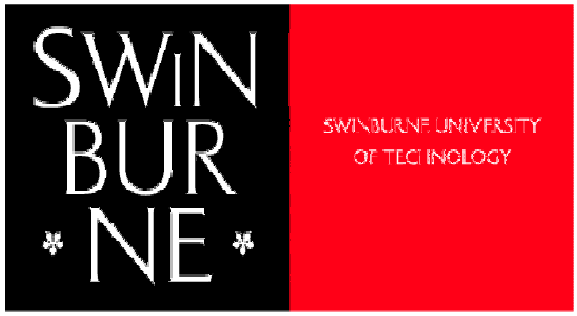
Electrospun Nanofibrous Scaffolds for Small Diameter Blood Vessels

Nasser Khalf Awad Abdel Mogeth

(M.Sc., Egypt)

A thesis submitted for the degree of PhD of Engineering



Faculty of Science, Engineering and Technology

Bio-mechanics and Tissue Engineering Group

Swinburne University of Technology

2017

Dedications

This thesis is dedicated to my brothers, sisters and my beloved Parents. Those people are my heroes and I feel very privileged to have them in my life.

Declaration

This thesis does not have any material that are accepted for the award of any other degree or diploma, except where due reference is cited in the context of the thesis. To the best of my knowledge, this thesis does not have any materilas that are priorly published by another scholar, except where due reference is cited in the context of the thesis.

Signature: Date:

8/03/2017

Acknowledgments

I would like to express my gratitude to my principal supervisor Prof. Yos S Morsi, for his encouragement, guidance and advice throughout the course of this research project. Without his clear supervision this work would not have been achieved.

I would also like to give the sincere and heartfelt thanks to my co-supervisor, Prof.Tong Lin, from institute for frontier materials (IFM) at Deakin University, for his great encouragement and support during my PhD study. His kindness to students, his hardworking, his positive attitudes towards difficulties, and his enthusiasm for science inspired me to strong interest in the research work. The help of my associate supervisor, Dr Cynthia Wong, from IFM institute at Deakin University for her beneficial assistance in the cell culture work.

I would greatly like to acknowledge Swinburne University of Technology for supporting my PhD research through the Swinburne University Postgraduate Research Award (SUPRA).

I must give my deep thanks to technician Labs in Swinburne University and Deakin University where I gained a lot from the research collaboration.

Finally, special thanks goes to my family for their love and continuous help.

Publications

Honors & Awards

- **Swinburne university postgraduate research award (SUPRA),** Swinburne university, 2014-2017.

Journal papers

- Nasser K. Awad, Sharon L. Edwards and Yosry S. Morsi. A Review of TiO_2 NTs on Ti Metal: Electrochemical Synthesis, Functionalization and Potential Use as Bone Implants. Material science and engineering C, accepted.

- Nasser K. Awad, Cynthia S. Wong, Yosry S. Morsi and Tong Lin. Electrospun Fibrous Scaffolds for Small diameter Blood Vessels: A review. in progress.

- Nasser K. Awad, Cynthia S. Wong, Yosry S. Morsi and Tong Lin, Preparation of SBS nanofibres with controlled elasticity and their endothelial cell culture behaviour. in progress.

Book chapters

- Md. Shamsul Arefin, Nasser K. Awad, Chandra Prakash, Rathore Anupam Shukla and Yosry S. Morsi. Ventricular Assist Device and Its Necessity for Elderly Population, IGI global 2015, pp.314-334.

Abstract

The ideal scaffolds for creating synthetic small diameter blood vessels have not yet been found. The limitations of implanting these scaffolds are thrombosis formation, intimal hyperplasia and aneurysmal dilation. Electrospinning technique fabricates polymer fibrous scaffolds with average fibre diameters ranging from 50 to 500 nm which is similar to those observed in extracellular matrix of native blood vessels. Therefore, it promotes the adhesion, proliferation and growth of cells. Herein, we classify the polymers deployed for the fabrication into synthetic polymers, natural polymers and hybrid polymers. Further, the biomechanical properties and the biological activities of the electrospun small diameter blood vessels including antithrombogenic ability and cell response are discussed. Polymer blends seem to be a strategic way to fabricate small diameter blood vessels because it conveys both suitable biomechanical properties contributed by synthetic polymers and favourable sites to cell attachment contributed by natural polymers.

In this study, elastic poly (styrene-butadiene-styrene) copolymer (65%:29.5%) and nonelastic poly (styrene-butadiene-styrene) copolymer (70%:30%) copolymer were electrospun with a ratios (E:N (5:0), E: N (4:1), E:N (3:2), E:N (1:1), E:N (2:3), E:N (1:4), E:N (0:5)) in a mixture of tetrahydrofuran THF: N, N-dimethyl formamide DMF (70:30 v/v) to obtain 17 wt% SBS concentration. Starch was sprayed over the aluminium foil prior to electrospinning to facilitate detaching the electrospun fibrous scaffolds from the foil by immersing the scaffolds on the foil in the water.

After that, the fibrous scaffolds were characterized by scanning electron microscopy (SEM), Panalytical X'pert Powder (XRD), Fourier transform infrared spectra (FTIR),

Thermogravimetric analysis (TGA), Differential scanning calorimetry (DSC), Instron tensile tester, Contact angle and air permeability. Cytotoxicity test was performed to measure the cytotoxicity of SBS fibrous scaffolds to endothelial cells. Cell culture were then performed on the electrospun scaffolds using human umbilical vein endothelial cells (HUVECs). Then, the cellular attachments and proliferation were investigated using MTS assay, SEM and confocal microscope.

Elastic SBS, nonelastic SBS blended elastic SBS at different ratios (E: N (4:1), E: N (3:2), E: N (1:1), E: N (2:3) and E: N (1:4)), and nonelastic SBS were successfully electrospun with an average fiber diameter of 2μm. The measured average pore size of SBS fibrous scaffolds were 8±0.01 μm and the scaffolds are all permeable to air. Blending nonelastic SBS to elastic SBS paved the way to tune the mechanical properties of SBS polymer while keeping the chemical composition stable.

SBS fibrous scaffolds are hydrophobic and it was essential to be treated by air plasma to become hydrophilic which in turn helped ECs to attach and proliferate on the fibrous scaffolds. SBS fibrous scaffolds have no cytotoxic effect on ECs. ECs attached and proliferated on SBS fibrous scaffolds regardless of their elasticity. ECs maintained its polygonal shape on SBS fibrous scaffolds and they are oriented along the fibres. SBS fibrous scaffolds with weight ratios 1:1 and 2:3 may show better cell viability than elastic SBS.

To the best of our knowledge, we have, for the first time, tuned the mechanical properties of the SBS fibrous scaffolds without changing its chemical compositions through blending nonelastic SBS to elastic SBS during the electrospinning process. Furthermore, we have, for the first time, studied endothelial cell culture for SBS fibrous scaffolds. SBS fibrous scaffolds exhibited a new cell

surface structure that promoted ECs adhesion and proliferation and it therefore paved the way for different tissue engineering applications.

Keywords: electrospun nanofibres, small diameter blood vessels, endothelial cells, thrombosis and antithrombogenic agents.

Table of contents

List of figures

List of tables

List of abbreviations

Polytetrafluoroethylene	PTFE
Poly (ethylene terephthalate)	PET
Vascular grafts	VGs
Extracellular matrix	ECM
Endothelial cell	EC
Smooth muscle cells	SMCs
Polycaprolactone	PCL
Poly (L-lactide-co-ε-caprolactone)	PLCL
Poly-lactic acid	PLA
polyurethane	PU
Polylactide	PLA
Cleavable chain extenders	CCEs
Hydroxyethyl lactate	EGLA
Bis (2-hydroxyethyl) terephthalate	BET
Polyurethane ester urea	PEUU
Mass flow rate	MFR
Polypropylene	PP
Recombinant human tropoelastin	rTE
Disuccinimidyl suberate	DSS
Ultimate tensile strength	UTS
Poly (L-lactic acid)-co-poly (ε-caprolactone)	P (LLACL)
Polydioxanone	PDO
Poly (D, L-lactide-co-glycolide)	PLGA

Thermoplastic polyurethane	TPU
Hexafluro-2-propanol	HFP
Trifluoroethanol	TFE
Dichloromethane	DCM
N, N-dimethylformamide	DMF
Trifluoroacetic acid	TFA
Fibroblast cells	FBCs
Mesenchymal stem cells	MSCs
Rat cerebral endothelial cells	RCECs
Po (3-hydroxybutyrate-co-3-hydroxyvalerate)	PHBV
Vascular endothelial growth factor	VEGF
Poly (ethylene glycol)-b-poly (L-lactide-co-caprolactone)	PELCL
Platelet-derived growth factor	PDGF
Vascular endothelial cells	VECs
Vascular smooth muscle cells	VSMCs
Chiton	Cs
Porcine iliac artery endothelial cells	PIECs
Human venous myofibroblasts	HVS
Polydimethylsiloxane	PDMS
N-isopropylacrylamide	(pNIPAm)
Poly (ester amide) s	PEAs
Arginine-glycine-aspartic acid	RGD
Hematoxylin and eosin	H&E
Methacryloyloxyethyl phosphorylcholine	MPC

Dipyridamole	DPA
Cholesterol-poly (e-caprolactone)	Chol-PCL
Cysteine-alanine-glycine	CAG
Small-caliber vascular grafts	SCVGs
Smooth muscle actin	SMA
Fused deposition modeling	FDM
Heparin	Hep
Small-diameter vascular graft	SDVG
Activated partial thromboplastin time	APTT
Thromboplastin time	TT
Prothrombin time	PT
Methacryloyloxyethyl phosphorylcholine-co methacryloyloxyethyl butylurethane	PMBU
Paclitaxel	PT
Styrene-butadiene-styrene	SBS
Elastic SBS	E
Nonelastic SBS	N

Chapter.1

Introduction

1.1. Background

Atherosclerosis is a condition characterised by the hardening and narrowing of arteries due to the formation of plaques which can lead to blocked arteries [1, 2]. Angioplasty and surgical bypass are common techniques used to treat this disease. In the case of surgical bypass, vascular grafts function to bypass the blocked area of the blood vessels, thus, enabling blood to flow to the rest of the tissue. However, blood vessel replacement has limitations since it is rather expensive [3]. Autologous vessels are usually the preferred choice for blood vessel replacement because it is the patient's own tissue. However, harvesting autologous vascular tissues could be difficult for some patients. This is because patient may have limited number of available vessels due to being used in previous surgeries or their vessels may not be of good quality due to ill-health. Therefore, synthetic vascular grafts made of expanded polytetrafluoroethylene (ePTFE, i.e., Gortex) or poly (ethylene terephthalate) (PET, i.e., Dacron) are developed. Since 1956, these materials have been clinically approved for large diameter blood vessels and the occurrence of thrombosis is negligible owing to the high flow of blood and low resistance [4, 5]. Although these materials are available and provided clinical efficiency, they have low ratio of patency when employed for small diameter blood vessel (< 6 mm). The reported patency rate is 40% after 6 months and 25 % after 3 years [6]. In addition, these materials are non-

degradable, which means the patient may be subjected to further cardiovascular surgery in the long term due to the inability of tissue growth and remodelling.

Since the current materials used in clinics have limitations, there are now a lot of research looking into using various types of polymer including synthetic polymers or natural polymers or polymer blends to create synthetic blood vessel. Various strategies have been used to prepare scaffolds such as self-assembly, drawing, template synthesis, phase separation, wet spinning, electrospinning [7,8] and their combination have also been attempted [9,10]. Scaffolds made from fibrous materials are highly desirable especially those from nanofibres. This is because extracellular matrix (ECM) of the tissues comprises three-dimensional fibrous structure in the range of 50-500 nm [11, 12]. It has been shown that scaffolds made from nanofibres are beneficial for cell attachment and growth [11, 12].

Most nanofibre scaffolds are prepared by electrospinning. Electrospinning enables the fabrication of fibrous scaffolds with fibre sizes ranging from nano- to micro-metre, which have physical properties close to that of natural ECM [11, 13]. For instance, the electrospun fibrous scaffolds have high porosity, pore-interconnectivity and large surface areas, providing suitable surface sites for the cells to adhere and proliferate [13, 14].

In previous studies on electrospun nanofibres for tissue engineering applications, the matter of producing nanofibers with different diameters , core-shell nanofibers and nanoparticles functionalized nanofibers have been thoroughly covered [1,8]. Little attention have been paid to the application of creating synthetic small diameter blood vessels and its biological response whether in vitro or in vivo. Therefore, this study summarises the progress of using electrospun nanofibres to develop small blood vessels, i.e. those with a diameter below 6 mm. The

biocompatibility studies both in vitro and in vivo are described. State-of-the-art techniques to functionalize nanofibrous tissue scaffolds for the purpose of promoting biocompatibility or decreasing blood clotting are also discussed.

1.2. Native blood vessels

The native blood vessels comprise three layers: intima (inner layer), media (middle layer) and adventitia (outer layer), as illustrated in Fig. 1 [15]. The intima layer is a monolayer of endothelial cell (EC). EC monolayer prevents the blood from clotting. The media layer consists of smooth muscle cells (SMCs). Adventitia layer comprises collagenous ECM and fibroblast. Collagenous adventitia adds rigidity to the blood vessel walls, while media layer provides them with elasticity [16]. Blood vessels dilate and contract in response to a signal from ECs or cytokines [17]. Intima, media and adventitia layers are separated from each other by layer of limiting membrane.

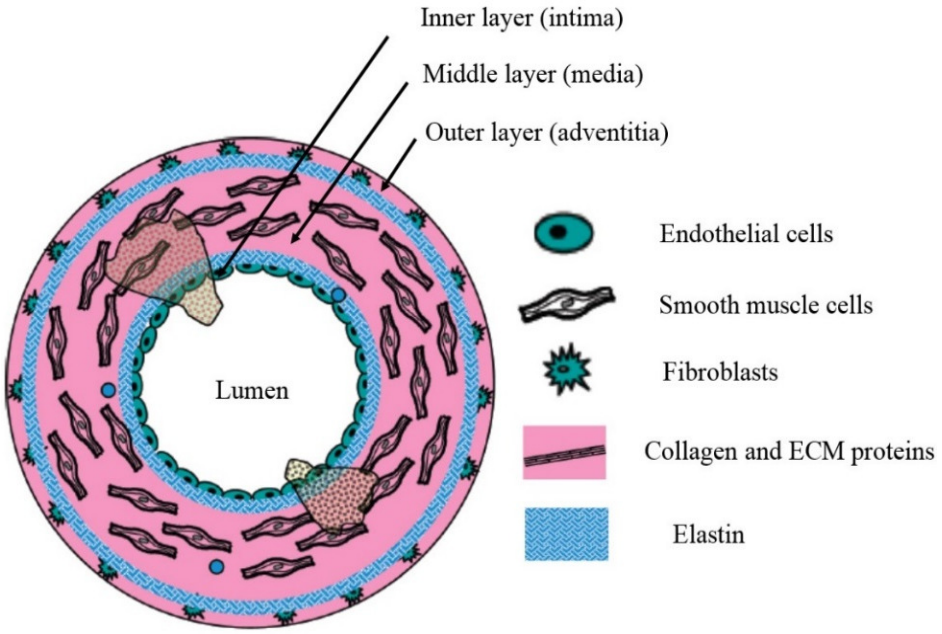


Fig.1.1: Schematic illustration of blood vessel structure [reprinted from Ref.15].

Table. 1 lists the mechanical properties of some native blood vessels [18-22]. There is a lack of consensus in the mechanical properties obtained for the same tissue

due to different evaluation methods. For example, the circumferential elastic modulus reported for saphenous vein varied significantly, from as low as 2.25 MPa to 43 MPa [18-20]. Similarly, a large variation was also observed for the longitudinal elastic modulus [18, 19].

Ideally, synthetic blood vessels should mimic the native blood vessels in both structure and functions. They should be biocompatible and bioactive, and have acceptable mechanical properties. Electrospinning technique contributes to the fabrication of synthetic blood vessel through its ability to create fibrous scaffolds. In the following section the technique and parameters of electrospinning are discussed thoroughly.

Table. 1 Mechanical properties of some natural blood vessels

Types	Elastic Modulus (MPa)	Ultimate stress (MPa)	Strain at failure (%)	Burst strength (mmHg)	Ref.
Saphenous vein (Circ.)	43	3	11	NA	[18]
Saphenous vein (Long.)	130	13	17	NA	[18]
Saphenous vein (Circ.)	4.2	1.8	242	1680-3900	[19]
Saphenous vein (Long.)	23.7	6.3	83	NA	[19]
Saphenous vein (Circ.)	2.25	4	180	1250	[20]
Left internal mammary artery (Circ.)	8	4.1	134	2000	[19]
Left internal mammary artery (Long.)	16.8	4.3	59	NA	[19]
Femoral artery (Circ.)	9-12	1-2	63-76	NA	[21,2]

Circ., circumferential; long., longitudinal; NA, not available.

1.3. Electrospinning technique

Electrospinning shows great potential in the fabrication of fibrous scaffolds for developing blood vessels. It can produce a seamless fibrous tube with fibre diameter controllable in either nanoscale or microscale. Electrospinning involves a physical process in which a viscoelastic solution is stretched under a high electrostatic

force. Fig. 1.2a schematically illustrates the basic setup for electrospinning, which contains a syringe with a syringe pump, a needle nozzle, a collector, and a high voltage power supply. Typically, a polymer solution is charged by a high voltage, and a Taylor cone is formed at the tip of the nozzle. A fine jet ejects from the tip of the Taylor cone when the electric field overcomes the surface tension. The charged jet then has an intensive interaction with the electric field formed between the nozzle and the collector, making itself undergo a whipping movement. Solvent evaporation from the jet leads to solidification of the jet into fibres, which are deposited on the collector forming randomly-oriented fibrous mats [23-26].

The fibre morphology of electrospun fibres can be influenced by factors of electrospinning process. These parameters include viscosity of the solution, electrical conductivity, molecular weight of polymer, applied voltage, flow rate of polymer solution, the spinning distance and ambient condition such as humidity and temperature [27, 28]. Research indicate that increasing polymer concentration, polymer molecular weight or solution viscosity lead to increase in fibre diameter [27, 28]. Increasing electrical conductivity of the polymer solution decreases fibre diameter [27, 28]. There is a contradiction in the case of applied voltage [27]. Some studies indicated that applied voltage had no influence on fibre diameter [29], while others showed that increasing the applied voltage during electrospinning resulted in an increase or decrease in fibre diameter [30]. The increase of solution flow rate increases fibre diameter. However, it should be noted that a reduced flow rate assists with solvent evaporation from the jet, which is essential for fibre formation.

Electrospinning technique can be used to fabricate tubular fibrous membrane, which is useful as scaffolds for blood vessel scaffolds (Fig. 2b). These scaffolds can be produced using different types of polymer [31].

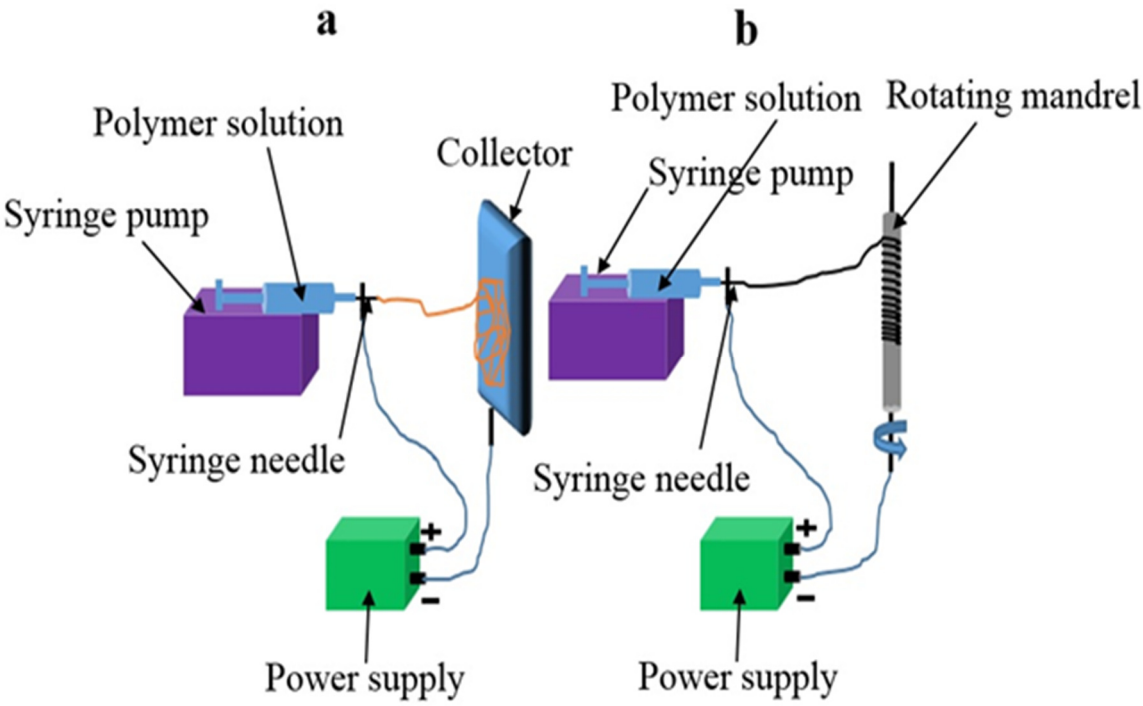


Fig.1.2: Electrospinning setups: (a) ground collector is used to collect fibres, (b) rotating mandrel is used to collect fibres.

1.4. Thesis objectives

The main aim of this PhD project is to fabricate artificial extracellular matrix (ECM) that guides cell adhesion, proliferation and spread. In order to achieve that, the following specific objectives were identified.

1) Electrospinning of elastic styrene-butadiene-styrene (SBS), nonelastic SBS-blended elastic SBS and nonelastic SBS fibrous scaffolds.

Hypothesis: Tuning the elasticity of SBS fibrous scaffolds without changing the chemical composition.

2) Characterising the morphology, surface chemistry, thermal properties and mechanical properties of the fibrous scaffolds.

   Hypothesis: assuring the formation of fibrous scaffolds with particular properties.

3) Performing cytotoxicity study for the SBS fibrous scaffolds using ECs.

   Hypothesis: Determining how toxic is the SBS fibrous scaffolds to ECs as basic biocompatibility evaluation.

4) Performing cell culture for the fabricated fibrous mats using ECs at different time.

   Hypothesis: Determining how the change of the elasticity influences ECs response in terms of attachment and proliferation.

5) Studying ECs morphology, attachment and proliferation.

   Hypothesis: To study the biocompatibility of the SBS fibrous scaffolds.

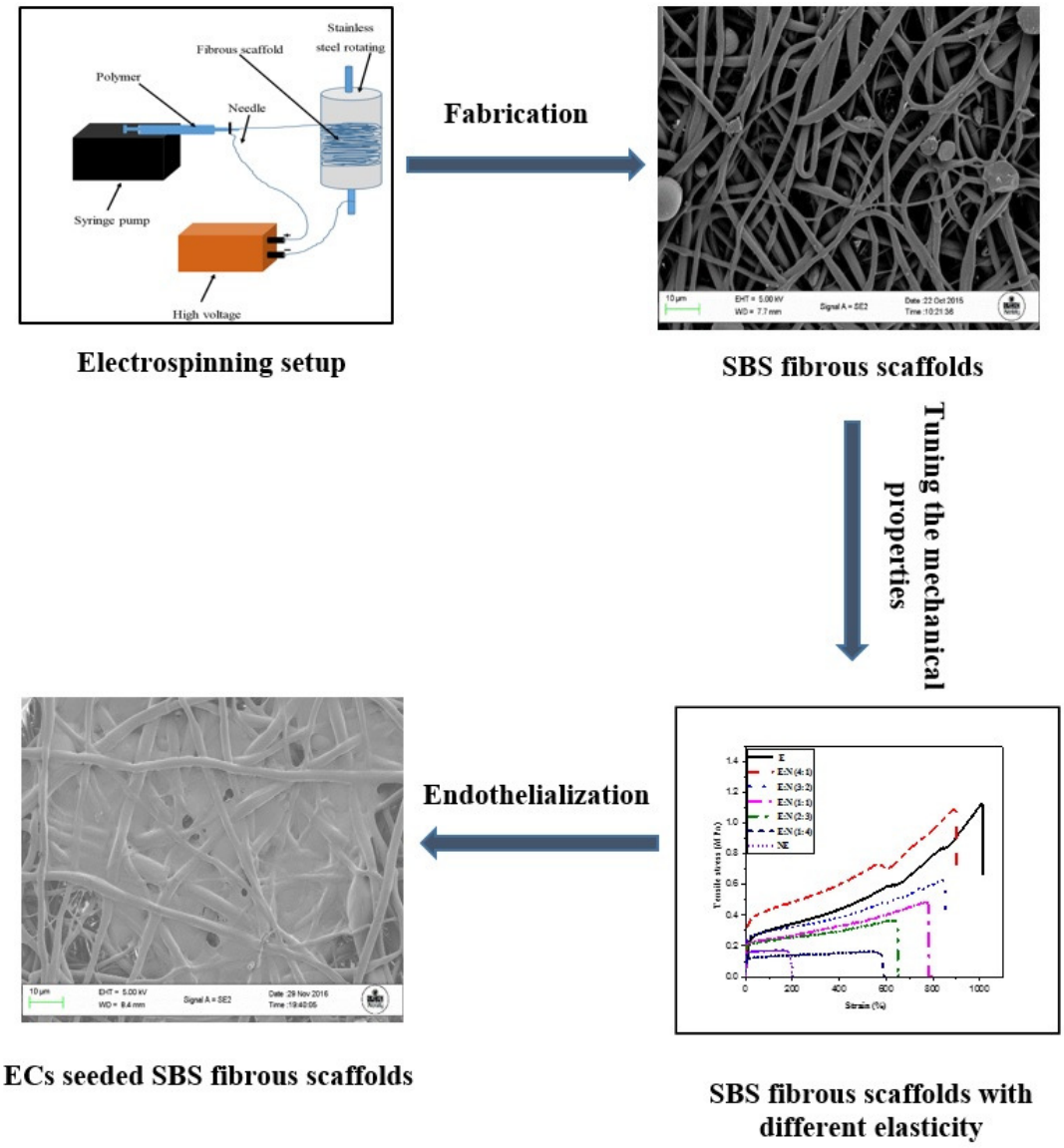


Fig.1.3: Graphical abstract for the study: step 1, fabrication of SBS fibrous scaffolds; step 2, tuning the mechanical properties of SBS fibrous scaffolds; step 3, evaluation of the in vitro endothelialisation of SBS fibrous scaffolds.

1.5. Thesis scope

As one of the pioneer study in the application of fibrous scaffolds in tissue engineered blood vessels, we focused on the fabrication and characterisation of the fibrous scaffolds and in vitro endothelialization of the fibrous scaffolds. In order to mimic the physical structure of ECM such as porosity and 3D fibrous structure,

polymer fibrous scaffolds were fabricated by electrospinning. Two copolymers of styrene-butadiene-styrene with different elasticity were chosen. Through mixing elastic SBS with nonelastic SBS at different ratios, the elasticity of the fibrous scaffolds can be controlled. The ECs that was tried, was Human Vein Umbilical Endothelial Cells (HVUECs). Cytotoxicity test of SBS fibrous scaffolds to ECs was performed as basic step for biocompatibility to evaluate how toxic are SBS fibrous scaffolds to ECs. In vitro ECs study was carried out to evaluate the biocompatibility of the fibrous scaffolds in terms of EC attachment and proliferation. The adhesion and proliferation of ECs to the fibrous scaffolds were evaluated by MTS assay, SEM and confocal microscope.

