exhibited a diffraction peak at 16.9°, which is the typical peak for a polyacrylonitrile polymer. Two equatorial peaks was showed which one was at 2θ = 29.5Å corresponding to a spacing of d ≈3.03 Å from the (1 1 0) reflection and another was at 2θ = 17.0Å corresponding to a spacing of d ≈5.3 Å from the (1 0 0) reflection. These peaks are common to the fiber diffraction pattern of PAN with hexagonal crystal

system [21]. The weak peak of PAN control showed from the diffraction pattern with the value of 2θ at 17.0°. This proves that fibers fabricated using electrospinning gives limited crystallinity. This low crystallinity leads to stretched PAN chains solidified rapidly after elongation, preventing crystal formation in the electrospun PAN fibers.

In contrast, PAN with Na_2CO_3 and NaOH surface treatment increase in crystallinity even after short timing treatment showed two diffraction peaks indexed with values of 2θ of 17.0° and 29.5°. The crystallinities of the NaOH-treated fiber are higher than PAN control and PAN after treated with Na_2CO_3. The main factor depends on the alkali concentrations selected for the treatment and the type of fiber studied [22]. Crystallinity factor will lead to the structural changes of PAN fibers after surface treatments, thus will influence the course of their entire degradation process.

The contact angle of PAN-modified surfaces treated via Na_2CO_3 and NaOH as well as the control is shown in Figure 2 (c). It indicated that PAN-treated by Na_2CO_3 and NaOH were more hydrophilic (significant lower contact angle) than found in untreated PAN. The contact angle of the PAN surface of the PAN was reduced from 115° to 88° and 64° after treated with Na_2CO_3 and NaOH, respectively. These results clearly indicate that untreated PAN fibers had the least attraction toward deionized water. However, this was improved upon by surface treatment. The water contact angle of the PAN surface gradually decreased after treating with Na_2CO_3 and NaOH. It is noteworthy that receding contact angle decreases for all PAN fibers after surface treatment.

3.4 Endothelial cell adhesion and proliferation

Once HUVECs were seeded onto both untreated and surface treated and coated PAN fibrous scaffolds in presence of fibrin, cell attachment and proliferation were evaluated at day 1 and day 3. Endothelial cell adhesion and proliferation to PAN fiber is presented in Figure 3. After day 1, HUVECs cultured on PAN fibers were rounded in phenotype for all fibers (Fig. 3 (a,d,g)). This is due to the short timing of culture. HUVECs seemed to be securely attached and spread on the surface in a flatten formation, regardless of the surface treatments and surface coating after day 3. It was evident that cells were more actively proliferating across the modified surfaces (Fig. 3 (e) and (h)) while cells remain separately on PAN control even for day 3. It suggests that HUVECs adhered to PAN treated with Na_2CO_3 and NaOH then coated with fibrin surface were higher than that found on the PAN control at the time points of 1 and 3 days. This was proven by staining after day 3 culture (Fig. 3(f,i)) which confirmed that not only confluent HUVECs layer was formed, but also cells oriented completely along the aligned fiber direction and exhibited an elongated morphology for both surface treated fibers coated with fibrin.

Absorbance of PAN treated with Na_2CO_3 and NaOH coated with fibrin increase by increasing time as shown in Fig. 3(i). However, PAN control increases absorbance slowly because of reduced toxic of fluorine coming from DMF which is PAN solution during fabrication of fibers. The MTT absorbance of PAN after treated with Na_2CO_3-fibrin coated is quite similar with PAN treated with NaOH-fibrin coated treated throughout the testing period. PAN treated with NaOH-fibrin coated preceding PAN treated with Na_2CO_3-fibrin coated as much as 4% on day 3 but almost same on day 5 and day 7.