1.6. Thesis value

It is hope that this study provides a strategy to develop synthetic ECM that is able to support ECs in terms of adhesion and proliferation and approach the development of tissue engineered small diameter blood vessel. To the best of our knowledge, that is the first time that SBS polymer is electrospun into nanofibres with controlled elasticity without changing the chemical integrity. Also, that is the first time that cytotoxicity and biocompatibility of SBS fibrous scaffolds to ECs are studied. The fabrication of SBS tubular fibrous scaffold with 5mm diameter is successfully achieved. Additionally, the fibrous scaffolds may provide a new type of cell culture surface for different types of cells that can be deployed for different applications of tissue engineering and cell based therapies.

Chapter.2

Literature review

2.1. Preparation, morphology and mechanical properties of nanofibrous scaffolds

Fabrication of polymeric fibrous scaffolds by electrospinning has been intensively investigated using various polymers including synthetic, natural and polymer blends (see Table.2) Synthetic polymers exhibit better mechanical properties than natural polymers. Blending two synthetic polymers or two natural polymers could result in enhanced mechanical properties.

Mechanical properties of artificial blood vessels have direct influence on shear stress as well as intimal hyperplasia when they are attached to native vessels. When there is a match between the mechanical properties of the artificial vessels and the native vessels, both shear stress and intimal hyperplasia could be avoided. This, in turn, will help to reduce the formation of blood clots within the artificial blood vessels. Furthermore, the artificial blood vessels should be durable enough to withstand the frequent blood circulation and the associated pressure.

2.1.1. Synthetic polymer nanofibre scaffolds

Various attempts have been sought to fabricate artificial blood vessels using synthetic polymers such as polycaprolactone (PCL), poly (L-lactide-co-ε-caprolactone) (PLCL), poly-lactic acid (PLA) , polyurethane (PU) , L-lactide-co-trimethylene carb **,** polypropylene (Moplen 462R PP) and polylactide (PLA 4060D) [32-46](see Table.2).

2.1.1.1. Poly (ε-caprolactone)

PCL based blood vessels with inner diameter of 2 mm and wall thickness of 650±15μm consisting a mixture of micro and nano fibres were fabricated [32]. The fabricated grafts demonstrated good mechanical properties. The longitudinal stress and strain to rupture were 4.1± 0.5 MPa and 1092 ±28% respectively. The burst pressure and suture retention strength were measured at 3280 ±280 mmHg and 936± 32 g, respectively. An average water leakage and an average blood leakage were evaluated to be 32.1±1.3 ml min^{-1} cm^{-2} and 0.87 ± 0.08 ml min^{-1} cm^{-2} at 120 mmHg respectively [32], as shown in Table.2.

In another study, PCL micro and nanofibres with average fibre diameters of 1.9 μm and 500-2500 nm respectively were used to fabricate small diameter blood vessels of 2 and 4 mm diameters. The fabricated blood vessels showed promising mechanical properties. Both tensile stress and strain stress of the fabricated vascular grafts (2-7.4MPa and 200-1200%) were higher than that measured for the native blood vessels (1.4 MPa and 100%). In fact, this property is advantageous since the mechanical characteristics might be greatly decreased in clinical conditions when the scaffold starts to degrade and the new natural tissues start to form [33].

Hu et al. [34] reported that the higher the rotation speed during electrospinning of PCL, the more aligned the fibres and the higher the elastic modulus. For instance, fibrous scaffold fabricated at 250 rpm experienced elastic modulus up to 10 MPa while that fabricated at 1500 rpm experienced elastic modulus up to 58 MPa.

Bilayered tubular electrospun fibrous scaffold comprised of pliable polymer PCL in the inner layer and stiff polymer PLA on the outer layer was fabricated layer-by-layer using electrospinning technique (Fig.3a; [35]). Fig.2.1b illustrates the electrospun PCL scaffold consisting of micro fibres and nano fibres with diameters of

1.5 to 6µm and 600±400 nm respectively and interconnected pores with 15 µm average pore size. The electrospun PLA scaffold consist of fibres with diameters 800 nm to 3 µm and interconnected pores with 10 µm average pore size (Fig.2.1C). The total porosity of PCL/PLA scaffold is approximately 79±4%. The soft layer of PCL polymer mimics the intima layer of natural blood vessel while the tough layer of PLA polymer mimics the adventitia layer of natural blood vessel. As shown in Table.2 the electrospun PCL/PLA fibrous scaffold showed acceptable mechanical properties, with Young's modulus of 30.9 ± 6.6 MPa that is almost 3 times higher than that of PCL scaffold (10.7 ± 0.3 MPa) [35].

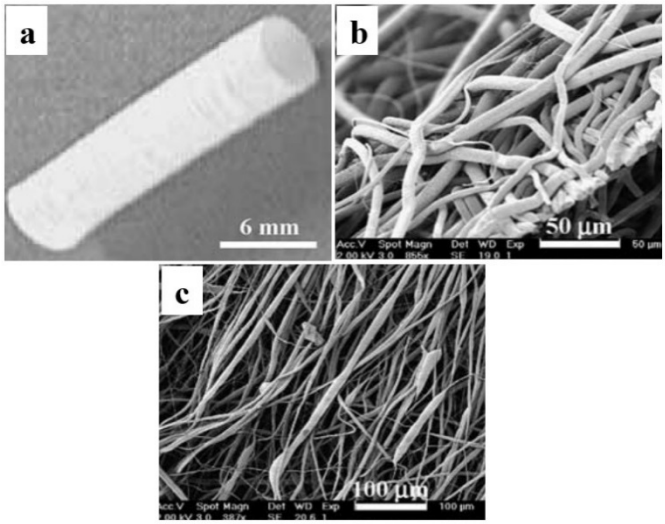


Fig.2.1: SEM photographs of (a) synthetic PCL/PLA blood vessel of 6 mm diameter, (b) the randomly oriented PCL inner layer, (c) the aligned PLA outer layer (reprinted from Ref.35).

Dynamic culture of PCL synthetic blood vessel using SMCs in bioreactor resulted in enhancement of the burst strength (1298±156 mm Hg) greater than that measured for the static culture (809±44 mm Hg) after 2 weeks [36].

Small diameter vascular graft has also been fabricated by electrospinning of PLCL. The diameter of the graft (2.3-2.5mm) and the wall thickness of the graft (50-340 µm) increased with the increase of electrospinning time from 10 to 100 min. It

was found that the thinner the wall thickness of the synthetic vascular conduit, the more compliant the conduit. For example, the stiffness parameter and diameter compliance of the thinnest graft (Inner diameter=2.3±0.1mm and wall thickness=49±5µm) was 6.8±3.1 and 18.7±11.2%/mmHgx10-2 while it was 76.2±18.0 and 2.0±0.2%/ mmHgx10-2 for the thickest graft (Inner diameter=2.5±0.1mm and wall thickness=336±21 µm) [37].

A combination of cell matrix engineering with electrospinning resulted in fabricating enhanced PLCL nano fibre (1.05±0.23 µm average fibre diameter). This fibrous scaffolds is seeded with SMCs. The resulted vascular grafts demonstrated enhanced mechanical characteristics [38]. The cell matrix engineered PLCL vascular grafts demonstrated comparable mechanical properties to native rabbit aorta, as well as, high self-sealed property, due to the elasticity of PLCL. The grafts exhibited tensile strength of 1.91±0.56MPa and 3.23 ± 0.57MPa and strain strength of 135% and 270% after 1 week and 4 weeks cell culture times respectively compared to the values obtained for native rabbit aorta (2.61±0.4MPa for tensile stress at a strain of 86.7%). Moreover, the PLCL grafts demonstrated better mechanical properties than GORE-TEX, which is currently used clinically as vascular grafts [38] (see Table.2).

2.1.1.2. Polyurethane

Another example of polymers that has comparable mechanical properties to native blood vessels is polyurethane (PU). PU has been used to fabricate vascular graft of 4 mm inner diameter, with an average fibre diameter of 732.72 ± 52.22 nm and porosity of 50-60. The tensile strength, rupture load and ultimate elongation obtained were 5.85 ± 0.62 MPa, 16.5 ± 1.1 N and 294.5 ± 19.4 % respectively [39].

Thermoplastic PU can be modified, for example, by adding pentenoyl chloride up to 20% and then cross-linked during electrospinning using UV irradiation. The

fabricated blood vessel of 1.6 mm diameter using the modified electrospun polyurethane fibres showed burst pressure of 550 mmHg and compliance of 12.1 ± 0.8 and 6.2 ± 0.3%/100 mmHg for uncrosslinked and crosslinked fibres respectively [40]. Thermoplastic PU can also be modified by replacing the aromatic diisocyanate MDI with aliphatic diisocyanate HMDI [41]. Further, the hydrolytic degradability of TPU can be increased by introducing cleavable chain extenders such as hydroxyethyl lactate (EGLA) and bis (2-hydroxyethyl) terephthalate (BET) instead of the chain extender BDO. TPU containing EGLA exhibited degradability rate two times faster than surgical poly (lactic acid) (PLA) while TPU containing BET showed slower degradability rate than PLA. From the point of tissue engineering, higher degradability of the fibrous scaffolds might give an opportunity for integrated native tissue to eventually form scaffold- free vessel. The modified TPU conduits demonstrated good mechanical characteristics with tensile stresses of both TPU containing EGLA and TPU containing BET (4.7±0.2 MPa and 17±1 MPa) were being less than the benchmark used in this study, Pellethane (20±4 MPa) [41].

Blood vessels of 1.3 mm and 4.7 mm were successfully fabricated from TIPS/ polyurethane ester urea (PEUU). The inner layer contains porous TIPS polymer casted using custom molds with the same inner diameter as the blood vessels and the outer layer has electrospun nanofibrous PEUU with fibre diameters of 743 ± 201 nm. The pore sizes of TIPS scaffold were 51 ± 3 µm and 123 ± 20 µm for the of 1.3 and 4.7mm diameters respectively while the pore size of PEUU nano fibrous (5.1 ± 3.2 µm) was the same for both sizes of vessels. The vessels showed comparable mechanical properties to the native blood vessel. Whereby, the elastic modulus was 1.4 ± 0.4 MPa, and ultimate tensile stress was 8.3 ± 1.7 MPa. The compliance and suture retention forces were found to be 4.6 ± 0.5 x10-4 mmHg-1 and 3.4 ± 0.3 N respectively [42].

Another way of creating PU synthetic vascular graft of diameter of 4mm and 48 mm is by spin casting the PU layer first to pattern microgrooves on the lumen area. Then, PU micro fibres were electrospun on the outer layer with average fibre diameter of 1.20 ± 0.31 µm (Fig.2.2). The fabricated blood vessel exhibited acceptable mechanical properties such as Young's modulus of 2.00 ± 0.40 MPa and strain stress of 300%. [43].

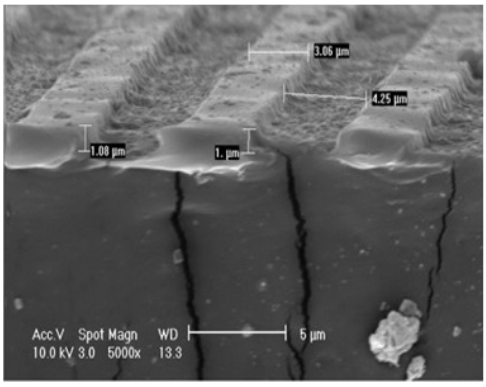


Fig.2.2: SEM image of polyurethane fibrous scaffold consists of micropatterned internal layer and electrospun micro fibres external layer; the ridge width, channel width and channel depth were 3.6 ± 0.2, 3.9 ± 0.1 and 0.9 ± 0.03 µm, respectively (reprinted from Ref.43).

2.1.1.3. Other polymers

Other polymers apart from PCL and PU were used to create fibrous scaffolds such as L-lactide-co-trimethylene carbonate [44], polypropylene (Moplen 462R PP) and polylactide (PLA 4060D) [45]. Different treatment for the polymers is employed either [44, 45].

Crosslinking L-lactide-co-trimethylene carbonate fibrous scaffold by γ-radiation enhanced the mechanical properties of the synthetic fibrous scaffold. The crosslinked scaffold exhibited Young's modulus matched the native human artery (0.4 to 0.8 MPa) [44].

Melt electrospinning technique was employed to manufacture synthetic blood vessel which avoided the drawbacks of using solvents. However, the influence of the mass flow rate on the structure of electrospun fibrous scaffold is major compared to the other fabrication parameters such as voltage and distance between the spinneret and the collector. Tubular vascular grafts were fabricated using polypropylene (Moplen 462R PP) and polylactide (PLA 4060D) after finding the appropriate mass flow rate (25 g/10 min and 2.16 kg at 230^{o}C) with an average fibre diameters up to 4.8 and 3 μm respectively (Fig.2.3). However, the random nature of the electrospun fibrous scaffolds might affect the response of the cells either positively or negatively [45].

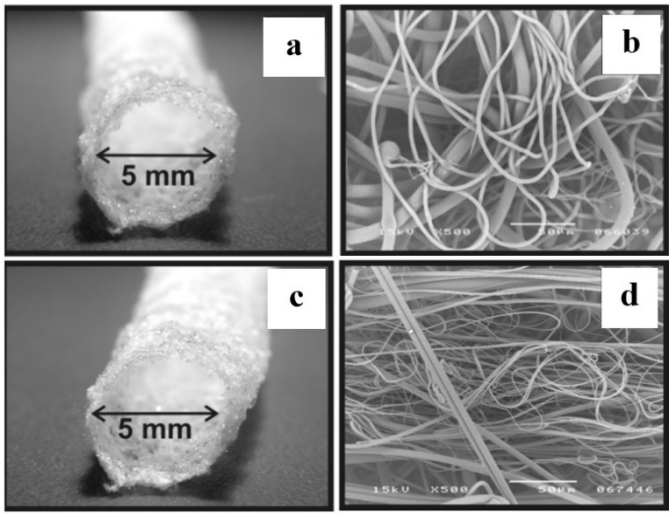


Fig.2.3: Tubular blood vessels fabricated by melt electrospinning: (a, b) polypropylene (Moplen 462R PP); (c, d) polylactide (PLA 4060D) (reprinted from Ref.45).

2.1.2. Natural polymer nanofibre scaffolds

Natural polymers, such as silk, collagen and elastin, have also been deployed to fabricate blood vessels. They have good biological properties in terms of cell attachment and proliferation. However, they demonstrated low mechanical properties, compared to synthetic polymers that is essential in synthetic blood vessel manufacturing [46-53].

2.1.2.1. Silk

Silk fibroin is a protein that can be obtained from the Bombyx mori silkworm. Research showed that silk has good biocompatibility, degradability and mechanical properties [46-48]. Electrospinning of silk fibroin was of great interest. However, it was done with other polymers to enhance electrospinnability or in organic solvents.

Silk-based vascular graft of 5 mm diameter and 0.15 mm wall thickness was fabricated (Fig.2.4). The electrospun scaffold demonstrated elastic modulus, ultimate tensile stress and burst strength up to the values of 2.45 ± 0.47 MPa, 2.42 ± 0.48 MPa and 811 mmHg respectively, which are comparable to native blood vessels [46].

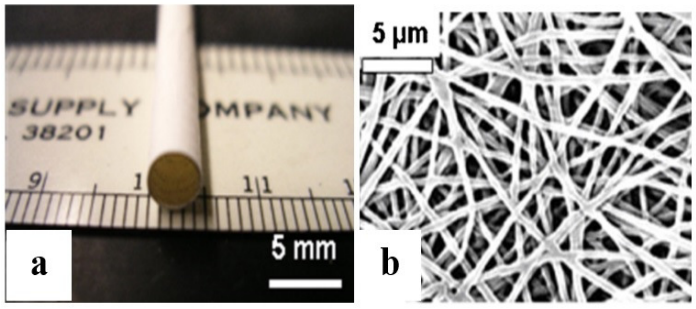


Fig.2.4: (a) Image of silk-based vascular graft (b) SEM photograph of silk-based fibrous scaffold (reprinted from Ref.46).

Zhou et al [47] illustrated optimised conditions for the fabrication of silk fibroin (SF) based blood vessel using electrospinning without the utilization of organic solvent. These fabrication conditions are 18 kV, collection distance of 18 cm, concentration of 37%, and flow rate of 0.15 mL/min. Methanol treatment of fibrous scaffolds after fabrication led to an increment of the tensile strength value from 0.36 MPa to 3.57MPa. The treated SF scaffold is more crystalline than untreated SF scaffold which was confirmed by DSC analysis and ATR-FTIR-ATR. The melting/decomposition temperature and enthalpy measured by DSC analysis increased by lengthening the methanol treatment to 15 min (for untreated sample T (^{o}C) and ΔH

are 279.7^{o} and 130.13 Jg^{-1}, for methanol treated sample T (^{o}C) and ΔH are 287.2^{o} and 138.44 Jg^{-1}. FTIR analysis indicated that the peak of β-sheet structure is clearer through longer methanol treatment (1699 $cm^{-}1$) [47, 48], see Table.2.

2.1.2.2. Gelatine

Collagen constitutes the major ratio of native blood vessel compositions [49]. Gelatine is a denatured form of collagen and has been used to electrospin scaffolds for blood vessels. Gelatine nanofibrous tubular scaffold was electrospun with an internal diameter of 5 mm and an average fibre diameter of 0.67μm [49]. However, gelatine is soluble in water. Crosslinking with glutaraldehyde is a way to prevent the dissolution of gelatine fibrous scaffold (Fig.2.5) [50]. The crosslinked scaffolds showed reasonable mechanical properties relative to natural collagen. For instance, gelatine scaffold crosslinked with glutaraldehyde exhibited a young's modulus of 33.8 MPa in the axial direction whilst natural collagen has Young's modulus of the value of 5-10 MPa [51]. The crosslinked scaffolds further showed excellent tensile strength in the axial direction to up to 2.9 MPa compared to that measured for human coronary artery of 60 KPa. However, the strain to failure of these scaffolds was lower than that measured for arteries (11.7% for crosslinked scaffolds and 35% for arteries) [52], see Table.2.

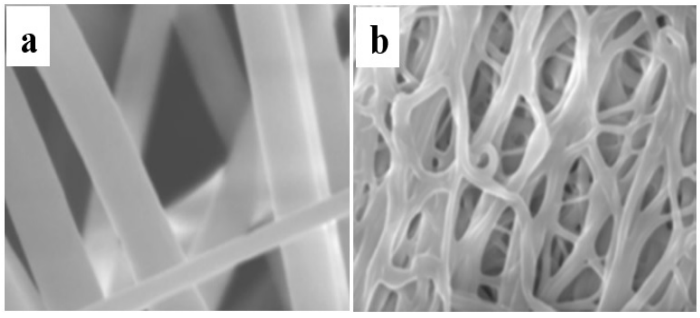


Fig.2.5: SEM photograph of gelatine tubular scaffold (a) before crosslinking (b) after crosslinking in 25% glutaraldehyde for 3 days (adapted from Ref.52).

4.1.2.3. Elastin

The existence of elastin in natural arteries increases the strain to failure and reduces the Young's modulus. Additionally, it reduces blood clotting as it is blood-contacting surface on stents and grafts. Recombinant human tropoelastin (rTE) is the monomer form of elastin which results in native elastin with crosslinking. rTE was utilized for the fabrication of electrospun blood vessel and crosslinked by disuccinimidyl suberate [53]. The electrospun rTE fibrous scaffold showed encouraging mechanical characteristics such as ultimate tensile strength of 0.36 ± 0.05 MPa, elastic modulus of 0.91 ± 0.16 MPa and burst pressure of 485 ± 25 mm Hg [53].

2.1.3. Nanofibre scaffolds from polymer blends

Polymer blends have been employed as material to fabricate blood vessels to obtain good mechanical properties and biocompatibility [54-62]. Blends comprising synthetic polymers only or natural polymers only or a combination of synthetic and natural polymers have been all attempted. This generally resulted in an enhanced mechanical and biological properties when compared to scaffolds produced from single polymers.

As examples of producing fibrous scaffolds from synthetic polymer blends [57-60], it was found that small diameter blood vessel fabricated from poly (L-lactic acid)-co-poly (ε-caprolactone) P (LLACL 70:30) (3 mm internal diameter) has mechanical properties closer to native abdominal aorta. The P (LLACL) fibrous scaffold demonstrated tensile strength of 3.9±0.3 MPa in the circumferential direction while native abdominal aorta showed tensile strength of 5.29 MPa in the same direction. Fig.2.6 a, b indicated that the integrity of the fibrous scaffolds is maintained for less than 2 month when immersed in PBS solution at 37^{o}C [54].

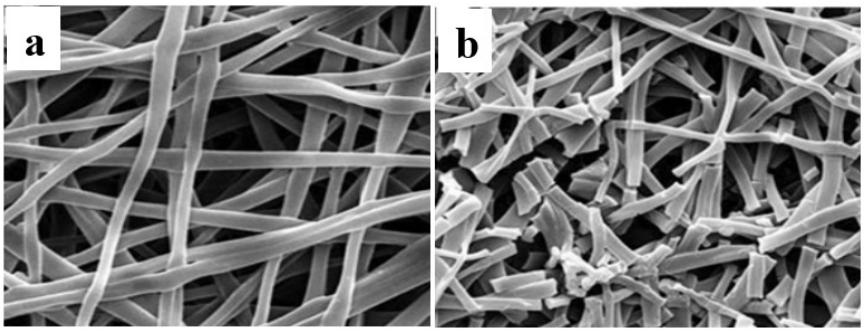


Fig.2.6: SEM images of in vitro degradation of P (LLACL) fibrous scaffold in PBS at 37^{o}C after (a) 0 time, (b) 2 months (reprinted from Ref.54).

Electrospun PU/PCL blend was used to construct small-diameter blood vessels (3 mm diameter, 0.5-2µm fibre diameter, 0.5-150 µm pore size).The thus-constructed vessels demonstrated sufficient mechanical properties (tensile strength: 18MPa, Strain: 375% and pressure strength: 590-600 mmHg) that meets the requirement of vascular graft applications [55].

PCL (25:75) blend demonstrated better biomechanical properties for cardiovascular graft applications than PLA/PCl (75:25), with tensile strength of 1.0±0.3 MPa versus 2.6±0.8 MPa, tensile strain of 7.4±2.3 % versus 1.8±1.2% and suture retention strength of 0.454 ± 0.047 versus 0.062 ± 0.025) [56].

Hybridization of silk fibroin with collagen for the fabrication of small diameter blood vessels (6mm diameter and 8mm length) enhanced the mechanical properties. The Silk fibroin-collagen fibrous composite showed burst pressure and strain at failure of 894.00±24.9 mmHg and 28.76±1.39% respectively while the pure silk fibroin fibrous scaffolds (SF) showed burst pressure and strain at failure of 575.67±17.47 mmHg and 27.12±2.63 % respectively [57]; (see Table.2).

In studies on synthetic-natural polymer blends [58-62], nanofibrous scaffolds comprises of gelatin/ PCL and collagen/ PLCL (average fibre diameter: 386.9±102.5 nm and 301.8±97.3 nm respectively) were tested in terms of their compatibility as

vascular prostheses [59]. Both gelatin/PCL and collagen/PLCL scaffolds demonstrated good wettability (contact angle=0^o). However, Young's modulus of collagen/PLCL scaffold increased from 1.77± 0.09 MPa to 5.99±0.80 MPa after 6 weeks transplantation in nude mice as a result of vessel-like tissue formation whilst Young's modulus of gelatin/PCL decreased from 1.49±0.06 MPa to 0.75±0.15 MPa after the same period of transplantation [58].

Polydioxanone (PDO)-elastin (50:50) blend demonstrated mechanical properties close to that measured for native femoral artery. Sell et al created a scaffold using PDO (100:0) that showed elastic modulus, ultimate stress and strain at failure of 19.98 ± 0.74 MPa, 5.57 ± 0.7 MPa and 206.33 ± 38.96 % respectively [59]. When the blend of PDO-elastin was (50:50), these mechanical properties were lower: 9.64 ± 0.66 MPa, 3.25 ± 0.24 MPa and 64.93 ± 3.97%. PDO-elastin (50:50) demonstrated characteristics that were closer to those measured for femoral artery (9 to 12 MPa, 1 to 2 MPa and 63 to 76%) [59].

Collagen-elastin-poly (D, L-lactide-co-glycolide) (PLGA) (45%-15%-40%) blend was utilized in fabricating small diameter blood vessel comprised of 4.75 mm diameter, 0.5 mm wall thickness and 477 to 765 nm average fibre diameter. The hybrid scaffold demonstrated tensile strength and young's modulus up to the values of 0.37 MPa and 0.85 MPa respectively [60].

Polylactide-silk fibroin-gelatin Composite based blood vessel (4.5 mm diameter, 0.5 mm wall thickness and 82% porosity) possessed breaking strength, strain, suture retention strength and burst pressure strength of 2.21 ± 0.18 MPa, 60.58±1.23%, 4.58 ± 0.62 N and 1596 ± 20 mmHg. These properties are comparable to native blood vessels [61, 62], see Table 2.

2.1.3.1. Layered scaffolds

Studies on blood vessel fabrication showed that mimicking the three walls of native blood vessels can be produced using layering technique of electrospinning [63-65].

Polycaprolactone, elastin and collagen have been used to fabricate tri-layered blood vessel resulting in compliance ranging from 0.8 to 2.8%/100 mm Hg and young's modulus ranging from 2.0 to 11.8 MPa. As shown in Table.2, the compliance and modulus measured for the tri-layered graft matches that of native artery [63.

Another polymeric blend, collagen-chitosan-thermoplastic polyurethane (TPU) was used to fabricate vascular graft blood vessel of 3mm in diameter. The fibrous scaffolds were electrospun in random and aligned orientations consisting of fibre diameter of 360±220 and 256±145 nm respectively. Both random fibrous scaffold and aligned fibrous scaffold showed average elongation at break strength and average tensile strength of 9.87 ± 1.77 % and 9.38 ± 1.04 MPa and 58.92 ± 15.46% and 14.93 ± 0.59 MPa respectively [64].

Additionally, collagen and chitosan can improve both the wettability and the biological properties of polymers. Collagen/chitosan/P (LLA-CL) (20:5:75) tubular scaffold was fabricated with attributes of 3 mm diameter, 1.1 ±0.5nm pore diameter, 409 ±120nm fibre diameter and 0.33 ± 0.09 mm wall thickness. This tubular scaffold provided ultimate stress , elongation at break , elastic modulus, burst press, compliance and contact angle of 16.9 ± 2.9MPa,112 ± 11%mmHg,10.3 ± 1.1MPa ,>3365 ± 6 mmHg ,0.7±0.4 mmHg and 110.5 ± 0.9^{o} [65].

Table.2 lists the polymers used for the fabrication of small diameter blood vessels and their relative mechanical properties. It is quite clear that the mechanical properties of polymer blends is better than individual synthetic and nature polymers.

Table.2: Polymers used for the fabrication of small diameter blood vessels, and their mechanical properties.