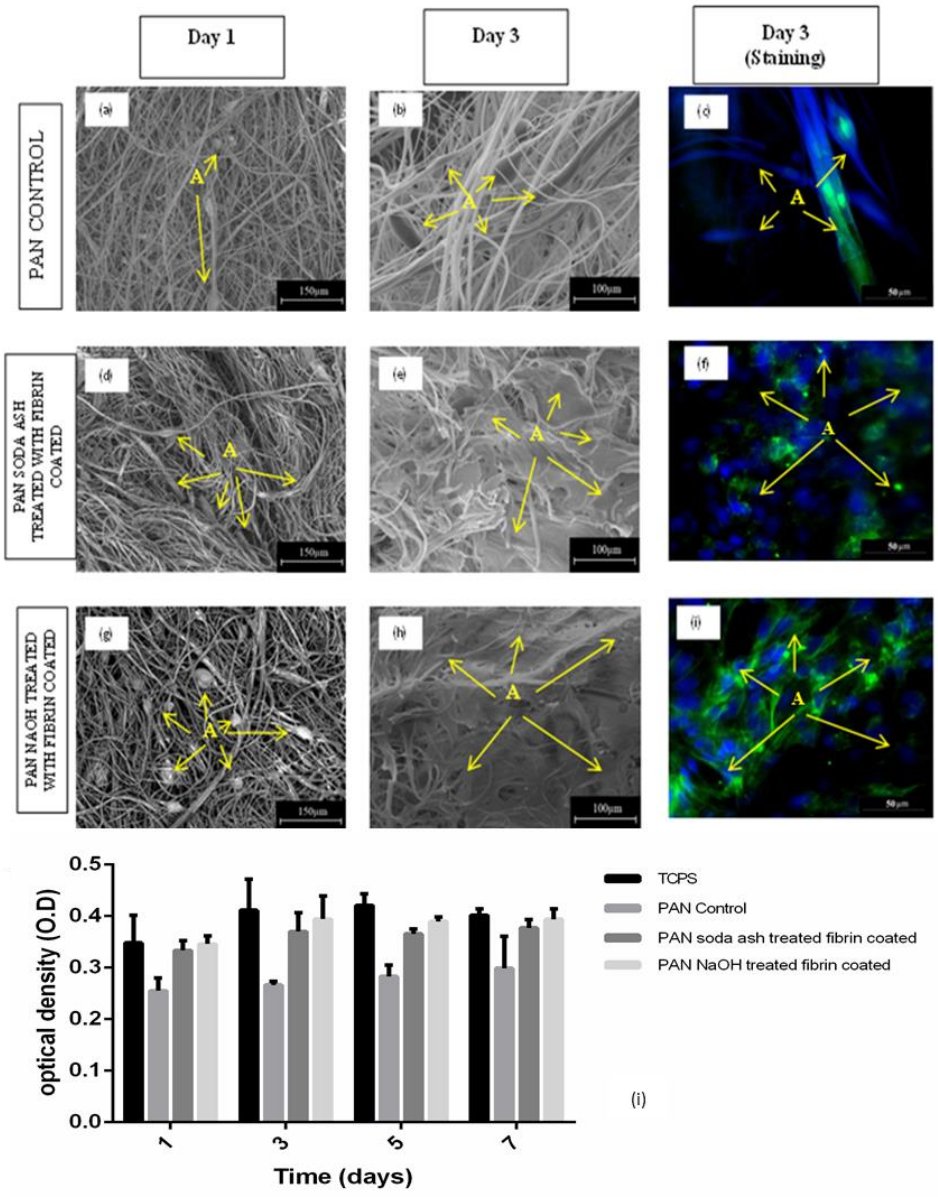


Fig. 3 SEM and images of HUVECs cultured on a untreated fiber as control (a,b,c); soda ash treated and fibrin coated (d,e,f); and NaOH treated and fibrin coated (g,h,i) after 1 and 3 days of study. Letters "A" indicate HUVEC cells. MTT test on cytotoxicity of PAN before and after surface treated to HUVECs also presented (i).

4. DISCUSSIONS

PAN fiber has been used for membrane applications [7]. In this study, PAN fiber was prepared to promote endothelial cell adhesion and proliferation. Surface treatment and bioactive coating of polymer fiber may lead endothelial cell proliferation thus encourages endothelial network or endothelialization, as presented elsewhere [23]. We have studied the effect of bioactive coating on PAN fiber to HUVEC responses, complimented previous study of Groth et al. that used PAN fiber for fibroblast cell for wound healing application [5].

It has been previously shown that the use of Na_2CO_3 and NaOH removes the natural surface wax thereby exposing the underlying texture of substrates [24]. This improves the wetting and wicking properties of the material which in turn increases the material surfaces for better surfacing coating [25]. In this experiment, it is observed that PAN fiber showed increased hydrophilicity after treating with Na_2CO_3 and NaOH thus improve coating of fibrin gel on PAN fibers. Bioactive protein; in this case was fibrin, has become positive surface charged at neutral pH and this will attract negative charge on the treated PAN sample [19]. Fibrin coating is able to attract HUVECs on PAN fiber since fibrin has directs cellular responses through specific receptor [26].

Many studies have already discussed about NaOH surface treatment on various substrates [14,24]. Afra et al. showed porous surface and rough morphology on PET substrate after surface treatment of 2 hours with NaOH [13]. A higher concentration of NaOH and long period of surface treatment will cause degradation of surface fiber [19] or grooved serrated surfaces. It has been reported that substrate with very high surface roughness decreases cell adhesion onto its surface [27]. Therefore, in this study the lower concentration for both treatments were used. There are no significant differences in morphology on the PAN surface after treating with both NaOH and Na_2CO_3 since the concentration for both treatments as low as 0.1 and 0.2 molar of $Na_2CO_3$and NaOH respectively.