Polymers	Solvents	Operating conditions						Mechanical properties				Ref.
		Polymer concentration (W/V %)	Voltage (kV)	Air gap (cm)	Flow rate (ml/h)	Spinning time (min)	Mandrel rotation speed (rmp)	Young's Modulus (MPa)	Maximum stress (MPa)	Maximum Strain (%)	Burst strength (mmHg)	
Synthetic polymer-based scaffolds												
PCL-PLA	$CHCl_3$	12.5	13	20	0.6	180	3600	30.9 ± 6.6	4.3 ± 0.2	47.0 ± 6.3		[35]
	$CHCl_3$/DMF	14	13	20	1.5	180	10800	10.7 ± 0.3	1.2 ± 0.1	260		
PCL	$CHCl_3$/ EtOH	15	20		12	6			4.8	600		[47]

PCL	$CHCl_3$/ EtOH	5-15	15-25		12-24		4500		2-7.4	200-1200		[33]
TIPS-PEUU	HFIP	8	10		1		250	1.4 ± 0.4	8.3 ± 1.7			[42]
PCL	$CHCl_3$/ EtOH	15	20		12	6			4.1±0.5	1092 ±28	3280 ±280	[32]
PLCL	HFP	9	15		1		500	1.2 ±0.3	3.23 ± 0.57	270	933 ±22	[38]
PCL	$CHCl_3$/ MeOH	5	18		2			17.44± 0.91	13.35 ±1.47	168.40 ±8.76		[48]
		5	11		8			21.00 ± 1.39	8.72±0.84	639.20 ± 24.15		
Natural polymer-based scaffolds												
Silk			10-11		0.9		3000	2.45 ± 0.47	2.42 ± 0.48		811	[46]
Gelatine	TFE	10	30		1.5	50	2	33.8	2.9	11.7		[49]

(rTE)	HFP	15	18.5	12.5	2		4400	0.91 ± 0.16	0.36 ± 0.05		485 ± 25	[53]
Hybrid polymer-based scaffolds												
PDO-elastin (50:50)	HFP	100 mg/ mL and 200 mg/ mL	22	12	4 and 8		500	9.64 ± 0.66	3.25 ± 0.24	64.93 ± 3.97		[60]
Collagen-elastin-PLGA	HFP	5-20	22	10	3		500	0.85	0.37			[61]
PLLACL coated with collagen	DCM/ DMF	10	10		1	15	150	16.6 ± 4.4	3 .9 ± 0.3	292 ± 87		[54]
PEUU-PMBU	HFP	15	10	15	1	45	250	3±1	342±43			[67]
PLA-Silk Fibroin-Gelatin	formic solution	13	30	13	0.2		1000		2.21± 0.18	60.58± 1.23	1596± 20	[62,63]
	$CHCl_3$/ EtOH	5	25	15	0.1		2000					
PCL-collagen	HFP	1	20	10	3		1000	2.7±1.2	4.0±0.4	140±13	4915± 155	[68]
PHBV-PCL	$CHCl_3$	1	20	15	0.5		3000	22 ±7	1.4 ± 0.3	30 ± 20		[69]

Collagen-chitosan-P(LLA-CL)	HFP/TFA		14	12-15	1			10.3± 1.1	16.9 ± 2.9	112 ± 11	>3365 ± 6	[66]
Lecithin-cholesterol-(Chol-PCL)	$CHCl_3$/DMF		18	15	3			35.92 ± 4.75	5.22 ± 0.50	107.15 ± 10.78		[70]

HFP; 1, 1, 1, 3, 3, 3-hexafluro-2-propanol, TFE; 2, 2, 2-trifluoroethanol, DCM; dichloromethane, DMF; N, N-dimethylformamide; TFA; 2, 2, 2-trifluoroacetic acid.

2.1.4. Biological studies on fibrous small-diameter blood vessels

The successful implantation of small diameter blood vessels is influenced by factors such as anastomosis (attachment of the artificial blood vessels to the native blood vessels), intimal hyperplasia resulting from the over-proliferation of SMCs inside the blood vessels and thrombosis formation due to blood clots. Endothelialization of the synthetic blood vessel is one way to overcome some of the limitations mentioned.

In an attempt to mimic native blood vessels, synthetic blood vessels have been seeded with cells including endothelial cells (ECs), fibroblast, smooth muscle cells (SMCs), and mesenchymal stem cells (MSCs). Research on tissue engineering using fibrous scaffolds have shown that cell attachment and proliferation is affected by parameters of the fibrous scaffolds such as the type of scaffold [72], alignment of the fibre [43], wettability [55] and collagen content of the scaffolds [54]. The responses of synthetic blood vessels to these cells are discussed in the following sections.

2.1.4.1 In vitro studies

2.1.4.1.1. Endothelial cells

ECs is a cell type that is commonly used to seed synthetic scaffolds for blood vessels. This is because ECs constitute the inner layer of native blood vessels and can prevent thrombosis formation [15-17 and 71-79]. ECs cell attachment and proliferation is affected by parameters of the fibrous scaffolds such as stiffness [66, 71], shear stress applied on the scaffold [72], wettability [55], collagen content [54, 73-76], alignment of the fibre [43, 64, 77], and the type of scaffolds [46, 53, 78, 79].

Research in the field of vascular grafts fabrication have reported that stiffness of the material can influence the cell-scaffold interaction. A reduction in ECs proliferation was shown when cultured on stiffer gel-based scaffold [71]. Gaudio et al. showed that Rat cerebral endothelial cells (RCECs) proliferated better on electrospun PCL scaffold

which was more flexible than electrospun PCL/po (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) composite blend. Additionally, there were more significantly more apoptotic cells on PHBV fibrous scaffold due to the stiffer nature of PHBV [66].

Small-diameter grafts made of PCL electrospun nanofibres with 2.4±0.1 mm inner diameter, 128±13µm wall thickness and fibre diameters on the inner and outer surfaces of 799±116 nm and 820±121 nm respectively were seeded with ECs. It was demonstrated that the gradual increase of shear stress from low (3.2 dyn/cm^2) to high (19.6 dyn/cm^2) applied on the endothelialized grafts in a custom-designed mock circulatory instrument could reduce the detachment of cells, increase cell elongation and alignment and actin fibres with the direction of flow. This protocol could be utilised to enhance the retention of ECs prior to implantation of the vascular grafts [72].

PU/PCL blend scaffold resulted in hydrophilic surface (contact angle: 126^o) which supported the adhesion, and proliferation of cow pulmonary artery endothelial cells when cultured for 5 days (optical density: 4% versus 1% for 1 day) [55].

Researchers showed that the addition of collagen to polymeric scaffolds can enhance EC interaction with the scaffolds. A bilayered fibrous vascular graft of poly (e-caprolactone) (PCL) and collagen blend of 4.75 mm with different fibre diameters (0.27 µm inner layer and 4.45 µm outer layer) enabled confluent endothelization on the inner layer lumen [73-75]. Collagen coated P (LLACL) fibrous scaffold by air plasma treatment assisted the adhesion, spread and proliferation of Human coronary artery endothelial cells (HCAECs) after 10 days of culture [54]. A vascular prosthetic of collagen/chitosan/P (LLA-CL) (20:5:75) promoted ECs interaction compared to pure P (LLA-CL) [65]. Encapsulation of vascular endothelial growth factor (VEGF) in the fibrous scaffold of chitosan hydrogel /poly(ethylene glycol)-b-poly(L-lactide-co-caprolactone) (PELCL) as inner layer and platelet-derived growth factor (PDGF) in the fibrous scaffold of emulsion poly(ethylene glycol)-b-poly(L-lactide-co-

glycolide) (PELGA)/ PELCL as outer layer by coaxial electrospinning could potentially control the proliferation of vascular endothelial cells (VECs).VECs proliferated faster to cover the lumen of the graft (optical density=1.4) after 3days of culturing [76].

Fibre alignment is another parameter that influences EC proliferation. Aligned nanofibres have been shown to enhanced BAEC cells response in comparison to random nanofibres when synthetic blood vessel were fabricated from absorbable poly-e-caprolactone (PCL) with 4.5 inner diameter and 400-500 nm average fibre diameter [77]. Polyurethane vascular graft with patterned lumen enabled the cells to migrate through these patterned channels, thus, enhancing the full endothelialization of the vascular graft (Fig.2.7; [43]).

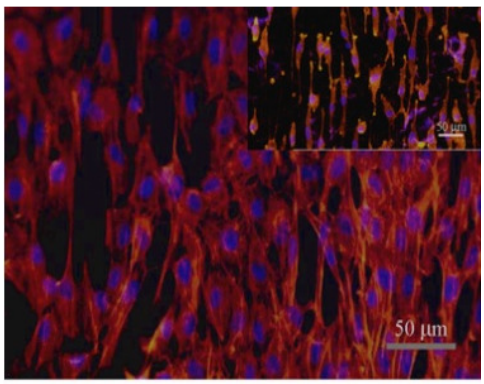


Fig.2.7: Endothelial cells cultured inside the micropatterned polyurethane-based synthetic blood vessel after 3 days (reprinted from Ref.43). Porcine iliac artery endothelial cells were cultured for 7 days on both random and aligned collagen-chitosan-thermoplastic polyurethane (TPU) scaffolds leading to almost equal cell viability on both scaffolds for PIECs (absorption index 1.6) [reprinted from Ref.64].

Similarly, ECs have also been cultured on natural polymer [46, 53, 78, and 79]. Human aortic endothelial cells cultured on silk fibrous mats have been shown to adhere and proliferate well on the fibrous scaffold. However, human aortic endothelial cells did not infiltrate the fibrous scaffold due to the small size of the pores [46]. Tubular scaffold fabricated from recombinant spider silk protein (pNSR32), polycaprolactone (PCL) and gelatin (Gt). At a ratio of 5:85:10 exhibited porosity, pore size, average fibre diameters and contact angle of

86.2±2.9%, 2423±979,166±85 nm and 45.7±13.7^{o} respectively. The proliferation of rat aortic ECs on pNSR32/PCL/G scaffold was higher than pure PCL and pNSR32/PCL after 7 days of culture , evidenced by a higher proliferation index of 26.8% compared to that of PCL and pNSR32/PCL (17.8% and 21.5%) respectively [78]. Using the same silk protein (pNSR32) and combining it with PCL and chiton also showed enhanced EC growth which may lead to release of higher concentration of NO within 7 days culture (40 µm/L versus 30 µm/L for untreated PCL) [79]. This is in return reduced the risk of thrombosis.

Another natural polymer, rTE was used to fabricate blood vessel and has been demonstrated to support endothelial cell adhesion and growth [53].

2.1.4.1.2. Smooth muscle cells

SMCs constitute the middle layer of native blood vessels and it provides the elasticity to the vessels so they can withstand the pulsatile forces from constant blood circulations [15-17].

The ability for SMCs proliferation interact with scaffolds can be affected by parameters such as the mode and the time of culture [36, 38], the type of scaffold [80], fibre alignment [81, 82], and collagen content of the scaffolds [58].

SMCs cultured in tubular fibrous scaffold of poly (lactide-co-ε-caprolactone) of 4mm internal diameter experienced better proliferation under a dynamic condition than in static culture. In addition, both collagen and DNA contents under dynamic culture were higher than the static culture after 2 weeks (11.5 µg/mg and 35 µg/mg for collagen respectively and 5.7 ±0.35 mg/uL and 7.5±0.2 mg/uL for DNA respectively) [36].

PLCL nano-fibre based vascular grafts seeded with SMCs showed good biological properties [38], with increasing cell viability dramatically by increasing cell culture time of grafts from 1 week to 7 weeks resulting in values of $5x10^5$ cells and $11x10^5$ cells

respectively. DNA content evaluation showed enhancement as a result of extending cell culture time from 1week (1.4±0.1 µg/µL) to 4 weeks (5.6 ±0.3 µg/µL) [38].

Poly (ester amide) s (PEAs) was used as novel approach for fabricating synthetic blood vessel. PEAs is difficult to electrospin. However, Srinath et al. have shown that adding 18-30% PCL can enhance electrospinnability. PEA-PCL fibrous scaffolds with an average fibre diameter of 0.4µm enhanced both the proliferation of SMCs and the expression of elastin after 7 days when compared to PEA discs and PCL fibrous scaffold with the same fibre diameter. Further, the elastin expression of PEA-PCL fibrous scaffold was 230% while it was just 50% for PEA films and 100% for PCL fibres [80].

It has been noted that the expression of cultured SMCs on electrospun PLLA fibrous scaffold covered polydimethylsiloxane (PDMS) can be either synthetic or contractile phenotype. Synthetic SMCs are able to swiftly proliferate and migrate, producing ECM components like collagen and elastin. Contractile SMCs are mature and do not produce ECM which is the case of healthy tunica media of natural blood vessels [81]. The alignment of fibres plays a role in that, random PLLA fibres tended to produce SMCs with synthetic phenotype and aligned fibres will exhibit SMCs with contractile phenotype [81].

SMCs seeded on the blended scaffold of Collagen/elastin/poly (D, L-lactide-co-glycolide) (PLGA) (45%/15%/40%) provided mitochondrial metabolic activity of 90% after 7 days of culture [60]. SMCs responded better to collagen/PLCL than gelatin/PCL providing bipolar spindle shape indicating a contractile phenotype with an optical densities of 0.3 and 0.2 respectively after 1 day of culture [58].

Apart from seeding SMCs on scaffolds, technology has enabled sheets of SMCs to be generated forming scaffolds made entirely from cells. Combination of cell sheet technology with electrospinning resulted in harvesting robust confluent cell sheet which is difficult to obtain by cell sheet technology alone [81]. Firstly, micropatterned polydimethylsiloxane

(PDMS) was covered by N-isopropylacrylamide (pNIPAm). Secondly, electrospun polycaprolactone (PCL) scaffold was mounted on the micropatterned PDMS and then cultured by human aortic smooth muscles cells for 4 days. Eventually, the confluent cell sheet detached from the PMDS substrate upon cooling to room temperature and rolled over mandrel of 3 mm diameter to form synthetic blood vessel with contractile SMCs [82].

2.1.4.1.3. Fibroblast

Fibroblast constitute the outer layer of native blood vessels along with collagen and it adds rigidity to the vessels [15-17]. So in mimicking the native vessels, fibroblast are tested in tissue engineered vascular grafts research.

Fibroblasts, being a major constituent of the outer layer of blood vessel, is another cell type that is examined on synthetic vascular graft. Fibroblasts from various species such as mouse and human have been seeded on different types of polymeric scaffolds. Fibroblast cell attachment and proliferation is affected by some parameters such as the type of the scaffold [39], the size of the pores of the scaffolds [35], and collagen content of the scaffolds [57,64,65].

PU graft demonstrated similar biocompatibility compared to the commercial PTFE vascular graft where the relative growth of mouse fibroblasts (L929) cells is almost 80% in both cases [39]. 3T3 mouse fibroblasts cells adhered well to the surface of a bilayered PCL/PLA scaffold after 4 weeks of cell culture as shown in (Fig.2.8). Human venous myofibroblasts (HVS) cells were concentrated in the outer layer of PCL rather than the inner layer of PLA, which possibly due to the pore size. However, the cell content was almost 64% compared to the native porcine pulmonary valve tissue indicating that the growth of the tissues was in progress [35].

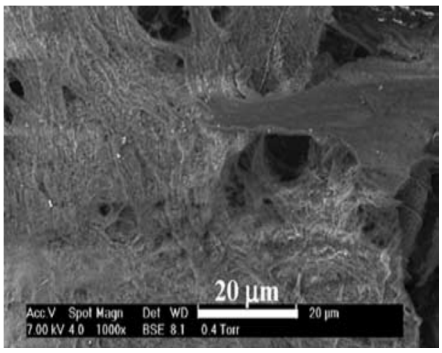


Fig.2.8: PCL/PLA nanofibrous scaffold cultured in 3T3 mouse fibroblasts cells for 4 weeks (reprinted from Ref.35).

Human dermal fibroblasts was cultured on PDO-elastin scaffolds for 1 day and 7 days and it was observed that these cells could infiltrate the surface of polydioxanone-elastin. When cultured on the pure PDO, the fibroblasts were only attached to the surface as shown in Fig.2.9. It is clear that PDO boosted the mechanical properties of the blend while elastin improved the elasticity and the cells interaction since it mimics the natural ECM [59].

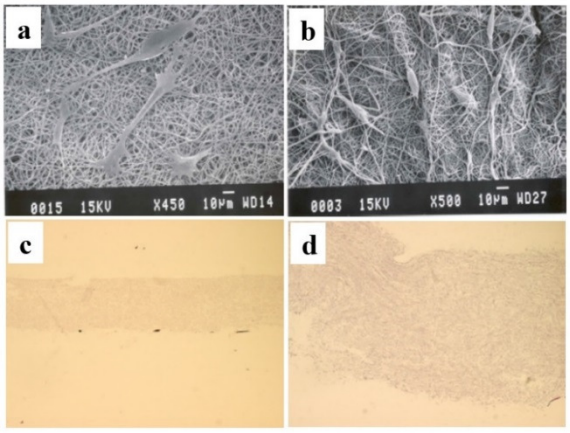


Fig.2.9: SEM micrographs of human dermal fibroblasts cells cultured on (a) pure polydioxanone and (b) polydioxanone-elastin (50:50) blend for 1 day, histology examination of human dermal fibroblasts cells cultured on (c) pure polydioxanone and (d) polydioxanone-elastin (50:50) blend for 7 days (reprinted from Ref.59).

3T3 mouse fibroblasts cells experienced good adhesion, spread and proliferation when seeded on Polylactide-Silk Fibroin-Gelatin fibrous scaffold for 21 days [64, 65]. The 3T3

fibroblast cells responded better on hybridized silk fibroin-collagen fibrous scaffold than pure silk fibroin fibrous scaffold [57].

2.1.4.1.4. Mesenchymal stem cells

MSCs have been of interest to vascular research due to its ability to differentiate to SMCs and ECs in vivo. It can be used to replace ECs that are injured or impaired due to SMCs overgrowth [85].

Porosity plays an important role in the interaction of MSCs with the scaffold. MSCs cultured on PCL scaffolds with various porosity showed the higher the porosity of polycaprolactone (PCL) scaffolds (30 µm and 5-6 µm average fibre diameter) the faster the formation of neoarteries. MSCs cells seeded on highly porous PCL resulted in tissue remodelling while seeding on less porous PCL led to proinflammatory phenotype [48].

TIPS/ polyurethane ester urea (PEUU) scaffold was tested using MSCs demonstrating cell density up to 92 ± 1% [42] in comparison to the control.

Electrospun poly (propylene carbonate) fibrous scaffold with 5µm average fibre diameter was used to fabricate vascular graft of 1.5-2 mm diameter and 0.3-0.4 mm wall thickness. MSCs were cultured on the vascular graft for 14 days and it showed acceptable response in terms of adhesion, proliferation and differentiation [83].

2.1.4.2. In vivo studies

Implantation of electrospun synthetic blood vessels in animal models is an important step to demonstrate that the vascular graft can function as intended in a living system. Various electrospun polymers were used for in vivo studies including PCL [35] and PU [46], see Table.3.

A tubular PCL scaffold of 2mm diameter fabricated from PCL fibrous scaffold of 1.90 µm average fibre diameter showed better patency rate compared to expanded polytetrafluoroethylene (ePTFE) grafts when investigated in rats for 24 weeks. The growth of

endothelial cells and fibroblast with extra cellular matrix (ECM) was faster in PCL scaffolds and angiogenesis formation was also observed [69].

The histological analysis of the synthetic blood vessel made from PCL electrospun fibrous scaffold showed that no thrombosis or aneurysm accompanied the implantation of blood vessels in an abdominal aortic rat after 12 weeks. Homogenous infiltration of cells along with degradation of the scaffold, ECM formation and full endothelilization were also observed Fig.2.10 [33].

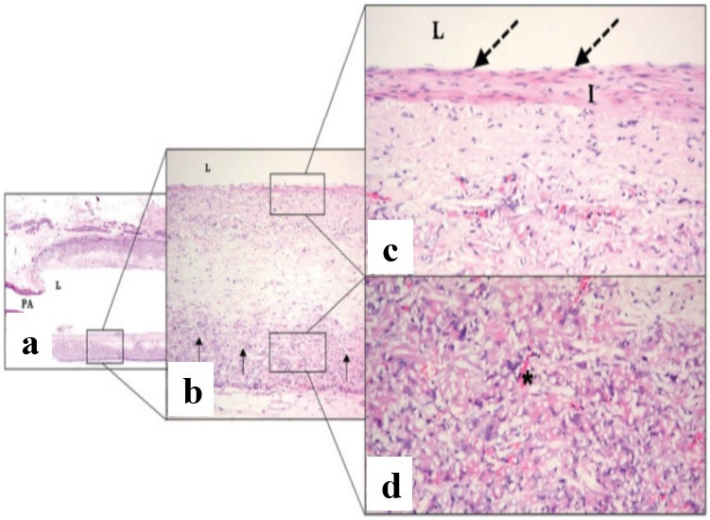


Fig.2.10: Histological analysis for PCL electrospun blood vessel implanted in an abdominal aortic rat for 12 weeks (a) 20 X, (b) 100 X, (c) and (d) 200 X magnification (reprinted from Ref.33).

PCL-based grafts have not been extensively investigated in vivo for its long term effect [51]. Sarra de Valence et al. showed that PCL grafts demonstrated good patency, endothelialization, and no thrombosis up to 6 months when implanted in the abdominal aorta of rat Fig.13 a, b. However, cell regression appeared at 12 and 18 months of implantation due to chondroid metaplasia formation that is responsible for calcification of the grafts (Fig.2.11 c, d; [32]).

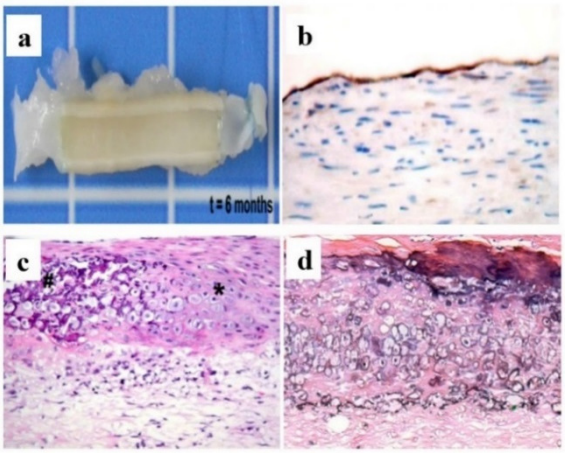


Fig.2.11: (a) Lumen area of explanted PCL graft with no thrombosis formation, (b) endothelial cells layer over the lumen of the graft observed by immunohistology with a brown CD34 staining, (c) the occurrence of chondroid metaplasia (*) after 6 months of implantation which in turn led to calcium deposition (#), (d) The invasion of chondroid metaplasia to the graft material and the osteogenesis formation in the intimal hyperplasia layer (reprinted from Ref.32).

Another way of increasing patency rate of small diameter blood vessels is to modify PCL scaffolds with arginine-glycine-aspartic acid (RGD) molecules. Small diameter blood vessel of 2.2mm inner diameter containing RGD enhanced both patency rate with no thrombosis observed after 4 weeks of implantation in a rabbit and SMCs and ECs infiltration. SMCs covered an average of 65.3±7.6% of PCL-RGD surface area after 4 weeks of implantation (Fig.2.12; [84]).

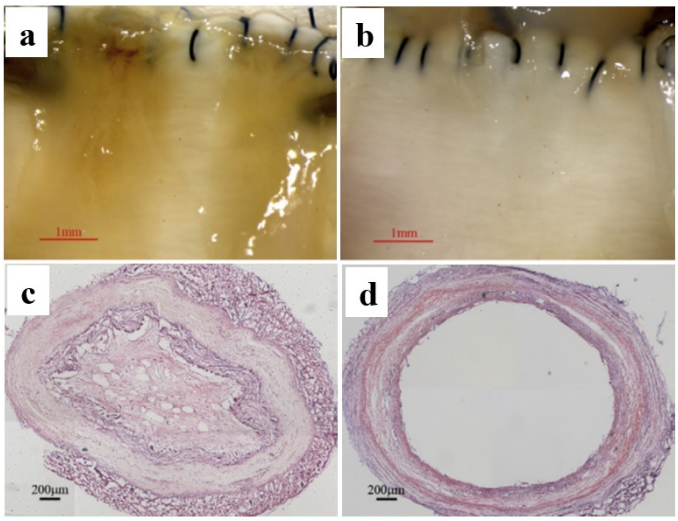


Fig.2.12: Investigation of explanted (a) PCL graft and (b) PCL-RGD graft by stereomicroscope and H&E cross-section staining after 4 weeks of implantation. Acute thrombosis was observed in the lumen of PCL (reprinted from Ref.84).

In vivo comparison study between PCL and polytetrafluoroethylene (ePTFE) was attempted for 16.5 months. It was evident that PCL characteristics is better than that of commercial ePTFE in terms of patency rate (100% versus 67%), compliance (8.2±1.0 %/100mmHg versus 5.7±0.7%/100mmHg), endothelialization (100±0.0 % versus 99.6±1.0 %), cellular-in-growth (32.1±9.2 % versus 10.8±4.0 %) and calcification (7.0±5.0 % versus 15.8±3.2 %). Therefore, this study paves the way for deeper analysis to commercially validate PCL based vascular grafts [85].

Comparison between gelatin/PCL scaffolds and collagen/PCL scaffolds has been carried out in vivo in mice for 6 weeks. The results of hematoxylin and eosin (H&E) staining after subcutaneous implantation showed that gelatin/PCL formed heterogeneous fibres with clear nondegradable scaffold, while collagen/PCL led to the formation of vessel-like tissues with homogenous surface and bands of collagen (Fig.2.13; [58]).

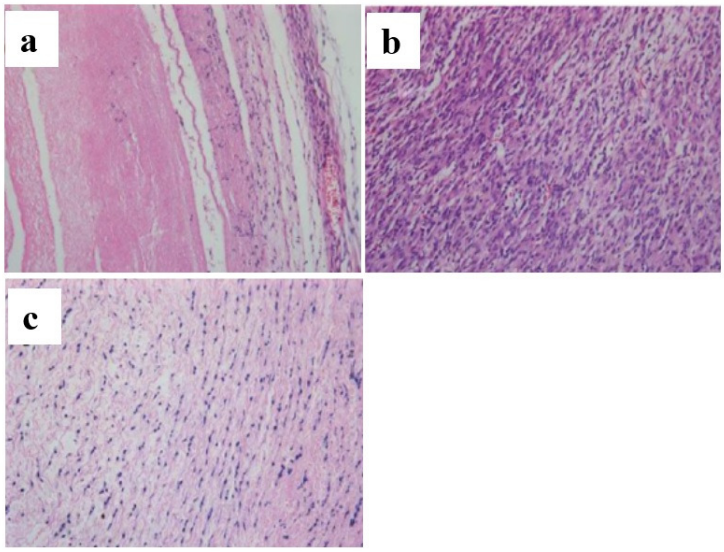


Fig.2.13: Histological photos (H&E staining) of gelatin/PCL (a) and collagen/PLCL (b) scaffolds after 6 weeks implantation in nude mice and newborn pig aortic blood vessel (c) as control (reprinted from Ref.58).

Scaffolds of 1.3 mm diameter made of electrospun biodegradable poly (ester urethane) urea (PEUU) modified with Nonthrombogenic 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymer were implanted in the abdomen of rats for 4, 8, 12 and 24 weeks. The MPC modified conduits demonstrated a patency rate of 92 % after 24 weeks of implantation which is much higher than that observed for unmodified conduits (40%). Neotissues comprise of collagen and elastin as well as smooth muscle cells (SMCs) and endothelial cells (ECs) were observed after implantation. Despite the fact that the constructed conduits demonstrated higher compliance ($4.5\pm2.0 \times 10^{-4}$ $mmHg^{-1}$) than native rat aortas ($14.2\pm1.1\times10^{-4}$ $mmHg^{-1}$), they become comparable to the native values after 4 weeks of implantation. Nonetheless, the conduits became stiffer after 24 weeks of implantation [86].

PU conduits demonstrated good biological characteristics when implanted in rats [46]. PU based tubular vascular graft was fabricated with 1.5 internal diameter, 70 µm wall thickness and 0.88 µm fibre diameter. The fabricated grafts showed 95% patency rate and no thrombin after implantation up to 6 months in inbred Sprague-Dawley rats. Myofibroblasts and myocytes showed good cell responses in terms of adhesion and proliferation. The ultimate circumferential tensile stress of the grafts was also evaluated to be 26.4 MPa [87].

Effect of porosity of PU grafts was investigated and it was found that fine mesh PU grafts with low porosity (53%) increased cell adhesion and proliferation at early stages than coarse mesh polyurethane grafts with high porosity (80%) when implanted in a rat model. However, cell number significantly improved in the high porosity polyurethane grafts. Both fine and coarse mesh grafts possessed the same biomechanical properties before and after transplantation. The tensile strength of the fine and coarse mesh grafts were of 20.2 $\pm$ 4.6 and 16.3 $\pm$ 0.9 MPa respectively, which was higher than that of native rat aorta 4.4±0.9 MPa [88].

In vivo study of replacing inferior superficial epigastric rabbit veins by P (LLACL) for 7 weeks implantation showed that the fabricated scaffold has good patency with no thrombosis observed [54].

Subcutaneous implantation trial of the PLA/SF-gelatin fibrous scaffold for 3 month in sprague-dawley rats, resulted in the formation of vascular network and the decrease of the shape of the scaffold. This indicated that the scaffold has good biocompatibility and biodegradability in vivo [61, 62]. Synthetic vascular graft (1 mm diameter) made of poly (L-lactic acid) micro fibres modified by poly (ethylene glycol) and hirudin showed integration and remodelling with host vasculature when implanted in carotid artery of female sprague-dawley rats. The elastic modulus increased from 3.5 MPa to 11.1 MPa as a result of increasing the implantation period from 1 month to 6 months [89].

Table.3 summarizes the cellular responses on small diameter polymeric blood vessels in vitro and in vivo.

Table.3: Polymers used for the fabrication of small diameter blood vessels, and their biostudies.

Polymers	Cell response		Ref.
	in vitro study	*in vivo* study	
Synthetic polymer-based scaffolds			
PCL-PLA	3T3 mouse fibroblasts cells covered the surface of PCL/PAL fibrous scaffold after 4 weeks. Human venous myofibroblasts (HVS) cells were concentrated in the outer layer of PCL-PLA scaffold.		[35]
PCL		Implanted in a rat revealing that endothelilization and extra cellular matrix (ECM) formation of PCL was faster than PTFE commercial grafts.	[47]
PCL		In vivo implantation in rat for 12 weeks showed that the blood vessels were completely endothelilized without thrombosis formation.	[33]
TIPS-PEUU	Cell culture resulted in density up to 92 ± 1% using Adult stem cells.		[42]
PCL		Good patency rate, no thrombosis formation and rapid endothelilization up to 6 months of implantation in abdominal rat aorta. However,	[32]

		calcium deposition appeared after that at longer term of implantation.	
PLCL	Smooth muscle cells (SMCs) were cultured for up to7 weeks.The viability of cells increased by increasing cell culture time (11x105 cells after 7 weeks).		[38]
PCL	Thicker fibre diameter based PCL graft enhanced the formation of immunomodulatory and tissue remodeling (M2) phenotype when MSCs cells were cultured.		[48]
Natural polymer-based scaffolds			
Silk	Human aortic endothelial cells and coronary artery smooth muscle cells experienced good proliferation.		[46]
(rTE)	Tropoelastin based blood vessel showed good endothelial cell response in terms of adhesion and proliferation.		[53]
Hybrid polymer-based scaffolds			
PDO-elastin (50:50)	Human dermal fibroblasts cells cultured on pure PDO and PDO-elastin blend for 7 days. Hybrid scaffold of PDO-elastin showed better cell response than pure PDO in terms of adhesion, proliferation and migration.		[60]

Collagen-elastin-PLGA	Ovine SMCs cultured on collagen/elastin/PLGA blend for 7 days demonstrating good cell viability (90%).		[61]
PLLACL coated with collagen	P LLA-CL-collagen vascular graft demonstrated good cell response when HCAECs are cultured.	P (LLA-CL)/collagen vascular graft demonstrated good patency without thrombosis formation when implanted in rabbit veins.	[54]
PEUU-PMBU	Rat smooth muscle cells were cultured on PEUU/ PMBU fibrous scaffold for 1 day resulting in diminishment of cell number (70-76%) compared to the control (TCPS) and pure PEUU.	Implanting the PEUU/ PMBU fibrous scaffold in rat abdominal aorta showed higher patency than PEUU.	[67]
PLA-silk Fibroin-Gelatin	3T3 mouse fibroblast cells cultured for 21 days on PLA/SF-gelatin showed good proliferation.	Subcutaneous implantation test in Sprague-dawley rat for 3 months resulted in biocompatibility of the graft.	[62,63]
PCl-Collagen	Bovine endothelial cells (bECs) and smooth muscle cells (SMCs) were cultured on PCL-collagen fibrous scaffold demonstrating confluent layer of ECs on the lumen of the graft.		[68]
PHBV-PCL	RCEC cells experienced apoptosis on PHBV because of its stiffness.		[69]
Collagen-Chitosan-P(LLA-CL)	ECs cells demonstrated good adhesion and proliferation on collagen-chitosan-P (LLA-CL) compared to pure P (LLA-CL).		[66]

Lecithin-cholesterol-PCL	MSC cells were cultured for 7 days on both pure Chol-PCL and lecithin-Chol-PCL for 7 days. MSCs proliferated better on lecithin doped Chol-PCL.		[70]

2.1.5. Functionalization of fibrous small-diameter blood vessel scaffolds

Pure fibrous scaffolds made into small diameter vascular grafts without any modifications may encounter thrombosis formation when transplanted in animal models. Therefore, modification of the fibrous scaffolds by antithrombogenic molecules such as hirudin, lecithin and heparin or antithrombogenic polymers such as nonthrombogenic 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymer or drugs such as dipyridamole (DPA) and aspirin, could significantly decrease thrombi formation, enhance endothelialization and further promote cell ingrowth.

Functionalization of fibrous scaffolds could be done either by covalent attachment of the antithrombogenic biomolecules or mixing the antithrombogenic biomolecules or polymers or drugs with the electrospun polymer during the electrospinning process. In this section, several ways of functionalizing fibrous scaffolds are discussed, focusing on the enhancement of mechanical properties and the biological properties of the fibrous scaffolds after functionalization.

2.1.5.1. Antithrombogenic biomolecules

Hirudin and poly (ethylene glycol) were used to modify poly (L-lactic acid) micro fibres. It has been shown that hirudin could reduce platelet aggravation and no thrombin activity appeared due to the existence of hirudin [89].

Lecithin/cholesterol-poly (e-caprolactone) (Chol-PCL) electrospun fibrous scaffold (average fibre diameter 0.5 to 1 μm) resulted in better hemcompatibility and cytocompatibility than net Chol-PCL due to the zwitterionic property of lecithin [70. The hemolysis ratio, which indicates the extent of lysed blood cells at the interface with the scaffold, is much lower in the lecithin /Chol-PCL (0.5%) than pure Chol-PCL (2.8 %) scaffold. Furthermore, the Lecithin/Chol-PCL conjugate demonstrated biomechanical characteristics including Tensile strength, Elongation at

break (%) and Young's modulus up to the values 5.22 ± 0.50 MPa, 107.15 ± 10.78 % and 35.92 ± 4.75 MPa. MSCs proliferated better on Lecithin/Chol-PCL (optical density 0.9 nm) than on pure Chol-PCL (0.65 nm) when cultured for 7 days [70].

Poly-ε-caprolactone (PCL) incorporated with peptide cysteine-alanine-glycine (CAG) was utilized to fabricate electrospun small-caliber vascular grafts (SCVGs) of 0.7 mm diameter and 7 mm length. The synthetic grafts were transplanted in the carotid arterial of sprague-Dawley rats for 6 weeks. It was found that CAG containing grafts achieved higher endothelization ratio (97.4 ± 4.6%) than pure PCL-based graft (76.7 ±5.4%) after 6 weeks of implantation. Therefore, thrombi formation would most likely be reduced. On the other hand, α-smooth muscle actin (ASMA) measured for a CAG/PCL graft (0.89 ±0.06) was significantly less than that of pure PCL graft (1.25±0.22).Therefore, it is speculated that CAG/PCL graft enhanced endothelilization while inhibiting intimal hyperplasia [90].

Heparin is the most common antithrombogenic molecule used to functionalise scaffolds. As such, there are numerous studies assessing the effectiveness of heparin.

Biomimetic small-diameter blood vessel (3 cm in length, 4 mm in inner diameter and 0.25 mm in thickness) was fabricated by electrospinning of gelatin-heparin (inner layer) and polyurethane (PU) (outer layer). The thus-fabricated vessel exhibited mechanical characteristics of breaking strength of 3.7 ±0.13 MPa and elongation at break of 110 ± 8%, indicating that the addition of heparin did not alter the properties of the scaffold significantly. The release rate of heparin was in the range of 18.5% (1day) to 33.0% (14 day). Consequently, the PU/Gelatin/Heparin based blood vessel appeared to have similar properties to native vessels and is hemocompatible as a result of heparin addition [91].

Combination of fused deposition modelling (FDM) with electrospinning for fabricating vascular conduit resulted in enhancement of the overall biomechanical and biological characteristics [68]. Poly-L-lactide (PLLA)/ heparin (Hep)/poly-ε-caprolactone (PCL) based blood vessel (5 mm diameter, 0.3 mm wall thickness, 6 cm length and 450±150 nm average fibre diameter) showed ultimate tensile strength of 1.58±0.07 MPa which is higher than that of electrospun poly-L-lactide (PLLA)/ heparin (0.72± 0.03 MPa) and human saphenous vein sample (SV) (1.15 ± 0.13 MPa) owing to the existence of PCL coil layer deposited by FDM. Further, live/dead assay and DNA content (4500 ng) showed high viability (>90% viable cells) and proliferation of human adult bone marrow mesenchymal stem cells (hMSCs) when cultured on PLLA)/ heparin/ PCL for 48 h [68].

Heparin-poly (ε-caprolactone) conjugate was employed for the construction of small-diameter blood vessel (Length=4 cm, Diameter= 2 mm) [92]. The PCL-heparin conjugate showed hydrophilicity (70^o) higher than pure PCL (10^o). Since the surface of PCL-heparin conjugate is negatively charged, it suppressed the adsorption of plasma protein like albumin and fibrinogen, which are both responsible for thrombi formation. Theoretically, the values of albumin and fibrinogen should be 250 and 270 ng.cm-2 respectively. Experimentally, the values of these proteins were 500 ± 32 and 560 ± 40 ng.cm-2 for pure PCL and 330 ± 21 and 340 ± 28 for PCL-heparin conjugate respectively. This implied that ECs achieved higher relative growth rate when cultured on PCL-heparin conjugate (160%) than pure PCL (100%). In vivo investigation was performed in dog's femoral artery using PCL-heparin graft for 4 weeks resulting in potent and compatible graft. This study suggested that the heparin containing graft might be suitable for vascular graft applications since it was able to suppress thrombus formation and promote ECs growth [92]. Along the same lines,

heparin was linked to the surface of poly (L-lactide) (PLLA) scaffolds by di-amino-poly (ethylene glycol) (PEG). This resulted in greater patency rate for heparin-PLLA scaffolds (85.7%) than untreated PLLA (42.9%) and also promoted ECs and SMCs infiltration in the nanofibrous scaffold [93].

In addition to conjugate heparin to the scaffolds, heparin can also be added into the polymeric solution to be electrospun so that it would be incorporated into the scaffolds. Electrospun heparin/poly(L-lactide-co-ε-caprolactone) (P(LLA-CL) fibrous scaffold demonstrated higher patency rate of 100% after 2 weeks of implantation in a canine model compared to P(LLA-CL) [94].Furthermore, pre-endothelilized heparin/P(LLA-CL) fibrous scaffold exhibited mechanical properties (tension of 95776 ± 193 g/g and elongation of 8.8 ± 1.7 mm) higher than that of pure P(LLA-CL) and even heparin/P(LLA-CL) [94].

Bilayered small-diameter vascular graft (SDVG) of 2.5 mm diameter and 6 cm length was constructed using heparin-conjugated polycaprolactone (hPCL) as the inner layer (0.15 µm average fibre diameter) and polyurethane (PU)-collagen blend as the outer layer (0.2-1 µm average fibre diameter).The constructed SDVG showed porosity and burst pressure of 45% and 300 KPa. In terms of its biocompatibility, in vitro culturing of L929 fibroblast cells on the inner and outer scaffolds of SDVG separately resulted in the cell's relative growth rates of 103.5% and 98.0% respectively. Moreover, in vivo transplantation in beagle dog model for almost 8 weeks led to no aneurysmal dilation, extravasation and stenosis [95].

In an attempt to mimic native blood vessel, a tri-layered electrospun small-diameter vascular conduit (1.5 mm diameter and 300 µm wall thickness), made of poly (ε-caprolactone) (PCL) and natural polymer chitosan (CS), was constructed by co-electrospinning. The PCL/CS conduit was loaded with heparin which was attached

to CS through ionic bonds. The internal layer of the conduit has higher concentration of CS (PCL/CS= 5/4 w/w) which in turn absorbed higher concentration of heparin. Heparin conjugation led to remarkable anticoagulation effect which was demonstrated by increasing activated partial thromboplastin time (APTT) to 180 s, thromboplastin time (TT) to 150 s and prothrombin time (PT) to 14 s. Additionally, platelets adhesion has been shown to decrease in PCL/Cs 5/4 w/w to 100 cells and in PCL/Cs 4/5 w/w to 200 cell. The PCL/Cs tube demonstrated acceptable tensile strength and young's modulus up to 9 MPa and 7.8 MPa respectively. In vitro culture using EC and SMC showed that heparin loaded PCL scaffold promoted the proliferation of EC by the secretion and stabilization of VEGF while inhibited moderately the proliferation of SMC by the activation of intracellular pathways. This result is encouraging because high proliferation of SMCs may lead to intimal hyperplasia especially at the initial stages and this can cause ECs to lose its function as well as narrowing the diameter of the vessels. Both ex vivo shunt and in vivo implantation in rat abdominal aorta for 1 month confirmed the in vitro results demonstrating the absence of thrombus formation and blood leakage [96].

2.1.5.2. Antithrombogenic polymer

Apart from using biomolecules to reduce the risk of thrombosis, antithrombogenic polymers such as MPC has been used to functionalise small diameter vascular grafts. Biodegradable poly (ester urethane) urea (PEUU) immobilized with nonthrombogenic 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymer was used to fabricate conduits of small diameter (1.3mm internal diameter) [91]. Firstly, the surface of PEUU fibrous scaffold was aminated with amine groups using radio frequency glow discharge in ammonia atmosphere. Following this, the amine sites reacted with the carboxyl groups of the MPC polymer through

condensation reaction to yield MPC functionalized PEUU fibrous scaffold. The MPC modified conduits showed reasonable biological activities when exposed to ovine blood since less platelet adhesion was observed compared to untreated conduits [86].

Another antithrombogenic polymer, (poly (2-methacryloyloxyethyl phosphorylcholine-co methacryloyloxyethyl butylurethane) (PMBU) was bioinspired by phospholipid and has been incorporated with PEUU to create small diameter blood vessel (1.3 mm internal diameter, 300 µm wall thickness and 500 nm average fibre diameter). The PEUU/15 % PMBU blend demonstrated Young's modulus, strain and compliance of 3±1 MPa, 342±43 % and 4.4±1.1 $X10^{-4}$ $mmHg^{-1}$ which were greater than that of pure PEUU (2±1 MPa, 388±58% and 2.9± 0.6 $X10^{-4}$ $mmHg^{-1}$. The blended scaffold of PEUU/15 % PMBU inhibited platelet deposition as well as inhibited rat smooth muscle cells growth in vitro (70-76% adhesion after 1day). On the other hand, in vivo study replacing rat abdominal aorta with PEUU/15 % PMBU scaffold showed that the fibrous scaffold only had a patency rate of 67% at 3 months. However, this was still higher than that of pure PEUU (40%) [67].

2.1.5.3. Drug loadings

Scaffolds can be made to impart antithrombogenic properties via the use of anticoagulants such as aspirin [97] and dipyridamole (DPA) [98].

Aspirin has been loaded in tubular fibrous scaffolds made of poly (caprolactone), which in turn enhanced the mechanical properties compared to pure fibrous scaffold of PCL, and decrease the aggregation of platelets, as shown in Fig.2.14. The elastic modulus increased from 2.9±0.1 MPa for PCL fibres to 13±2 MPa for 10% aspirin loaded PCL fibres [97].

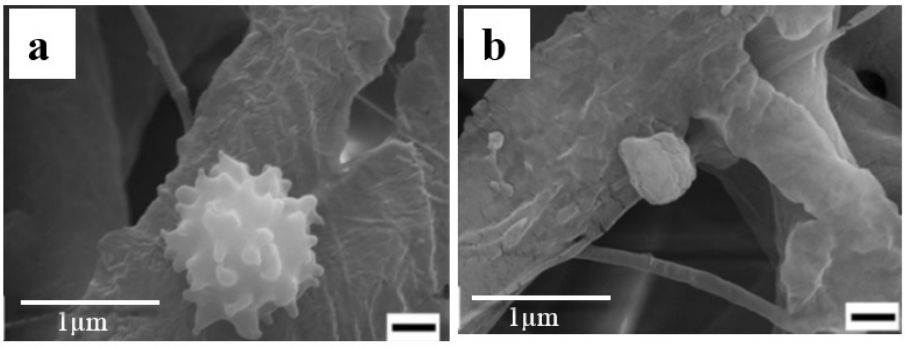


Fig.2.14: Platelets adhesion on (a) pure PCL fibrous scaffold and (b) 10% aspirin loaded PCL fibrous scaffold (reprinted from Ref.97).

In another study , the incorporation of dipyridamole (DPA) into biodegradable elastic polyurethane urea (BPU) fibrous scaffold during electrospinning of small-diameter blood vessel (1.5 diameter, 150µm wall thickness, 520 ± 100 to 650 ± 160 nm average fibre diameter) led to enhancement in both the biomechanical properties and the biocompatibility. BPU+10% DPA provided tensile strength of 7.4±0.1 MP (versus 3.4 ± 0.4 for pure BPU) and strain of 107±20 %. Additionally, BPU+10% DPA inhibited platelets adhesion (Thrombin-antithrombin complex concentration: 0.6 µg/mL against 1 µg/mL for pure BPU) and SMC cells proliferation while it enhanced EC cells proliferation after 7 days culture [98].

2.1.6. Conclusion

Synthetic polymer, natural polymer and hybrid polymer-based scaffolds have been intensely used for the fabrication of small-diameter blood vessels. Electrospinning technique has advantages over other techniques used for the fabrication of fibrous scaffolds because it has the ability to produce nanosized fibres ranging from 50 to 500 nm in diameter. These fibre sizes are similar to that of natural ECM of native blood vessels.

Synthetic polymers demonstrate good biomechanical properties. The mechanical properties as well as the cell responses of these fibrous polymeric scaffolds vary based on the elasticity of the polymer, the thickness of the fibres and the treatment employed before and after the fabrication including sterilization by α- or ɣ- radiations. Natural polymer have also contributed to the fabrication of small diameter blood vessels demonstrating good biocompatibility. Hybridization of polymers provides a strategy for the combination of good mechanical properties and cell interaction with the scaffold materials. Several polymer blends whether synthetic polymer blends or synthetic-natural polymer blends or natural polymer blends have all been employed successfully for the fabrication of small diameter blood vessels.

In addition to the previous attempts, the incorporation of antithrombogenic biomolecules such heparin and lecithin led to an enhancement in the overall properties along with the suppression of platelet adhesion. Therefore, it is conceivable that a match between a synthetic blood vessels and native blood vessels in terms of mechanical properties and biological function can be achieved by appropriate hybridization of polymers along with suitable antithrombogenic functionalization.

Chapter.3

Materials and methods

3.1. Overview

This chapter discusses the materials and techniques used in the thesis. In a brief description, elastic poly (styrene-butadiene-styrene) copolymer (65%:29.5%) and nonelastic poly (styrene-butadiene-styrene) copolymer (70%:30%) copolymer were electrospun with a ratios (5E:0N, 4E:1N, 3E:2N, 1E:1N, 2E:3N, 1E:4N, 0E:5N) in a mixture of tetrahydrofuran THF: N, N-dimethyl formamide DMF (70:30 v/v) to obtain 17 wt% SBS concentration. Starch was sprayed over the aluminium foil prior to electrospinning to facilitate detaching the electrospun fibrous scaffolds from the foil by immersing the scaffolds on the foil in the water.

After that, the fibrous scaffolds were characterized by scanning electron microscopy (SEM), Panalytical X'pert Powder (XRD), Fourier transform infrared spectra (FTIR), Thermogravimetric analysis (TGA), Differential scanning calorimetry (DSC), Instron tensile tester, Contact angle and air permeability. Cell culture were performed on the electrospun scaffolds using human umbilical vein endothelial cells (HUVECs). Then, the cellular attachments and proliferation were investigated using MTS assay, scanning electron microscope (SEM) and Laser scanning confocal microscopy (LSCM). Fig.3.1 shows a schematic diagram that summarizes the experimental procedures used in this thesis.

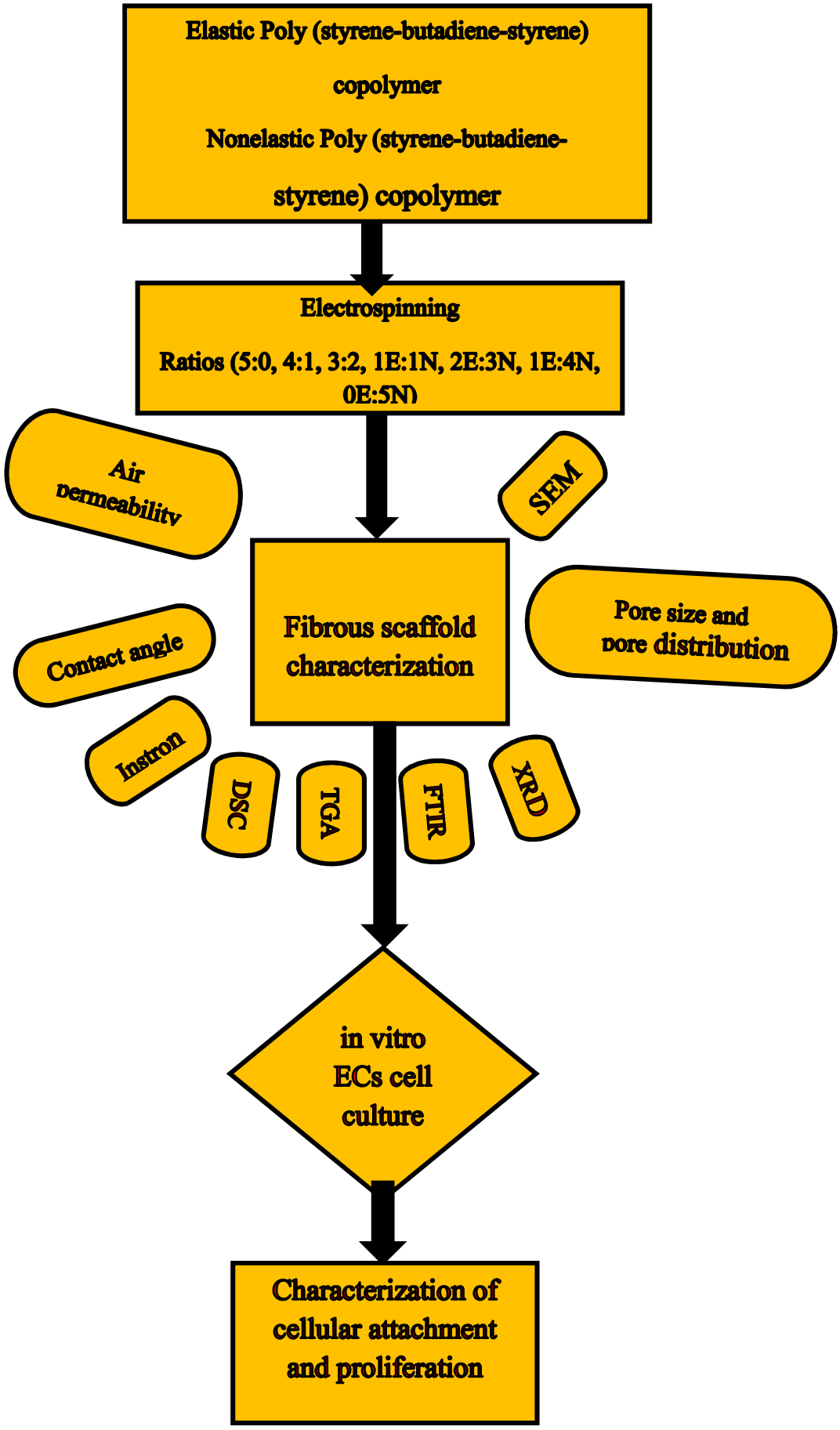


Fig.3.1: Schematic diagram showing the experimental procedures.

3.2. Materials

Poly (styrene-butadiene-styrene) (SBS, KRATON® D 1102 B Polymer with a polystyrene content of 29.5%) was purchased from Kraton Company (United States), Poly (styrene-butadiene-styrene) with a polystyrene content of 30%, tetrahydrofuran (THF), dimethylformamide (DMF) and ethanol were purchased from Aldrich. Starch was purchased from Erawan marketing co LTD Bangkok, Thailand. All chemicals were used as received. For cell culture, Phenazine methosulfate (PMS) Phosphate buffer saline (PBS), paraformaldehyde were purchased from Sigma Aldrich PTYLTD Castle Hill-NSW-Australia, MTS Reagent was purchased from promega PTYLTD-South Sydney business hub-Alexandria-NSW-Australia and endothelial cell growth medium 2 was got from Banksia scientific Co PTYLTD-Bulibmba-QLD-Australia. Human umbilical vein endothelial cells (HUVECs) was obtained from Promocell Company. Fetal bovine serum (FBS), Trypsin 0.25-EDTA, MEM EARLES, Alexa Fluor® 568 Phalloidin and DAPI (4, 6-Diamidino-2-Phenylindole, Dilactate) were purchased from Life Technologies Australia Pty Ltd.

3.3. Electrospinning of fibrous scaffolds

3.3.1. Solution preparation

Elastic SBS, nonelastic SBS-blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS are dissolved in a mixture of tetrahydrofuran THF: N, N-dimethyl formamide DMF (70:30 v/v) to obtain 17 wt% SBS concentration. The dissolution process is performed at room temperature using magnetic stirrer.

3.3.2. Electrospinning

SBS solution is filled out in a plastic syringe with stainless steel needle with 0.5 inner diameter. Electrospinning is conducted with an applied electric field of 20 $kVcm^{-1}$ keeping constant the needle to collector distance at 17 cm. The polymer

solution is fed at rate of 3 ml/h using syringe pump and the electrospun fibres are collected on rotating drum. The average humidity and temperature during experiments are 36 % and 20 ºC. To fabricate tubular fibrous scaffolds, 5 mm diameter stainless steel rod was used as collector.