Even though the morphology of PAN fiber after surface treatment gives not much difference but the diameter of fibers is affected. This is due to the transformation of nitrile to carboxylic group of the PAN surface may result in a decrease of fiber diameter. However incomplete exhaustion of nitrile group for Na_2CO_3 surface treatment gives an advantage to Na_2CO_3 compared to NaOH surface treatment in terms of mechanical properties since fiber diameter is strongly related with strength of the fiber [28]. Tensile strength of PAN fiber after treated with Na_2CO_3 is higher than NaOH surface treatment but still lower than PAN before surface treatment. This is because exposure the fiber to the heated surface treatment makes the fiber gives an emission and auto exhaustion to the environment [29]. In theory, tensile modulus decreases because of the random PAN fiber would become gradually aligned during uniaxial tensile test. This also leads to decreasing PAN fiber diameter after surface treatment, causing decrease in tensile strength and the fiber will easily fracture.

Alteration in physical characteristics of fibers will influence tensile properties due to increasing shrinkage and density of fibers [14]. The decreasing result for tensile modulus of PAN after treated with Na_2CO_3 and NaOH shows that PAN surface is relatively ductile due to exhaustions of nitrile group. This also led to the changes of crystallinity because acrylonitrile is a reactive chemical that polymerizes spontaneously when heated in the

presence of a strong alkali. Result of mechanical properties depends on the material used. Instead of synthetic fiber, previous study used natural fiber for base material [29]. Alteration in ductility of specific material after removal of impurities modified mechanical result [28]. However, tensile strength in this experiment before and after surface treatment is still in the range of coronary artery which is from 1.40 MPa to 11.14 MPa [23].

Cytotoxicity test on PAN fibers after surface treatment with Na_2CO_3 and NaOH shows these fibers are suitable for growth of HUVECs, attaining a significant level of increase in cell viability until day 7 of culture. This shows that the fibers with fibrin assisted increase proliferation of HUVECs. The SEM micrographs of HUVECs on PAN control and PAN treated with Na_2CO_3- fibrin coated and NaOH scaffolds obtained on day 1 of culture showed a normal morphology of cell growth on the fibers. HUVEC cells disconnects to individual cells, round phenotype and non-proliferating for all fibers. This is due to the shorten time of culture. This cell behavior is same with previous report [8], where fibers have low surface density and large inter fiber which did not encourage adhesion of the cells across the nearest fibers in short time seeding. HUVECs attach and spread more on PAN substrate after surface treatments than PAN control by day 3. In particular, the HUVECs were numerous and well-spread, forming monolayer cell on the PAN treated with NaOH scaffolds while reaching sub confluence. Some cells aggregated along the fibers on PAN treated with Na_2CO_3 as confirmed by double-staining with Alexa Fluor 488 for the cell cytoskeleton (i.e., actin filaments) and with Hoechst 33258 for nuclei.

These observations suggested significance increase of HUVECs adhesion due to better hydrophilicity after PAN fiber surface treatment process subsequently coated with fibrin. It also shows that fibrin was immobilized on the PAN, which is important for strong cell adhesion [30]. Fibrin contains many integration-binding sites and this reason makes it easier for cell to adhere on coated PAN fiber [31]. Formation of focal adhesions only occurs if the ligands can withstand cell contractile forces as actin stresses fibers and focal adhesions are critical for cell survival. This indicates culturing cell on native substrates proves that cell attachment, spreading and growth enhanced, depending on the substrate, which has been reported elsewhere [32]. SEM and staining micrographic observations support the trend of HUVECs proliferation quantified by the MTT assays.

5. CONCLUSIONS

This study attempted to attach HUVECs on PAN via two different surface treatments with soda ash and NaOH, then coated with fibrin gel. PAN treated with NaOH gives more attachment of HUVECs compared to the PAN treated with soda ash and PAN control sample. However, the attachment of HUVECs on PAN after soda ash surface treatment can be another option for wet chemical treatment rather than common NaOH since soda ash surface treatment gives improvement in wettability and mechanical properties. The presence of carboxyl functions on PAN fibre after surface treatment can be an advantage since fibrin can be covalently coupled via a simple wet chemistry. This is another successful finding of PAN fibre on cells after another research proving on fibroblast cells.

***Acknowledgement**: This work was supported by Malaysian Ministry of Higher Education (MOHE) grant (vote# 4F124) and Universiti Teknologi Malaysia Tier-1 Research grant (vote # 03H12). I. Sukmana is supported by Hibah Kompetensi Y2017grant from Kemenristekdikti, Indonesia.*

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