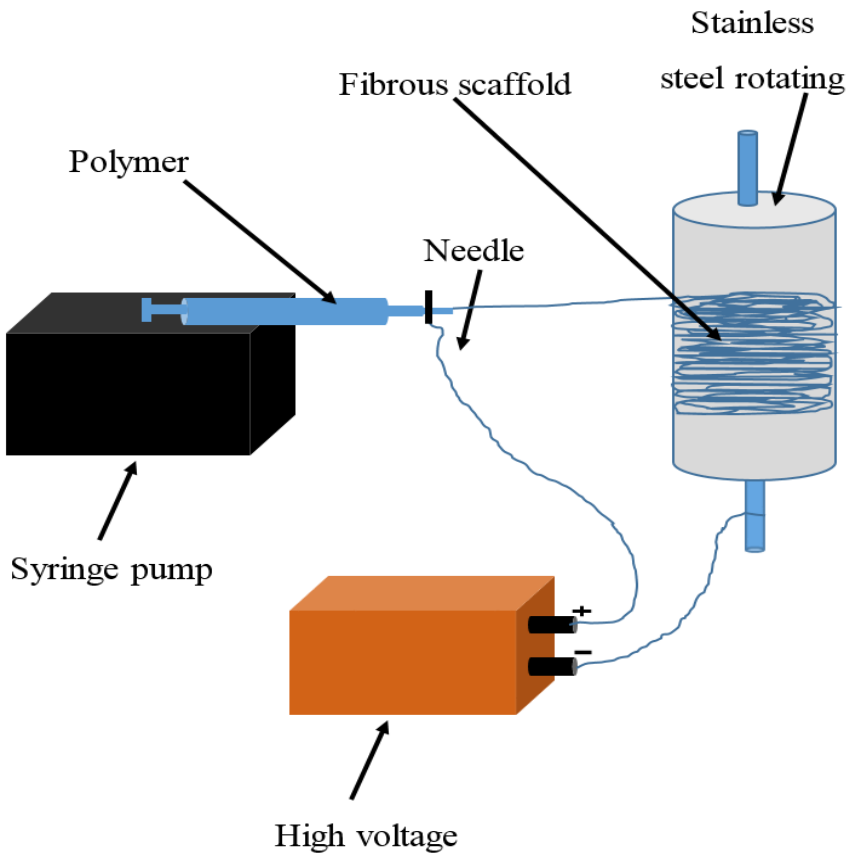


Fig.3.2: Shows the electrospinning process.

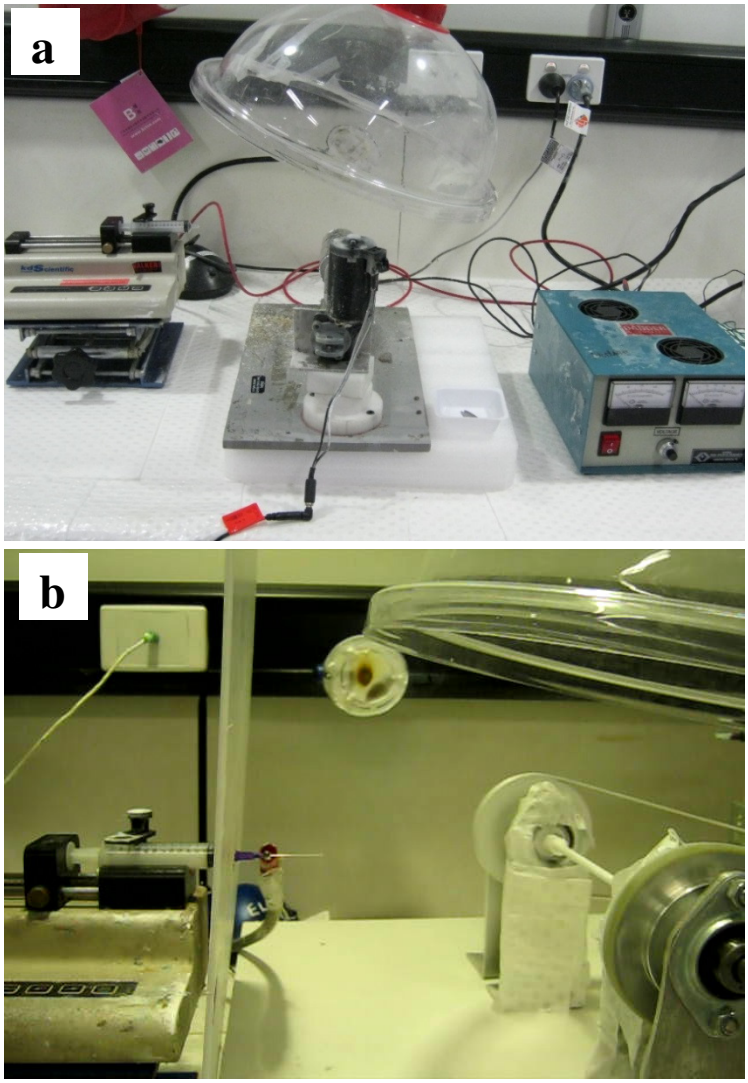


Fig.3.3: Shows images of electrospinning setups: (a) electrospinning setup to fabricate nanofibrous sheet (b) electrospinning setup to fabricate tubular fibrous scaffold with 5 mm diameter.

3.4. Scaffold characterization

3.4.1. Morphological characterization

Scanning electron microscope can image the surface of a specimen by scanning it through high energy beam of electrons. The beam of electron is produced by an electron gun. The electron beam travels through vertical pathway in the microscope in a vacuum atmosphere. The beam is focused by electromagnetic fields

and lenses toward the specimen. The sample emits signals once is bombarded by the beam of electrons. The signals include secondary electrons, backscattered electrons, and characteristics x-ray, and light, specimen current and transmitted electrons. Secondary electrons and bespattered electrons are used to image the samples. Fig.3.4 illustrates the working mechanism of SEM. Electrospun fibre mats are coated with gold using magnetron sputter and their morphology is investigated using scanning electron microscopy with a voltage of 5 kV.

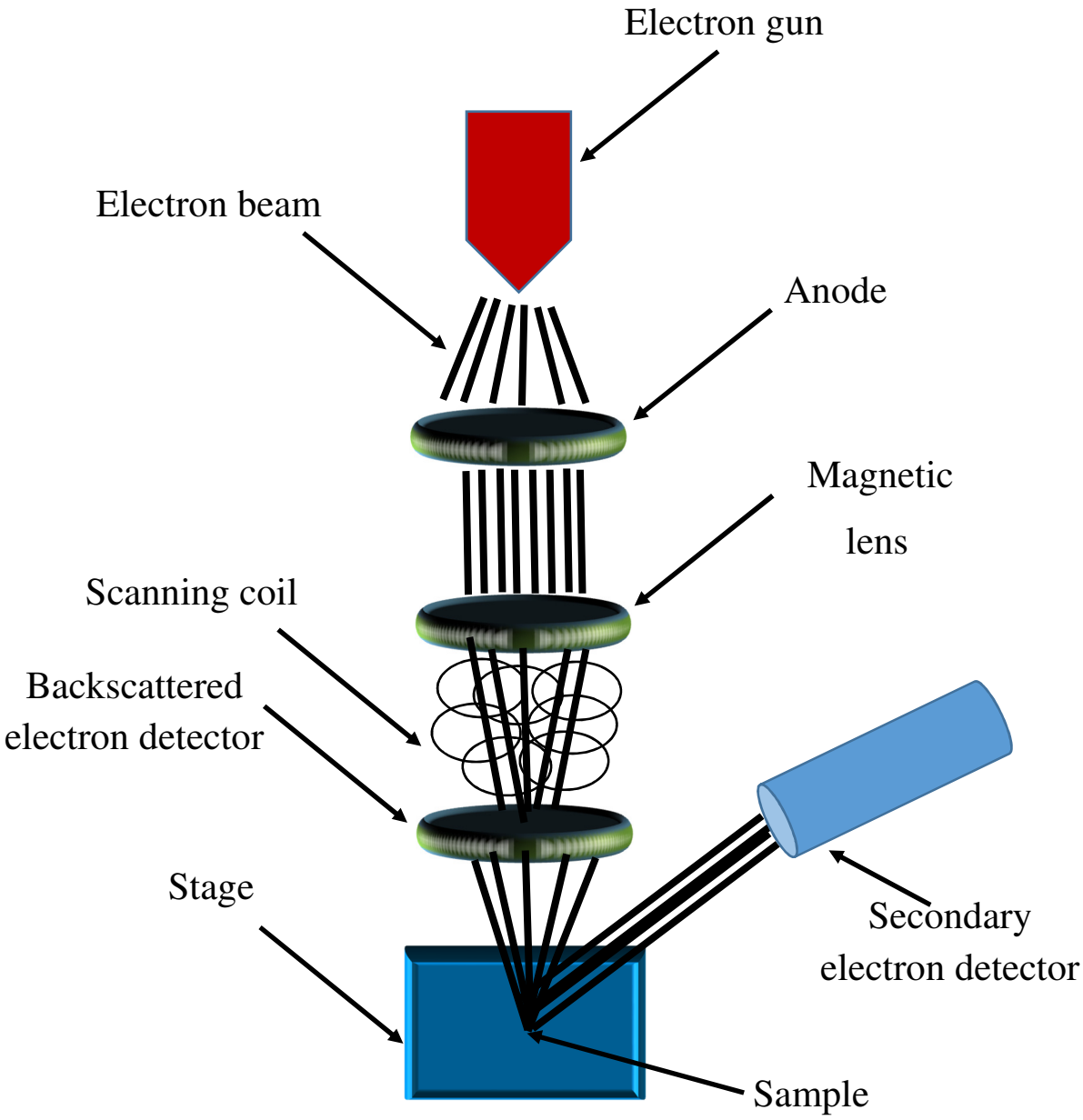


Fig.3.4: schematic diagram of electron microscopy.

3.4.2. Pore size and pore distribution

Electrospun fibrous scaffolds were punched into 14 mm diameter pieces for porometery measurements. A quantachrome porometer was used to measure pore size and pore distribution. Porofill was used as a wetting solution in porometery measurements. Fig.3.5 represents the porometer used in this study. The test is repeated three times for each sample.

Fig.3.5: represents quantachrom porometer used in this study.

3.4.3. Chemical composition and Crystallographic characterization

3.4.3.1. X-ray diffraction (XRD)

X-ray diffraction is a non-destructive technique that is used to obtain information about the chemical composition, crystallography and physical properties of a material. The working mechanism of XRD is based on illuminating a sample with electromagnetic waves that has wavelength as similar as the atoms ($1A^o$). The x-ray beams consists of a bundle of waves that can interact with each other producing interference. The waves of x-ray beam can also react with the atoms producing interference. The interference happens at a particular angle from the atomic planes. The resulted scattering is signature of a crystal structure. Bragg's law is applied on the interference resulted from the incident rays and scattered rays of every atom in the crystal structure.

$n\lambda=2d \sin \theta$……Eqn. (1)

Where n is any integer, λ is the wavelength of the incident x-ray beam, d is the distance between the atomic layers of a crystal structure, and θ is the angle of an interference resulted from incident x-ray beam and scattered x-ray beam. Fig shows a schematic illustration of Bragg's law. In this study, the crystallography of the fibrous scaffolds are characterized by Panalytical X'pert Powder (XRD).

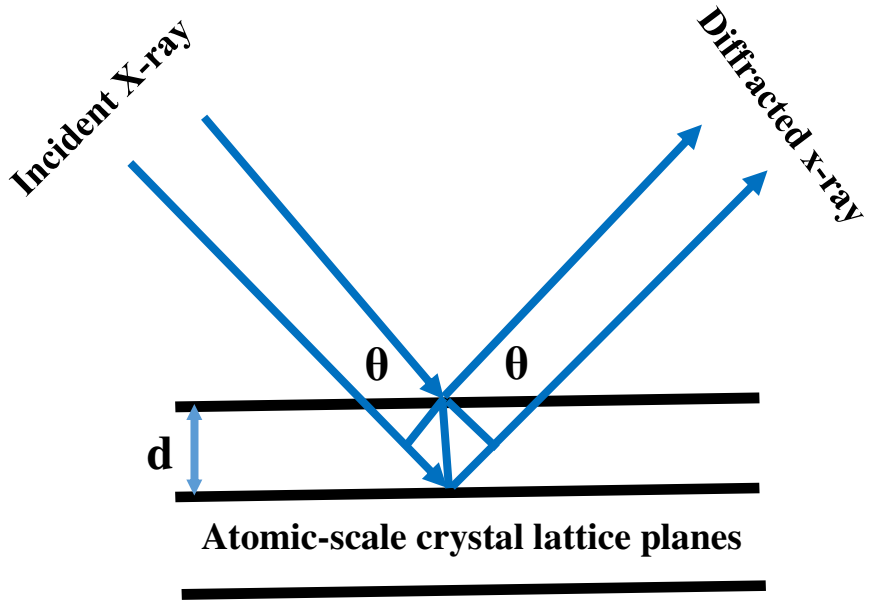


Fig.3.6: schematic diagram of theory of Bragg's law.

3.4.3.2. Fourier transform infrared spectra (FTIR)

FTIR is used in analytical or physical chemistry to identify a material through the spectrum that emits or absorbs. The spectrum is the result as a function of wavelength. FTIR spectrum of the fibrous scaffolds are measured using a Bruker VERTEX 70 instrument in ATR mode at a resolution of 4 cm^{-1} accumulating 32 scans. The spectra were obtained within 32 scans at 4cm^{-1} resolution. All tests were performed in a controlled environment (20±2^{o}C and 65±2% relative humidity). The data were analysed using opus 5.5 software.

Fig.3.7: PIKE pressure clamp mounted on ATIR mode.

3.4.4. Thermal characterization

3.4.4.1. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) analysis were performed for the fibrous scaffolds with a Mettler Toledo TGA/STDA851. The fibrous scaffolds were put in a ceramic pan and the test is carried out in air flow at a heating rate of 10 ^{o}c/min. the loss of the mass against temperature chart was got to identify any distinct peaks during decomposition.

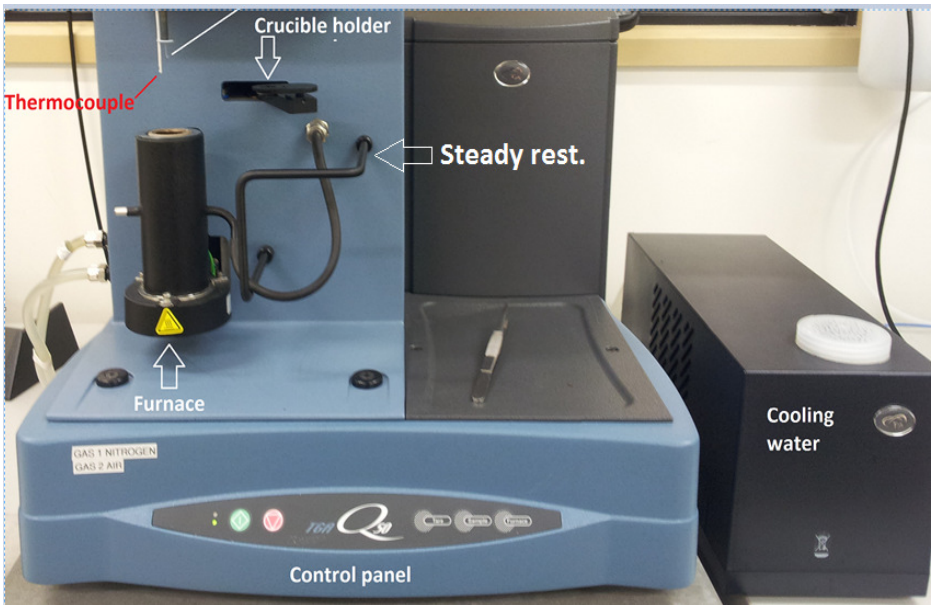


Fig.3.8: Shows TGA instrument composed of furnace that heat crucible.

3.4.4.2. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry was carried out using a Mettler Toledo DSc821 with star software version 9. Samples of the fibrous scaffolds with 5 mg weight were placed in aluminium pan with 13 mg weight. The instrument is adjusted to alternating DSC mode. The measurements occurred at heating rate 10 ^{o}C/min in a nitrogen atmosphere. Prior to performing the experiments, the samples were dried in a vacuum for 72 h at room temperature in the existence of phosphorous pentoxide. The thermal properties were measured and analysed from the first scan. Fig a, b show the DSC instrument with the entire furnace.

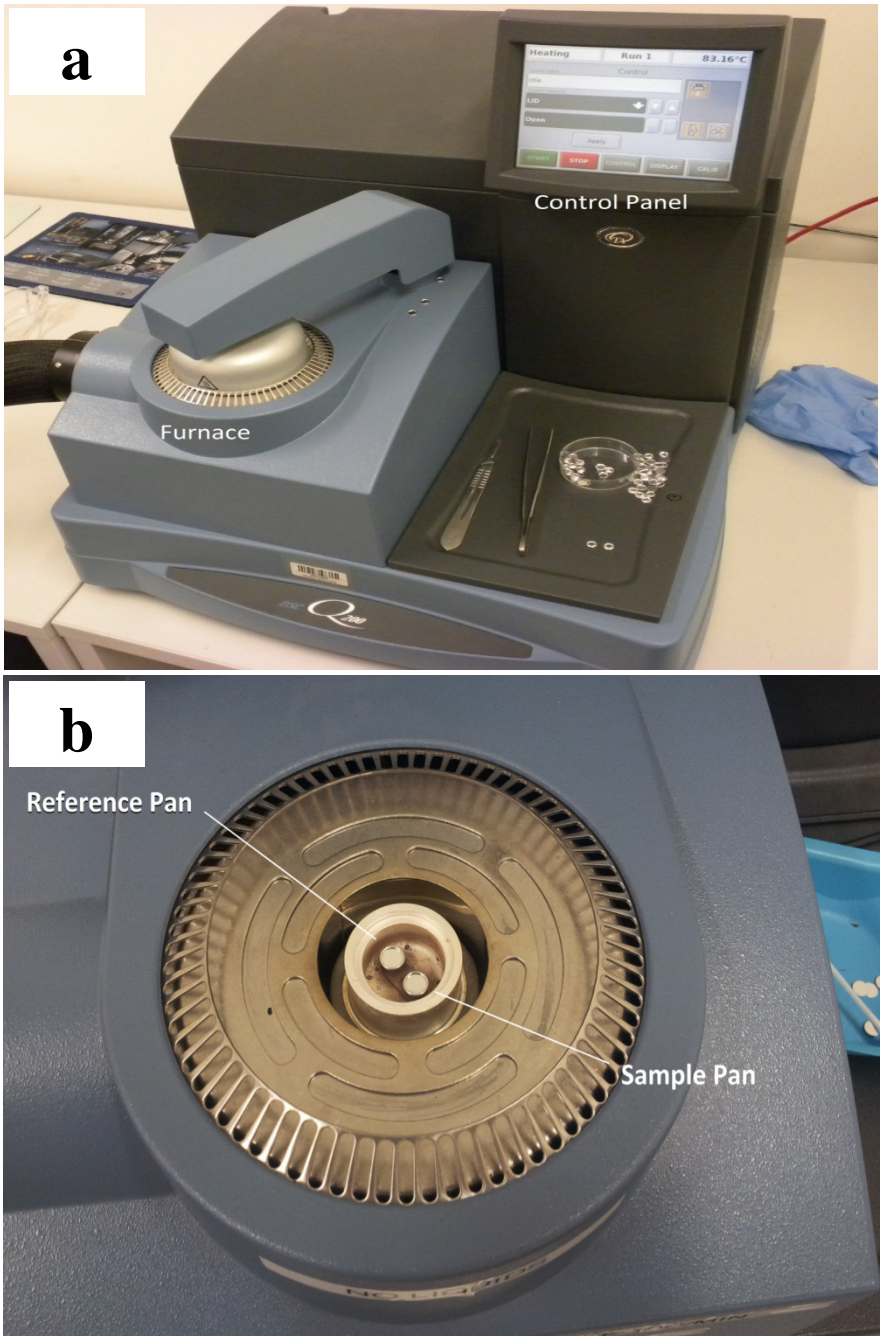


Fig.3.9: Shows DSC instrument composed of furnace that heats sample.

3.4.5. Contact angle measurements

Water contact angles were recorded using a KSV model CAM101 contact angle meter (KSV instrument Ltd, Finland, Fig.3.10). Static sessile drop method is utilized to measure water contact angle of the electrospun fibre mats. The fibrous scaffolds were cut into 1x2 cm and fixed on glass slides. Water drops (2 µL) are deposited on the surface of the electrospun fibre mats by a syringe (0.71 mm diameter) and the image were taken at rate 30 frame per second after deposition onto sample surface. Water drops images were captured by an Olympus DP70 high resolution microscope. Then, the contact angle is determined by software. The test is repeated 3 times for each sample. All tests were done in a controlled atmosphere (20±2 ^{o}C and 65±2 % humidity).

Fig.3.10: shows CAM 101 KSV Contact Angle Meter.

3.4.6. Air permeability measurements

Air permeability tests were carried out on samples of the electrospun fibre mats using FX3300 air permeability tester with the aperture number 5 under 98 Pa according to the standard (SIST EN ISO 9237-1999) as shown in Fig. The test is repeated 5 times for each sample. All tests were done in a controlled atmosphere (20±2 ºC and 65±2 % humidity).

Fig.3.11: FX3300 air permeability tester.

3.4.7. Mechanical characterization

Mechanical tests were carried out on rectangular samples of the electrospun fibre mats with an area of 10x20 mm and a thickness of 200 µm using Instron tensile tester at a test rate of 10 mm/min on 100 N load cell. Load-unload test was also performed for the samples at 10 % strain. In case of load-unload tests, the area under the hysteresis is calculated and demonstrated the dissipated energy. The test is repeated 3 times for each sample.

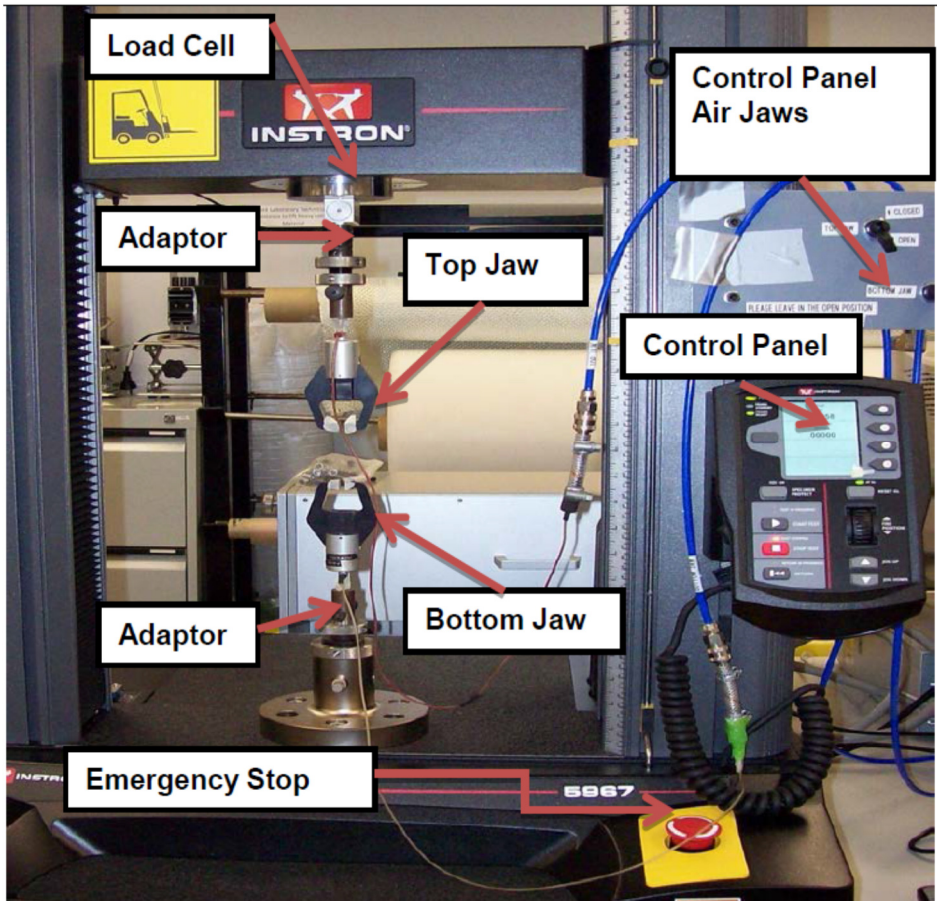


Fig.3.12: shows instron 30 KN tensile tester.

3.5. Cell culture related techniques

3.5.1. Plasma treatment of fibrous scaffolds

A lab-scale air plasma system was used to treat the fibrous scaffolds. The energy applied was 30 W. Each sample was treated from both front and rear surfaces separately for 5 min. Fig.3.13 shows the air plasma chamber used to treat the samples.

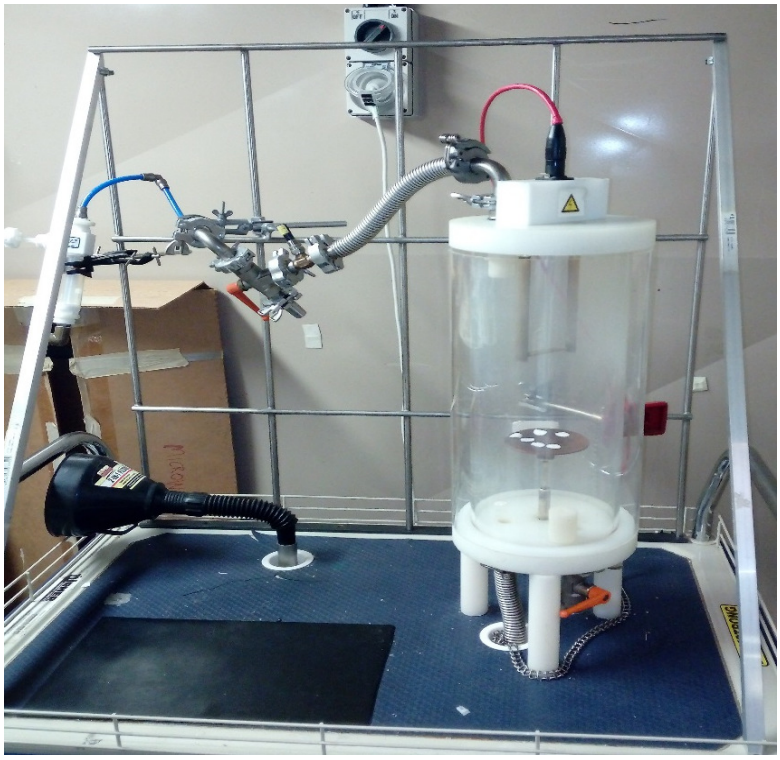


Fig.3.13: Air plasma technique.

3.5.2. Sterilization of SBS fibrous scaffolds

All samples in this work used for cell culture were sterilized using ethanol 70% and UV. They are firstly put individually in 24 well tissue culture plates and washed for 30 min in 70% ethanol on an orbital shaker and then washed for second time in 70% ethanol on the shaker for 24 h. Then, they are rinsed for 5 min in 70% ethanol on the shaker. Eventually, they are washed three times by PBS and irradiated with UV for 1 h before cell culture.

3.5.3. in vitro endothelial cells (ECs) culture

Human umbilical vein endothelial cells (HUVECs) were cultured in human endothelial cell growth medium 2 supplemented with 0.02 ml/ml Fetal calf serum, 5 ng/ml epidermal growth factor (recombinant human), 10 ng /ml basic fibroblast growth factor (recombinant human), 20 ng/ml insulin-like growth factor (Long R3IGF), 0.5 ng/ml vascular endothelial growth factor (recombinant human), 1µg /ml ascorbic acid, 22.5 µg /ml heparin and 0.2 µg /ml hydrocortisone at room temperature and then incubated in the humidified incubator at 37 ° C with 5 % CO_2. Medium was replaced every 2 days. When the cells reached confluence, they are subcultured. Subculture of the cells was carried out using PBS to wash the cells. Then trypsin was used to detach the cells from the T75 flask and then equal volume of media is added to inactivate the trypsin. After that, PBS was used to wash the remaining cells in T75 flasks and collected with trypsin and media. Then, the solution is centrifuged at 220 rcf for 5 min. After centrifugation, the resulted pellets were suspended in 1 ml media. Specific number of ECs was required for various studies. In order to do that, cells were counted using haemocytometer and trypan blue. The basic idea of this method is that alive cells become shiny and clear under optical microscope because they did not absorb trypan blue while dead cells become blue because they absorb trypan blue. The solution is made up from 10 µl cell suspension and 10 µl trypan blue. Then, 10 µl from this solution is pipetted onto a haemocytometer and covered with coverslip on place. Every square of the haemocytometer represents a volume of 1ml. The cells of the four corner squares are counted and the cell number in the cell suspension is calculated based on the following formula:

Cell number/ml=the average count per square x dilution factorx10^4

3.5.4. Seeding fibrous scaffolds with ECs

$1X10^4$ cells in a volume of 50 µl of cell suspension was seeded onto the sterilized fibrous scaffolds in 24 well tissue culture plates. After 3 h, 950 µl of ECs media was added to each well containing the fibrous sacffolds. Then, the seeded samples were incubated for 3h, 3days and 7days in a humidified incubator with 5% CO_2 and 37 ^OC. The media is changed every 2 days. The number of cells attached to the fibrous scaffolds was measured by MTS assay that is described later.

3.5.5. Cytotoxicity

Fibrous scaffolds were soaked in 1 ml of human umbilical vein endothelial cells and incubated for 3h, 3days and 7days in a humidified incubator with 5% CO_2 and 37 ^OC. After that, the fibrous scaffolds were removed from the media within the time points 3h, 3 days and 7 days. The media is then used to grow human umbilical vein endothelial cells in a 24 well tissue culture plates using a calculated density of $2X10^4$ cells/well for 3 days. The number of cells after 3days were measured by MTS assay that is described later.

3.5.6. MTS assay

MTS assay is a colorimetric method to measure cell proliferation. The working mechanism of MTS assay is that MTS tetrazolium compound reacts with cells and is reduced to colour formazan compound that is soluble in cell culture media. Live cells only are capable of such reaction as they contain dehydrogenase enzymes to cleave the formazan. The colour is detected in density to determine the live cell number. During the culture period, fibrous scaffolds at different time setups were collected for MTS assay. The fibrous scaffolds were rinsed with PBS to detach dead cells and were then transferred to new 24 well culture plates with 300 µl phenol red free media and 100 µl of MTS solution for 1 h in 37 ^oC incubator. After 1 h of

incubation, 100 µl of the mixture was collected to measure the absorption at 490 nm on a microplate reader. MTS standard curves were setup to convert absorbances obtained from the assay to cell number. The absorbances were calculated by subtracting the zero wells (phenol red free media) from all of the other records. The cell number attached to the fibrous scaffolds were calculated on the basis of the standard curves. All the cell culture experiments were performed in triplicate and presented in the form of averages and standard deviations.

3.5.7. Preparation of EC seeded scaffolds for characterization

The fibrous scaffolds were fixed in 4% paraformaldehyde overnight. Then, it was washed by PBS three times to remove the remaining of paraformaldehyde. Finally, it was stored in PBS in a fridge for further use.

3.5.7.1. SEM

The fibrous scaffolds were washed by deionized water to remove PBS. Then, it was washed by a series of ethanol starting from 40 % up to 100 %. After that, it is kept to dry in a fume hood overnight. Finally, it was coated with gold for investigation under SEM.

3.5.7.2. LSCM

Laser scanning confocal microscopy (LSCM, Leica TCS SP5, and Germany) equipped with argon (458 nm, 476 nm, 488 nm, 496 nm and 514 nm) and 405 Diode (405 nm) lasers was used to investigate the fibrous scaffolds containing endothelial cells. The fixed cells on fibrous scaffolds were stained with Phalloidin Alexa 568 (dilution ratio 1:100, invitrogen) for 1h and with DAPI (dilution ratio of 1:100, invitrogen) for 10 min at room temperature. Then, the fibrous scaffolds are rinsed in PBS three times to remove the remaining of the fluorescent dyes. The fibrous scaffolds were imaged under confocal microscope (Fig.3.14). The excitation

wavelengths of DAPI and Phalloidin are 358 nm and 578 nm respectively. The emission wavelengths of DAPI and Phalloidin are 461 nm and 600 nm respectively.

Fig.3.14: Laser scanning confocal microscopy.

3.6. Statistical analysis

Values (at least triplicate) were averaged and shown as means ± standard deviation (SD). Every experiment was carried out three times. Statistical analysis was performed for ECs attachment and proliferation using one-way anova test by SPSS for windows (SPSS Inc., copyright 1989-2002) version 11.5.0.

Chapter.4

Preparation and characterization of SBS fibrous scaffolds

4.1. Overview

Tissue engineering research has played a major role in regenerating, maintaining and improving the function of the organs that have degenerated or are influenced by pathological conditions [99]. Polymer-based scaffolds can be used in tissue engineering applications with polymer selection based upon the desired physical and chemical properties [100].

In this sense, musculoskeletal tissue engineering such as skeletal muscle [101], ligaments and tendons [102] and blood vessels [103] need biocompatible scaffolds that are mechanically compliant, and able to contract and dilate by external stimulus. Therefore, the mechanical properties of the scaffold are important in simulating native tissue repair and contribute to the regeneration of the tissue [104].

Thermoplastic elastomers like Styrene-butadiene-styrene (SBS) have been used in many industrial applications due to its excellent strength following repetitive deformation [105, 106]. Tailoring the molecular structure of SBS and its morphology permits us to tune its mechanical properties from a thermoplastic elastomer to a tough thermoplastic [107, 108].

SBS copolymer synthesis has been attempted for tissue engineering applications and has been patented with similarities to polyurethane as a candidate for artificial organs [109]. Various studies indicate that SBS has a large elongation at break (1200 %) [106, 108] enabling it to withstand deformation. Blood vessel scaffolds

require a polymer that is able to contract and dilate according to blood cycles without permanent deformation. In this regard, some studies about SBS are described below.

SBS is commercially available with a triblock structure of styrene and butadiene under the trade name Kraton (Kraton, Houston, TX). SBS is not biodegradable but it is bioresorbable when functionalized [109]. SBS has been absorbed in vivo through phagocytosis [110]. Biodegradation of SBS can be inferred by graft copolymerization [111, 112]. SBS cannot be sterilized by heat or steam due to its low glass transition temperature. Even so, various schemes of sterilization are employed for SBS including ethylene oxide gas, gamma radiation and electron beam sterilization [113].

SBS is naturally hydrophobic and the surface energy can be modulated using copolymerization and irritation. Therefore, the biocompatibility of SBS can be enhanced in terms of cytotoxicity [114, 115] and hemocompatibility [116]. SBS has a high electrical resistance and therefore a low dielectric loss. This makes SBS suitable for blending, copolymerization and crosslinking to biodegradable polymers using electrospinning [117, 118]. Various systems of blending SBS with biodegradable polymers are reported including chitosan [114], alginate, poly (γ-glutamic), polyaspartic acid, poly (vinyl alcohol) [119], and polycaprolactone [117].

SBS scaffolds have been prepared using electrospinning with nonwoven structure [120]. It has been electrospun to both random and aligned orientation. Two type of SBS with styrene to butadiene ratios of 80:20 and 60:40 have been used resulting in different mechanical properties. The highest butadiene ratio demonstrated lower elongation at break with a value of 382±75 %, compared to 442±85% for the lower butadiene ratio. SBS fibrous scaffolds showed hydrophobic nature with water conact angle value 135^{O}. Incorporation of carbon nanotubes (CNT) with SBS fibres

led to an increment of the mechanical properties. The elongation at break of 80% butadiene increased up to 501±80% while the 60% butadiene increased up to 1480±155%.

Triazolinediones (TAD) has been used to tailor the thermal and mechanical properties of SBS, resulting in an elongation at break from 90 to 700% and modulus from 100 to 120 KPa depending on the TAD ratio. The glass transition temperature of unmodified SBS and TAD modified SBS are 15 and 39 °C respectively [108].

Epoxidation with peroxyboric acid may be used to enhance SBS wettability and protein adsorption. Using this method, elongation at break decreased and strength increased; the tensile strength and elongation for SBS treated for 60 min , 90 min , and 120 min were 0.25MPa, 0.46MPa, 0.68MPa and 863%,648%, 626% respectively [121].

In this chapter, the synthesis of SBS fibrous scaffolds, morphology, surface chemistry and mechanical properties were studied. To the best of our knowledge, we have, for the first time, tailored the mechanical properties of SBS fibrous scaffolds without changing its chemical compositions through blending nonelastic SBS to elastic SBS during the electrospinning process.

4.2. Experimental procedures

Elastic SBS, nonelastic SBS and nonelastic-elastic SBS blend have all been electrospun. The electrospun fibrous scaffolds were characterized using different techniques such as SEM, XRD, FTIR, TGA, DSC, tensile testing, wettability and air permeability. Detailed description of materials and method are found in chapter three.

4.3. Results and discussion

4.3.1. Morphology of SBS fibrous scaffolds

The morphology of the electrospun polymer fibres is affected by many processing parameters including initial polymer solution (solvent properties, concentration of polymer solution, and molecular weight of a polymer), control of jet formation, and solvent evaporation (flow rate of a polymer, inner diameter of a needle, temperature and applied voltage), and collection set-ups that control random or oriented fibres [122,123].

For the sake of obtaining uniform SBS fibrous scaffolds, elastic SBS polymer was electrospun to different polymer concentration, from 12 wt% to 17 wt %. Fig.4.1a-d shows SEM images of elastic SBS electrospun at different concentrations from12 wt %-15wt % leading to fibrous structure at 15 % but not uniform enough with some beads. To enhance the electrospinnability of 15 wt% polymer solution, the electrospun parameters were changed as shown in Fig.4.2, NaCl was added to 15 wt% as shown in Fig.4.3. However, these attempts did not lead to uniform fibrous structure of 15w%. Therefore, SBS polymer was electrospun with 17 w% which led to uniform fibrous structure without beads as shown in Fig.4.4.

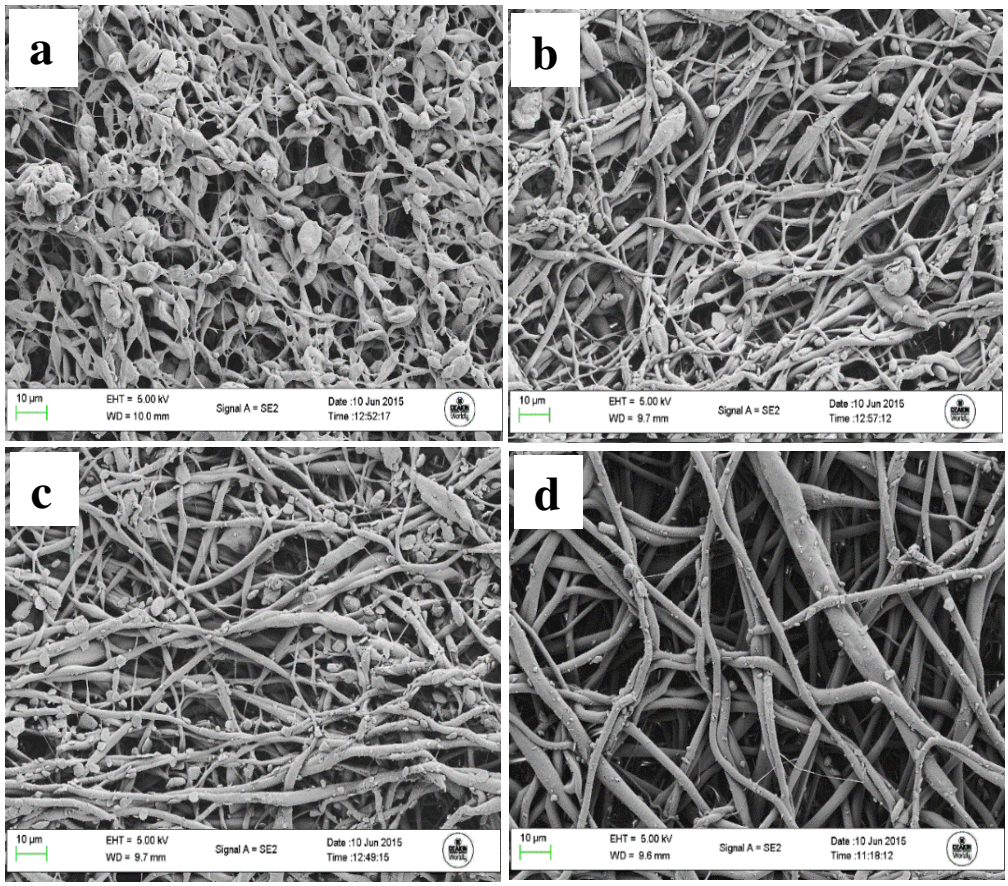


Fig.4.1: SEM images of electrospun elastic SBS at different concentrations: (a) 12 wt%, (b) 13 wt% , (c) 14 wt%, (d) 15 wt%, rate of 5ml/h , voltage of 25 KV, and distance of 13 cm.

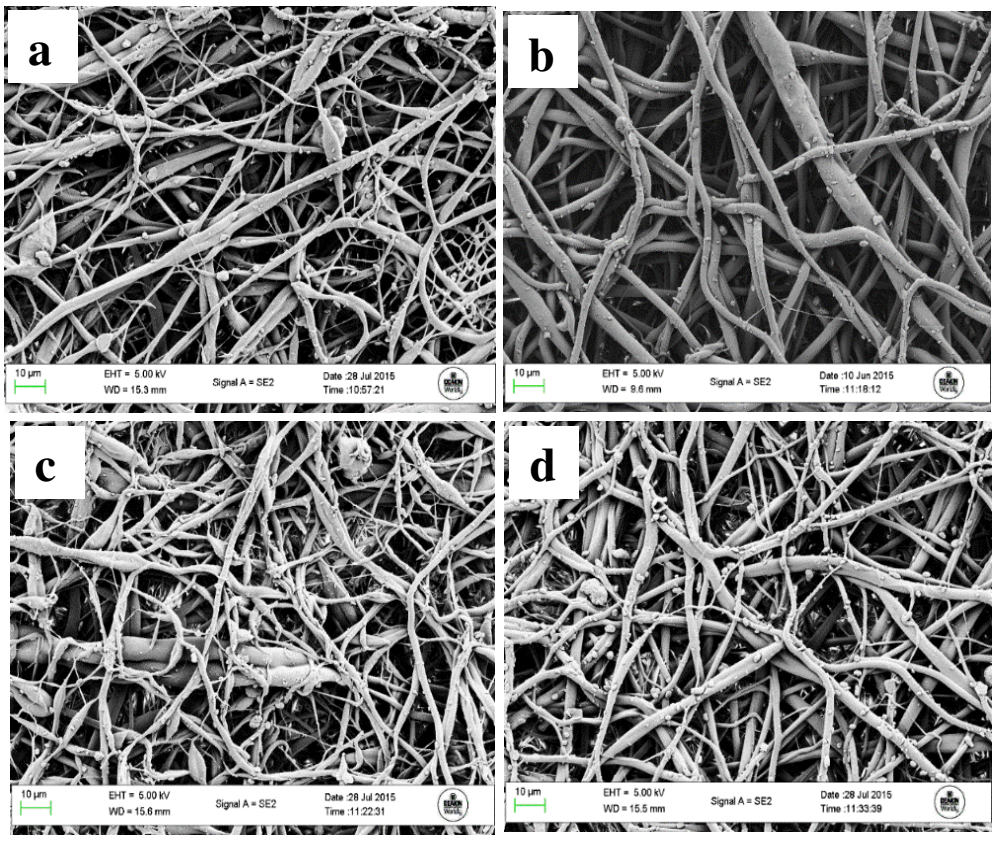


Fig.4.2: SEM images of elastic SBS electrospun with 15 wt% at different parameters: (a) 15 wt% SBS at d=12 cm and V=22 KV, (b) 15 wt% SBS at d=12 cm and V=25 KV, (c) 15 % SBS at d=20 cm and V=22 KV and (d) 15 wt% SBS at d=30 cm and V=25 KV.

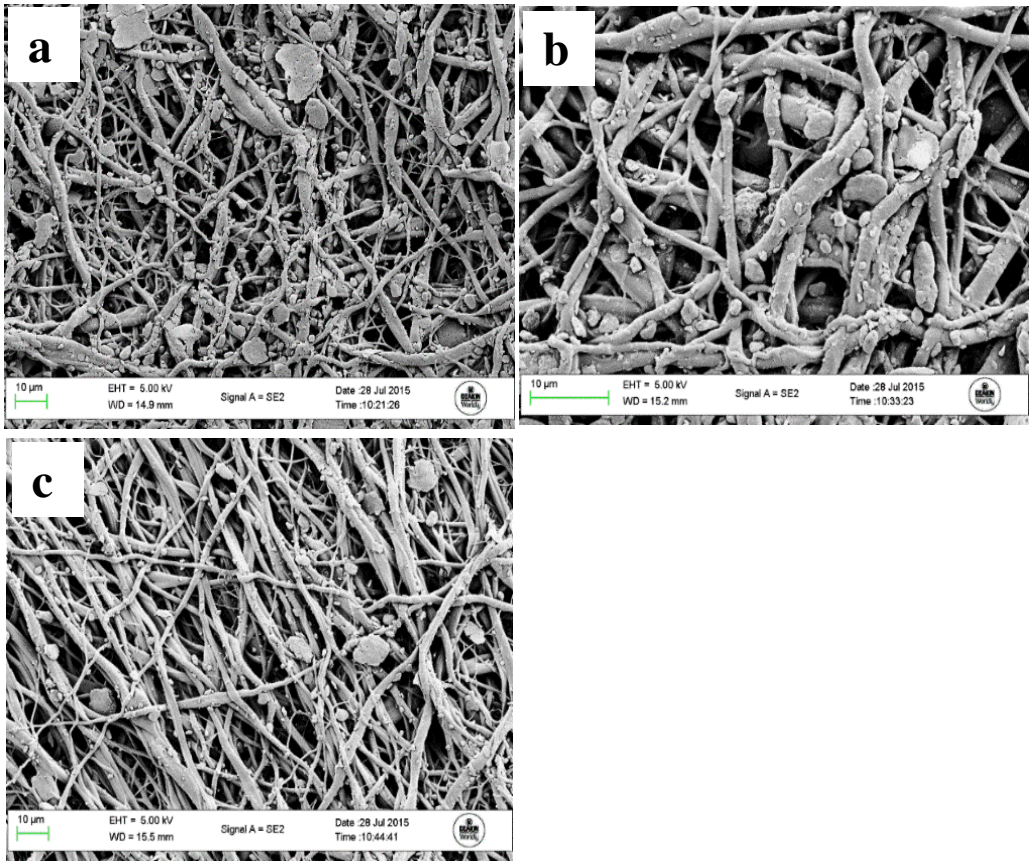


Fig.4.3: SEM images of elastic SBS electrospun with 15 wt % added NaCl at (a) 0.5 mg, (b) 1mg and (c) 2 mg, d =12 and voltage=22KV.

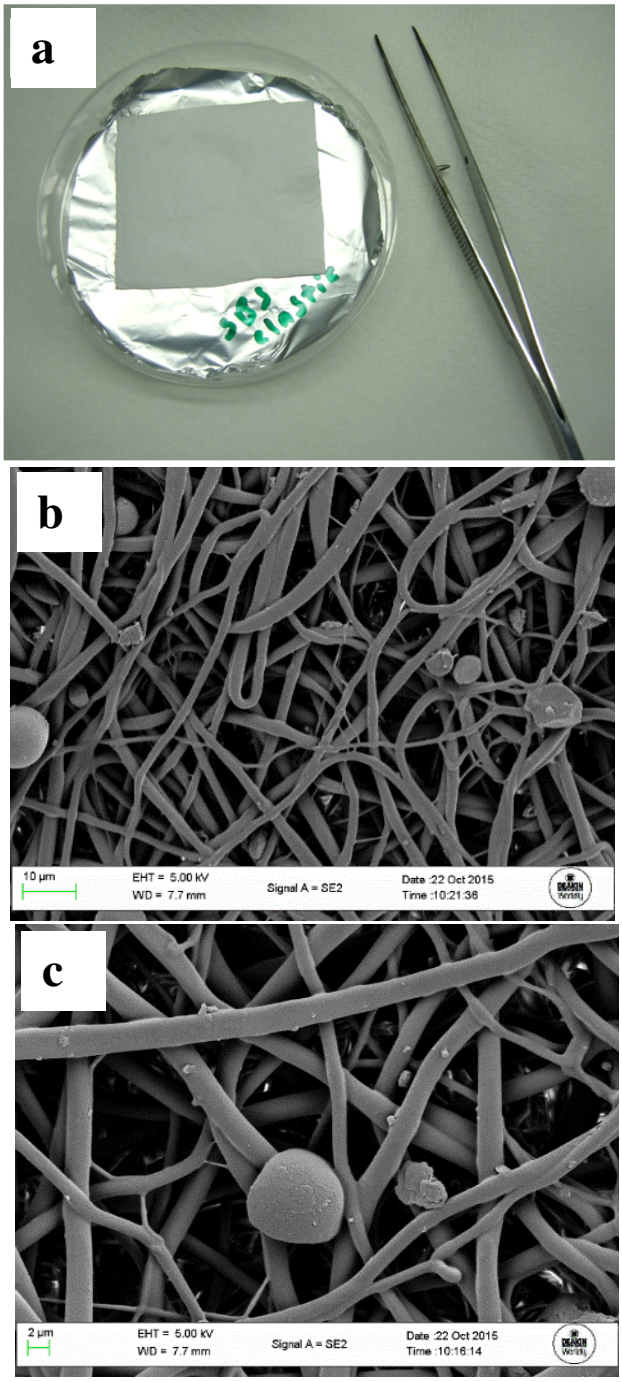


Fig.4.4: (a) macroscopic image of elastic SBS fibrous scaffolds electrospun with 17 wt% (a, b) SEM images of elastic SBS electrospun with 17 wt% at d=17 cm, rate=3 ml/h, and voltage=20 KV at different magnification.

Fig.4.5 (a-g) shows the morphology of the elastic SBS, nonelastic SBS-blended elastic SBS and nonelastic SBS. Since 17 w% of SBS led to uniform fibrous scaffolds, nonelastic SBS was blended with elastic SBS at this concentration. Fig.4.5a-g shows that all the fibrous scaffolds are uniform without any beads. The fibre diameters are calculated for all of the fibrous scaffolds as shown in Fig.4.6; leading to mean fibre diameter of 2 µm. It is to note that the average fibre diameter of electrospun SBS was reported before of the value of 2.5 µm [106] and 4.6 µm [120].

Fig.4.7 shows the distribution of the fibre diameter in the fibrous scaffolds. The fibres of 0 to 1.5 µm diameter account for 60%, the fibres of 1.5 to 3 µm diameter account for 70% , the fibres of 3 to 4.5 µm diameter account for 20 % and the fibres of 4.5 to 6 µm diameter account for 15 %.

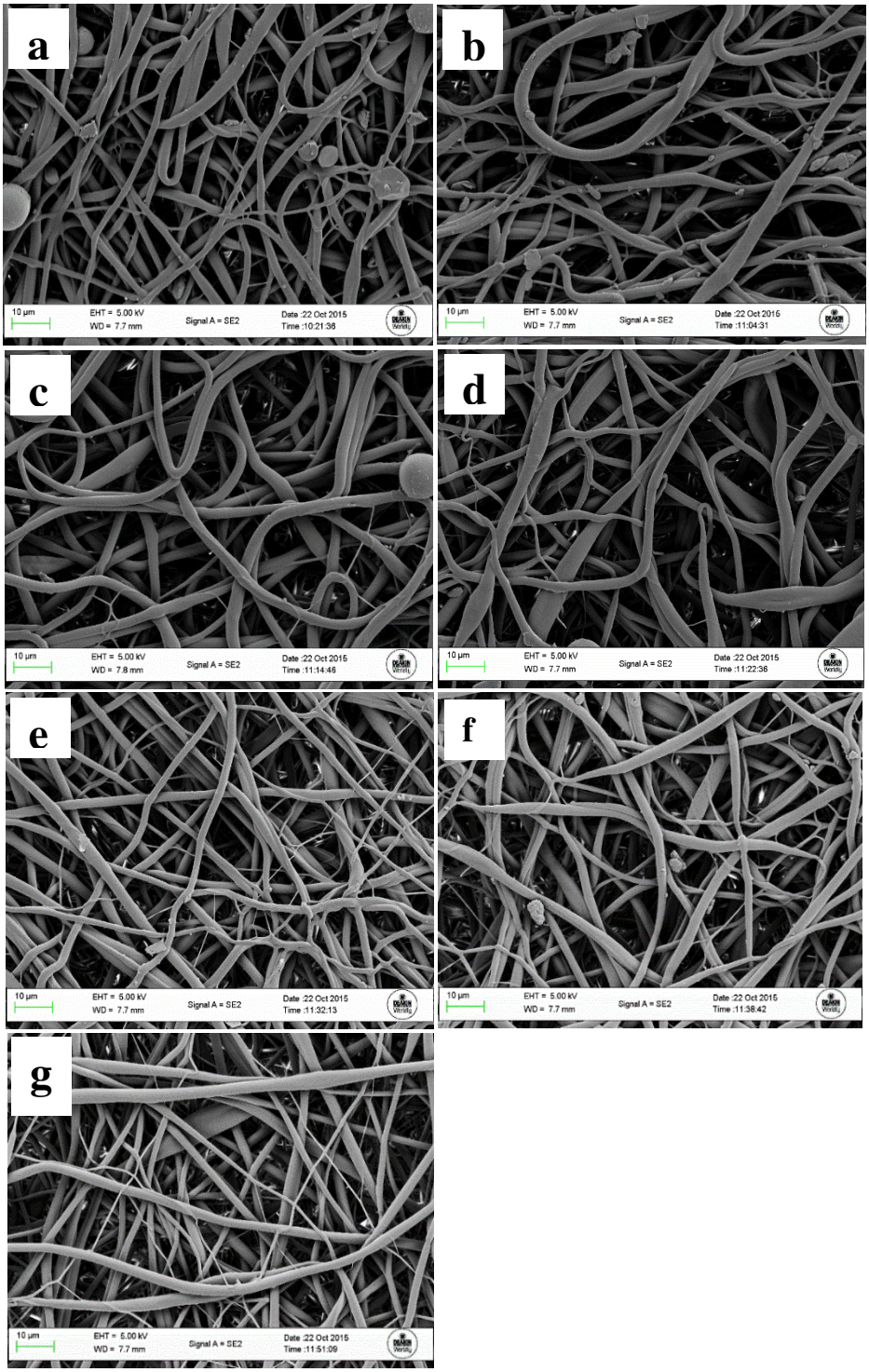


Fig.4.5: SEM images of (a) elastic SBS, (b-f) elastic SBS to nonelastic SBS (4:1, 3:2, 1:1, 2:3, 1:4) and (g) nonelastic SBS fibrous scaffolds electrospun with 17 wt% at d=17 cm, rate=3 ml/h, and voltage=20 KV respectively.

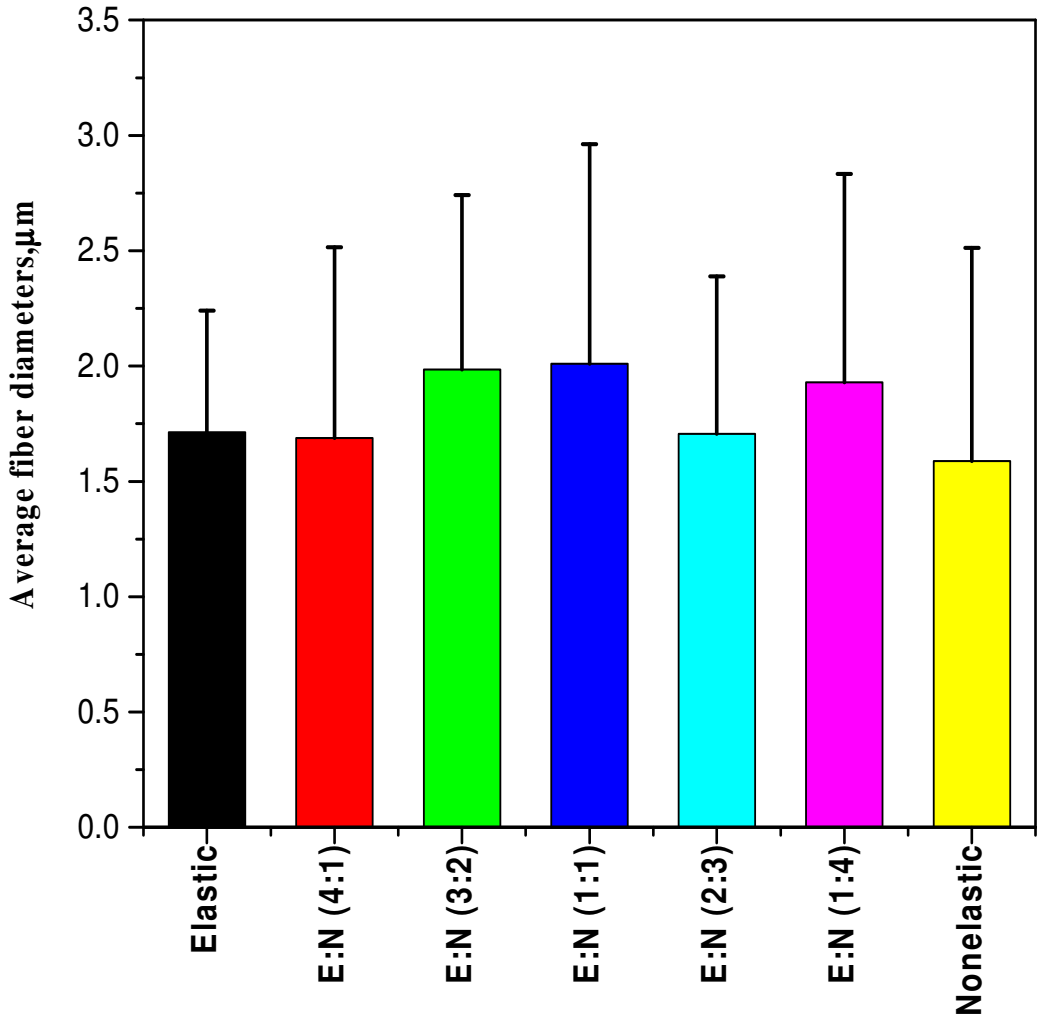


Fig.4.6: Average fiber diameters of elastic SBS, elastic SBS to nonelastic SBS (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds electrospun with 17 wt% at d of 17 cm, rate of 3 ml/h, and voltage of 20 KV, respectively.

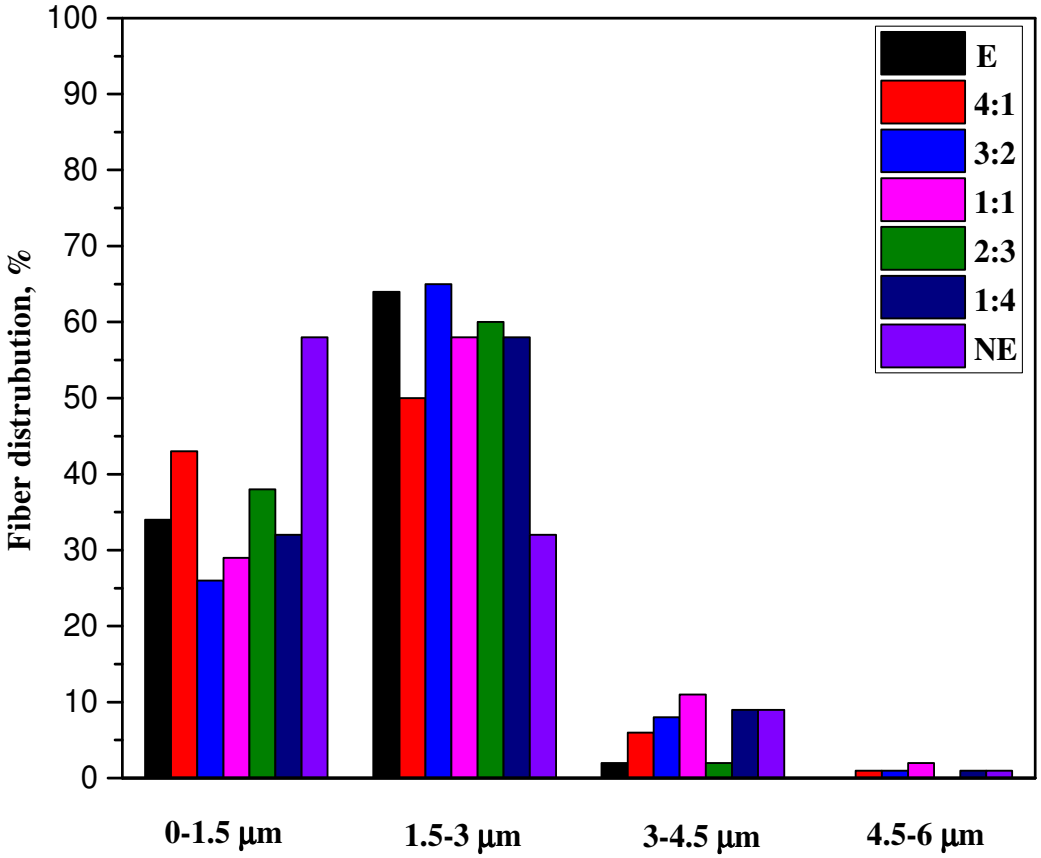


Fig.4.7: Fibre diameter distribution of elastic SBS, elastic SBS to nonelastic SBS (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds electrospun with 17 wt% at d of 17 cm, rate of 3 ml/h, and voltage of 20 KV, respectively.

4.3.2. Pore size and pore distribution of SBS fibrous scaffolds

For tissue engineered scaffolds, it was found that microporous or nanoporous structures of the interconnected pores could facilitate the transport of nutrients as well as the exchange of gases which in turn help cellular growth and regeneration of tissues [124]. Pore size and pore distribution analysis were characterized using an automated capillary flow porometer and the results are shown in Fig.4.8 and Fig.4.9. It is clear that the pore size distribution is between 6 µm to 9 µm with an average pore diameter of 8±0.01 µm.

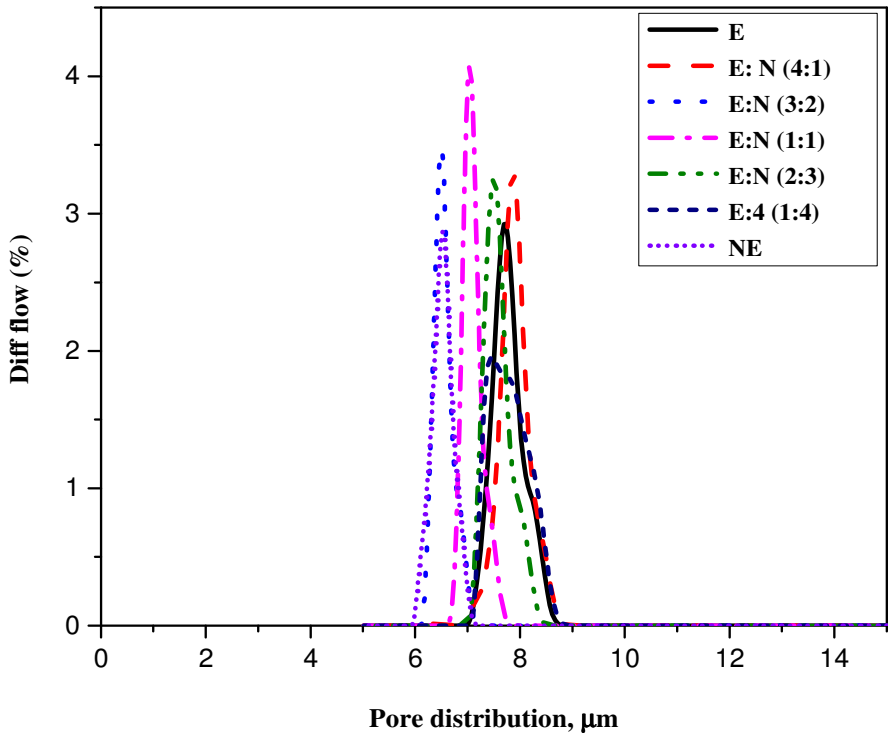


Fig.4.8: Pore distributions of elastic SBS, elastic SBS to nonelastic SBS (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds electrospun with 17 wt% at d=17 cm, rate=3 ml/h, and voltage=20 KV respectively.

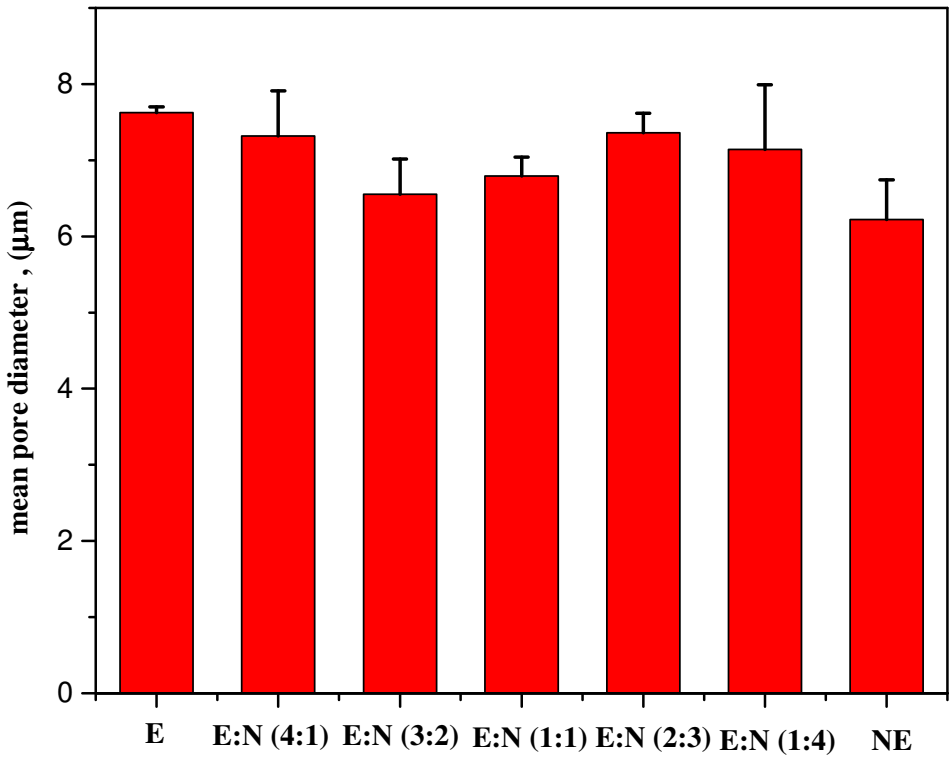


Fig.4.9: Average pore size of elastic SBS, elastic SBS to nonelastic SBS (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds electrospun with 17 wt% at d of 17 cm, rate of 3 ml/h, and voltage of 20 KV , respectively.

4.3.3. Surface analysis of fibrous scaffolds

4.3.3.1. XRD measurements

Fig.4.10 shows the XRD chart for the elastic SBS, nonelastic SBS-blended elastic SBS, and nonelastic SBS fibrous scaffolds. It is quite clear that the composition of the elastic SBS did not change after blending with nonelastic SBS. Fig.4.10 shows two peaks, one at 2θ=10° and the other at 2θ=20°. Glassy atactic polystyrene usually shows a scattering peak at 10° along with the main amorphous halo at 20° [122]. Amorphous polybutadiene shows a single peak at 19.5° [123]. XRD chart is consistent with the mixture of polystyrene and polybutadiene.

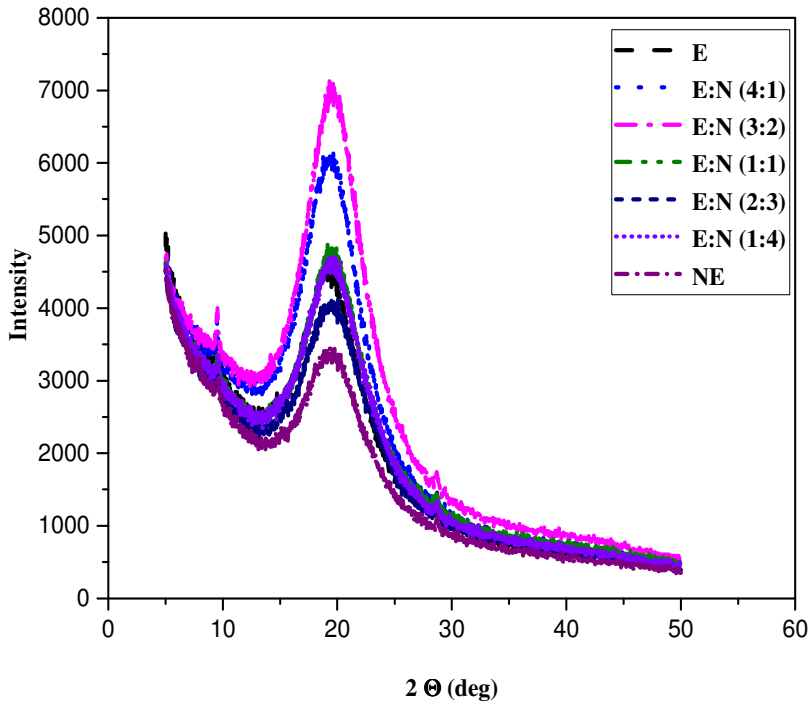


Fig.4.10: XRD curve for the elastic, elastic to nonelastic (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic fibrous scaffolds.

4.3.3.2. FTIR measurements

An FTIR infrared spectrum is a chemical fingerprint of a sample, providing with absorption peaks, which match the frequencies of vibration of the bonds between the atoms that compose the material. Since every material has unique arrangement of atoms, no two materials can produce the same infrared spectra. Therefore, infra-red spectra can result in qualitative analysis and quantitative analysis as well because the size of the peaks indicate the amount of material used. FTIR was performed on elastic SBS, nonelastic SBS blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS. Butadiene segments inside the fibrous scaffolds give CH_2 peak at 1449 cm^{-1}, C=C peak at 964 cm^{-1}, =CH stretching peak at 3005 cm^{-1} and C-H stretching peak at 2916 cm^{-1} and 2844 cm^{-1} [125,126]. Styrene segments inside the fibrous scaffolds gave C-H peak at 3060 cm^{-1} and aromatic C-C stretching peak at 1601 cm^{-1} (fig.4.11;[126, 127] .It is clear that mixing nonelastic SBS with elastic SBS did not change the FTIR response which in turn indicate the chemical composition is stable.

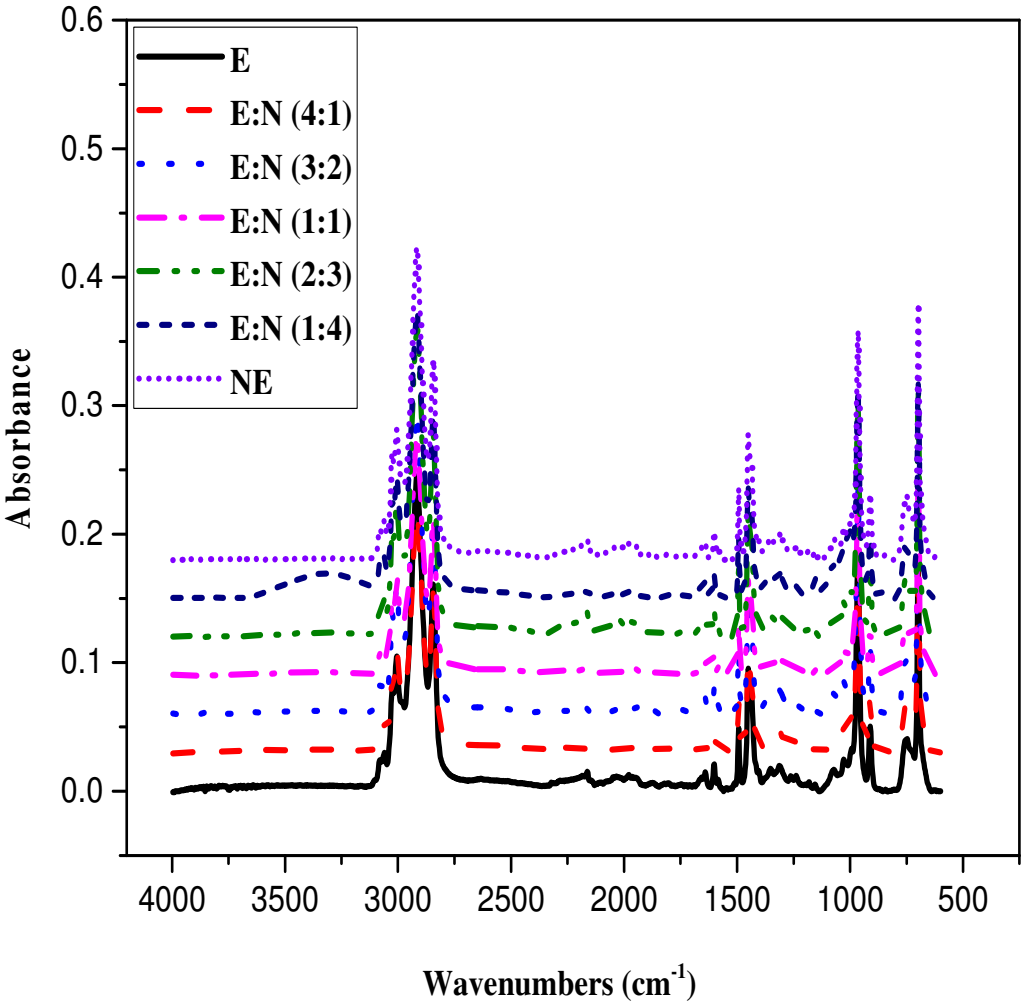


Fig.4.11: FTIR curve for the elastic, nonelastic SBS blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds.

4.3.4. Wettability of fibrous scaffolds

Surface wettability is an important property in tissue engineering field which contribute towards the substrate biocompatibility [128]. SBS fibrous scaffolds demonstrate hydrophobic properties with an average water contact angle (WCA) value of 117 º for elastic SBS and 115 º for nonelastic SBS. The average value of WCA for the mixed ratios of elastic SBS and nonelastic SBS varies and it does not depend on elasticity, rather, it relates to the topology of the fibrous scaffolds (fig.4.12; [30]).

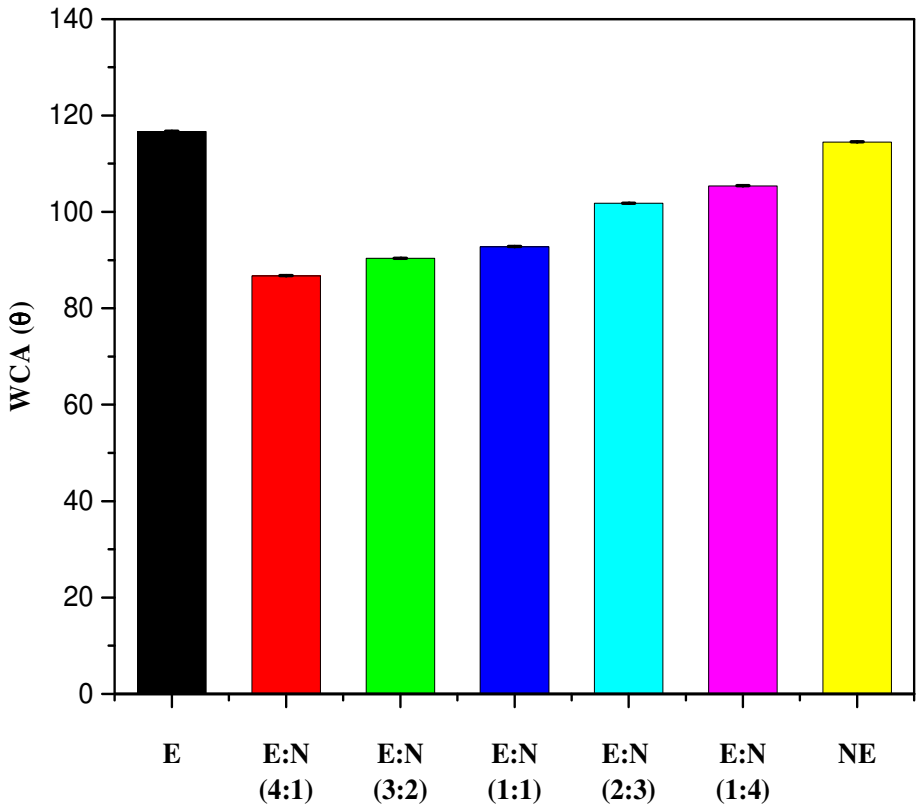


Fig.4.12: Water contact angle measurements (WCA) of elastic SBS, nonelastic SBS blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds electrospun with 17 wt% respectively.

4.3.5. Air permeability of fibrous scaffolds

Air permeability test was carried out to find whether SBS fibrous scaffolds are permeable to air or not because this property is important for nutrients infiltration for cells when cell culture is performed. Air permeability test demonstrated that the SBS fibrous scaffolds are all permeable to air regardless of elasticity. Elastic SBS showed an average air permeability value of 2.7 ± 0.6 $cm^3/cm^2/s$, while nonelastic SBS showed an average value of 3.5 ± 0.3 $cm^3/cm^2/s$ as shown in fig.4.13.

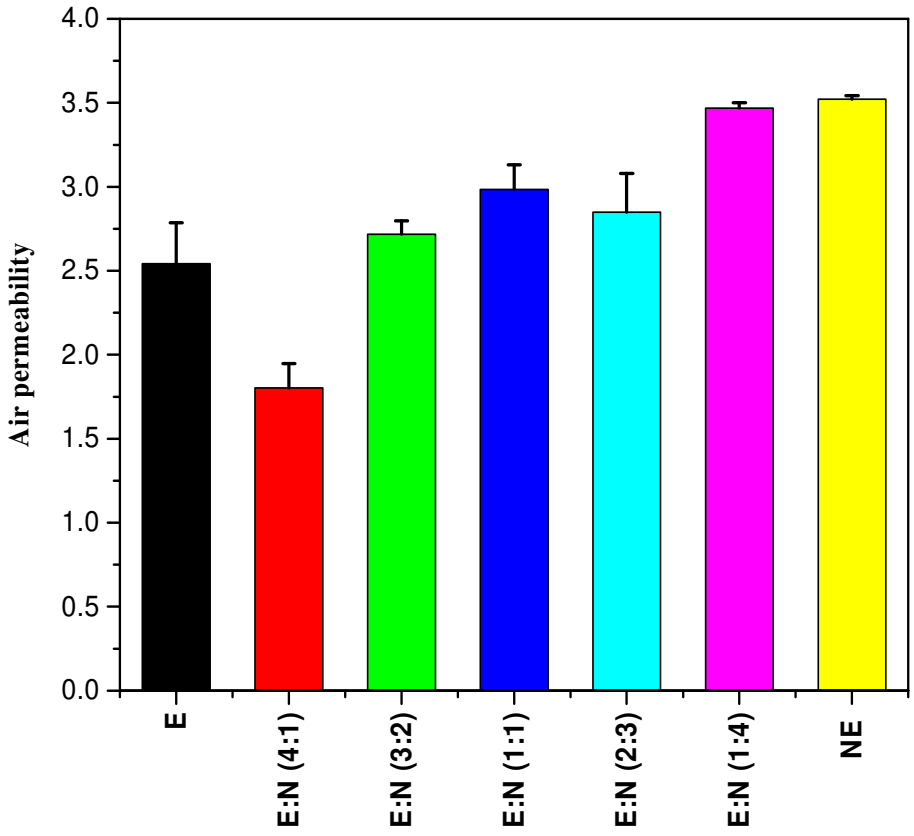


Fig.4.13: Air permeability curve of elastic SBS, nonelastic SBS-blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds electrospun with 17 wt%, respectively.

4.3.6 Thermal analysis of fibrous scaffolds

4.3.6.1. Thermogravimetric analysis (TGA)

Fig.4.14 illustrates the TGA curve of the elastic SBS, nonelastic SBS-blended elastic SBS and nonelastic SBS. The melting temperature for all of the fibrous sacffolds was approximately 500 ^{o}C. TGA result confirms the XRD result in relation to the unchanged chemical composition of elastic SBS after blending with nonelastic SBS.

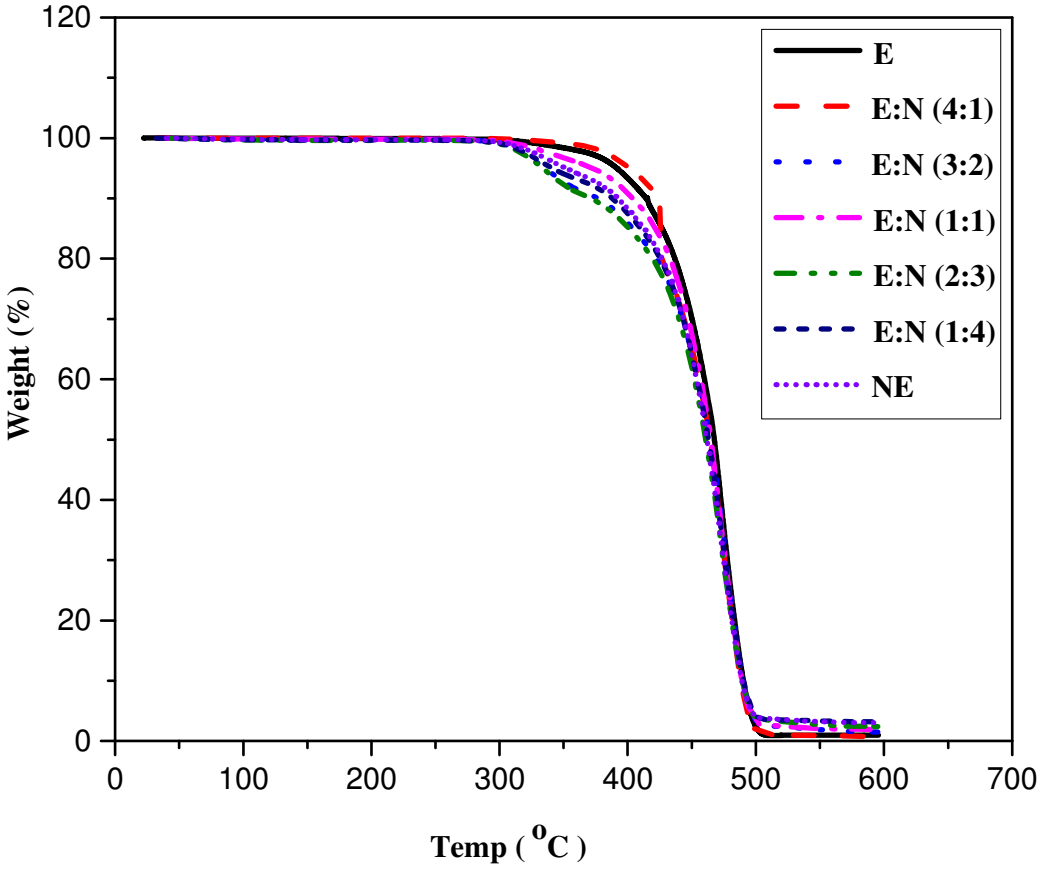


Fig.4.14: TGA curve for the elastic SBS, elastic SBS to nonelastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds.

4.3.6.2. Differential scanning calorimetry (DSC) analysis

Fig.4.15 illustrates the DSC curve of the elastic SBS, nonelastic SBS -blended elastic SBS and nonelastic SBS. The glass-transition temperature of the whole scaffolds was approximately 400 ºC. DSC result agrees with both XRD and TGA regarding the stability of the chemical composition of elastic SBS after blending with nonelastic SBS.

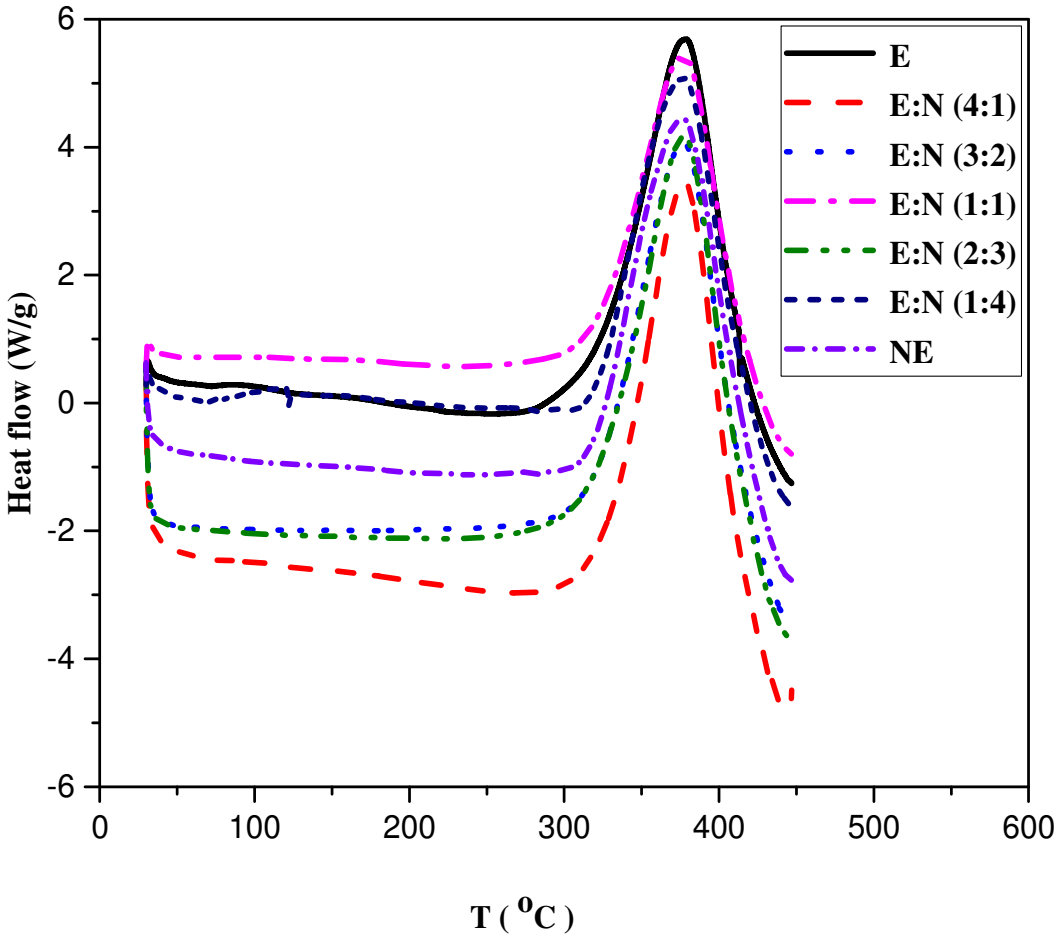


Fig.4.15: DSC curve for the elastic, elastic to nonelastic (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic fibrous scaffolds.

4.3.7. Mechanical properties of fibrous scaffolds

Mechanical properties of synthetic blood vessels are rather important since it affects the mechanical match with the native blood vessels. Fig.4.16 illustrates the strain-stress curve of elastic SBS, nonelastic SBS-blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds. The results showed that elastic SBS fibrous scaffold has the highest strain % of 916.23± 0.84 %. Blending nonelastic SBS to elastic SBS fibrous scaffold decrease the strain up to 616.99± 0.44 % in case of the ratio 1:4. Elastic SBS fibrous scaffolds have been shown to exhibit high strain (%) compared to any synthetic fibrous scaffolds reported so far [106]. Table.4 shows the mechanical properties obtained for elastic SBS, nonelastic SBS-blended elastic SBS and nonelastic SBS fibrous scaffolds electrospun with 17 wt%. Fig.4.17 demonstrates that strain (%) increased as the increase of elastic SBS ratio to nonelastic SBS ratio in the scaffolds. For example, Strain (%) of nonelastic SBS is 204.02± 0.01 while it is 889.59± 0.17 in case of elastic SBS to nonelastic SBS (1:4).

The mechanical hysteresis is calculated by the area under the curve of each loading-unloading cycle. The dissipated energy is proportional to the deformation and decrease as the number of cycles increase. Elastic SBS with low butadiene content demonstrated a narrow hysteresis compared to nonelastic with the higher butadiene content (0.7385 MJ/m^3 for elastic against 0.8284 MJ/m^3 for nonelastic) (fig.4.22). Note that, this behaviour has been previously reported for SBS copolymer [120].

For all of the samples, the hysteresis decrease for the first two cycles and then becomes constant for the rest of the cycles, similar to the reported behaviour for styrene-butadiene obtained in solvent casted or extruded films [120].

This phenomenon is known as Mullins effect and occurs due to the rearrangement of the macromolecular network [129].

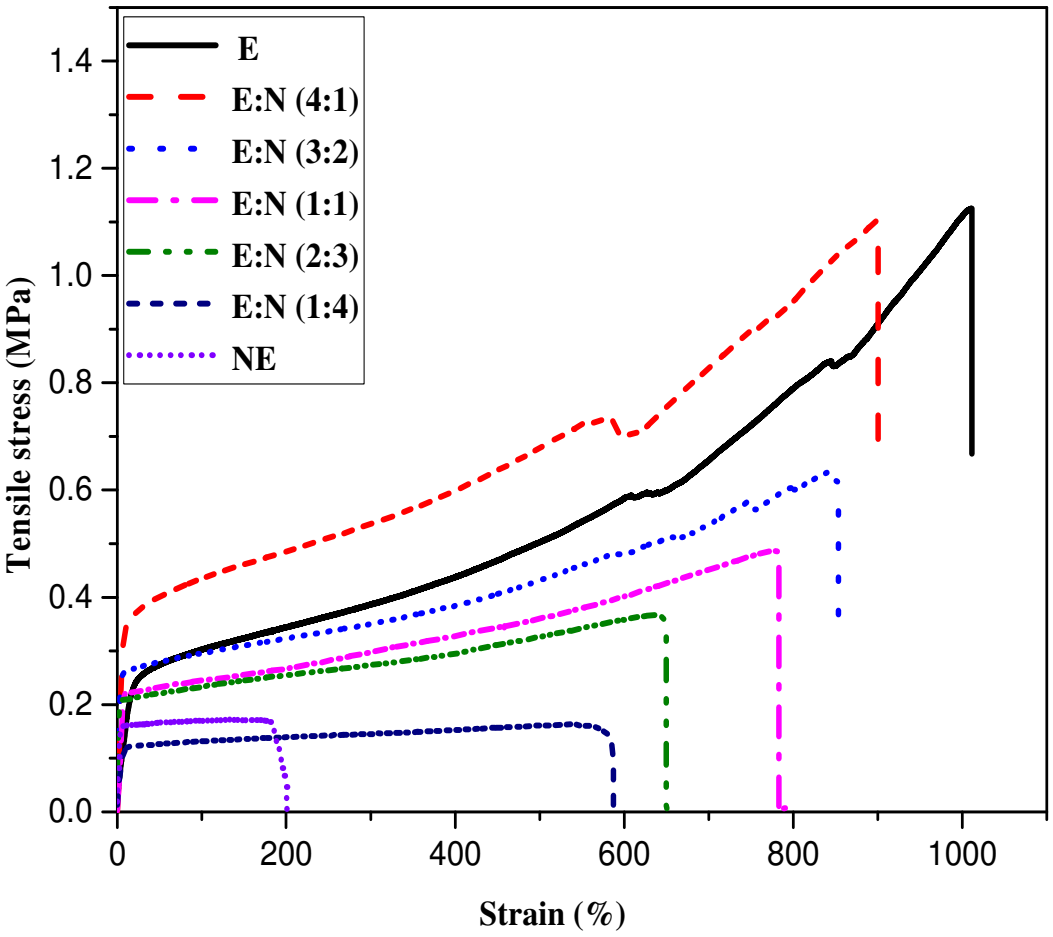


Fig.4.16: Strain-stress curve of elastic SBS, nonelastic SBS-blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds electrospun with 17 wt% respectively.

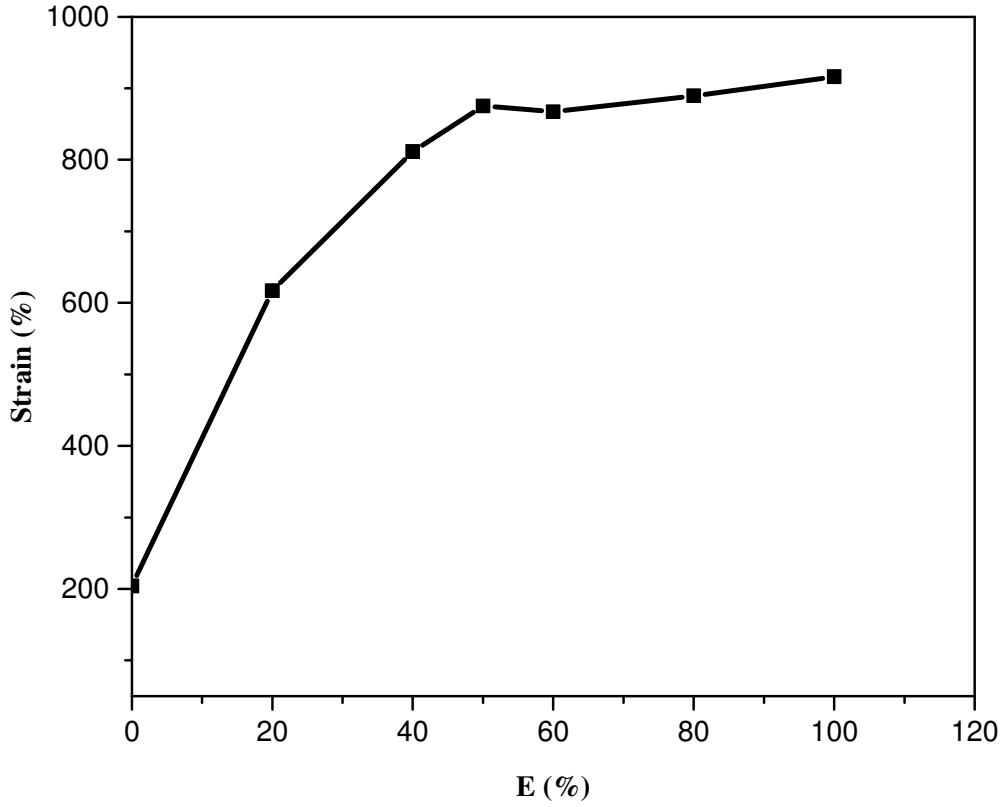


Fig.4.17: Strain %-elastic % of elastic SBS, nonelastic SBS-blended elastic SBS and nonelastic SBS fibrous scaffolds electrospun with 17 wt% respectively.

Table.4: shows the averaged values of tensile modulus, tensile stress and tensile strain of elastic SBS, nonelastic SBS-blended elastic SBS with ratios (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS fibrous scaffolds.

Sample	Tensile modulus (MPa)	Tensile stress (MPa)	Tensile strain (%)
E	0.162 ± 0.04	0.95 ± 0.22	916.23± 0.84
E:N (4:1)	0.16 ± 0.01	1.08 ± 0.03	889.59± 0.17
E:N (3:2)	0.07 ± 0.01	0.66 ± 0.07	867.32 ± 0.84
E:N (1:1)	0.06 ± 0.01	0.35 ± 0.30	875.16 ± 0.68
E:N (2:3)	0.04 ± 0.01	0.01±0.00	811.52 ± 1.45
E:N (1:4)	0.03 ± 0.01	0.01±0.00	616.99± 0.44
NE	2.69 ± 3.07	0.01±0.00	204.02± 0.01

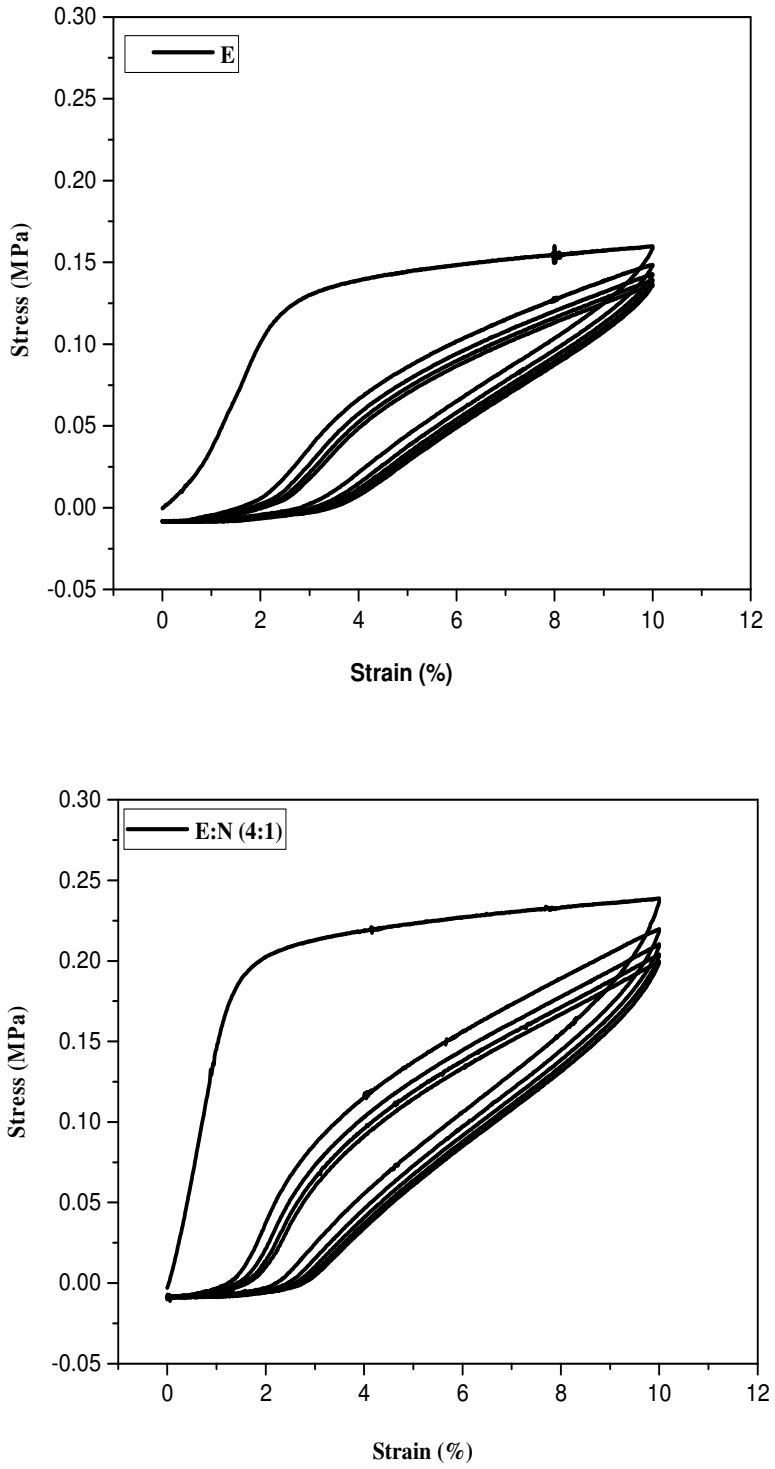


Fig.4.18: load-unload stress-strain curve of elastic SBS, nonelastic-blended elastic SBS (4:1). 5 cycles for maxim strain 10% is shown.

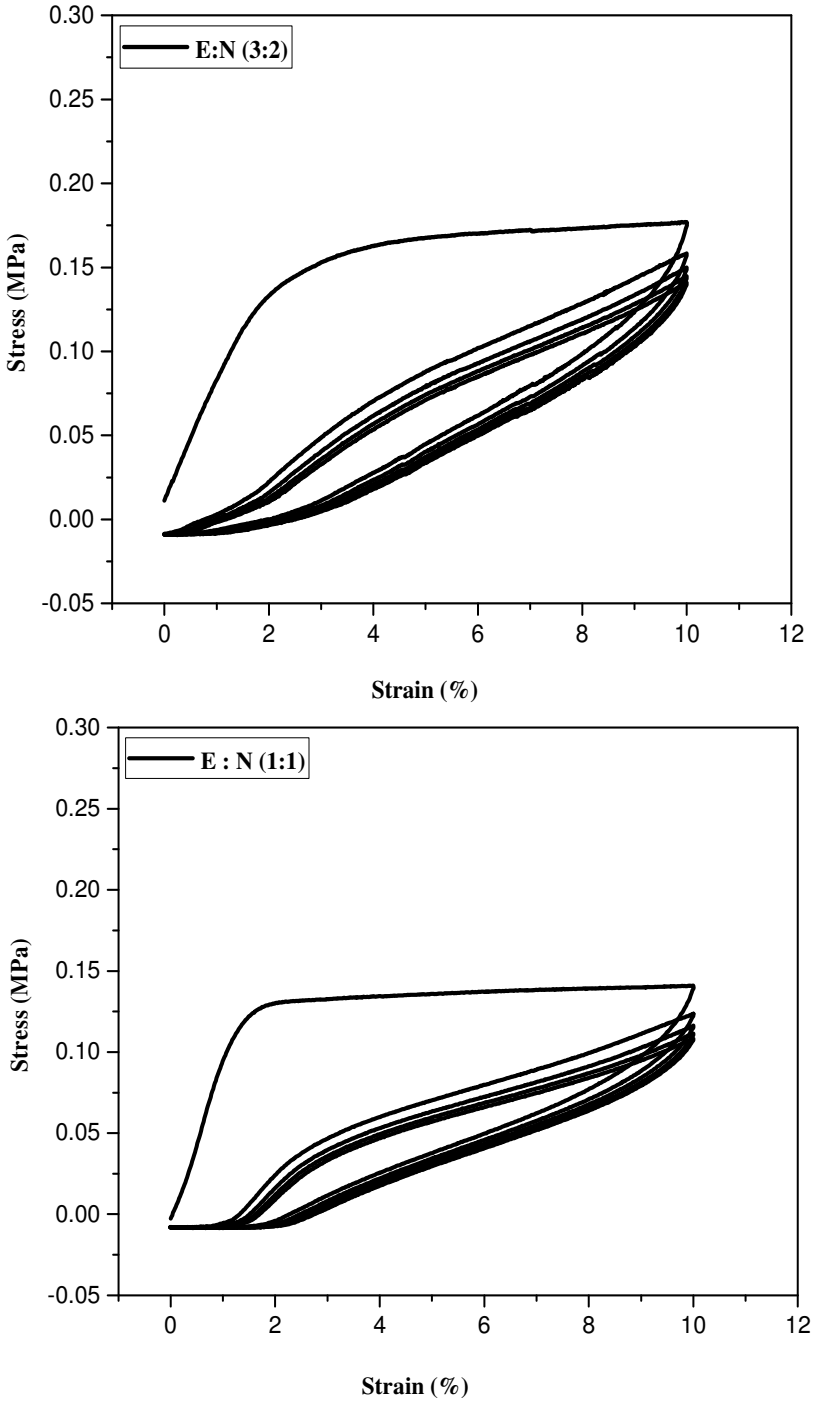


Fig.4.19: load-unload stress-strain curves of nonelastic-blended elastic SBS (3:2 and 1:2). 5 cycles for maxim strain 10% is shown.

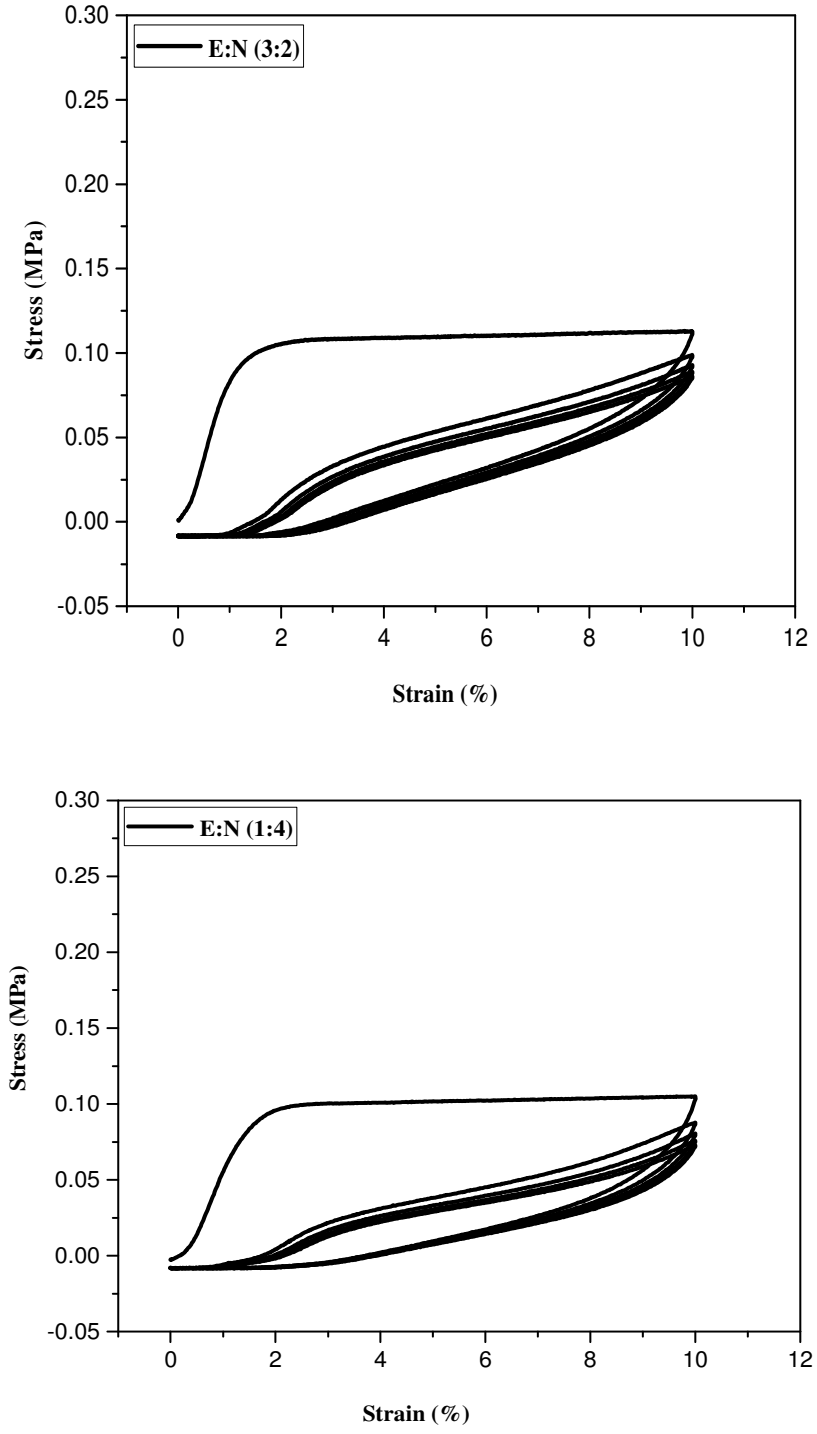


Fig.4.20: load-unload stress-strain curves of nonelastic-blended elastic SBS (2:3 and 1:4). 5 cycles for maxim strain 10% is shown.

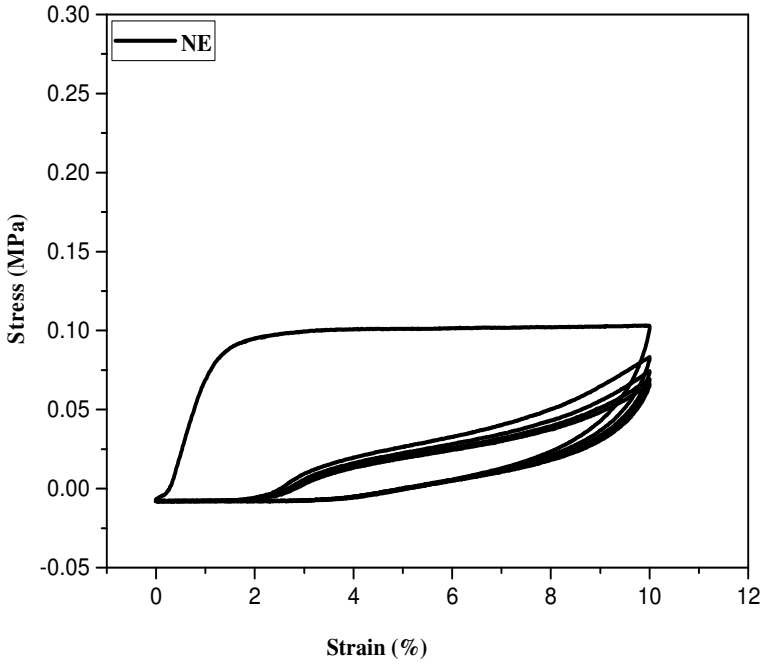


Fig.4.21: load-unload stress-strain curves of nonelastic SBS. 5 cycles for maxim strain 10% is shown.

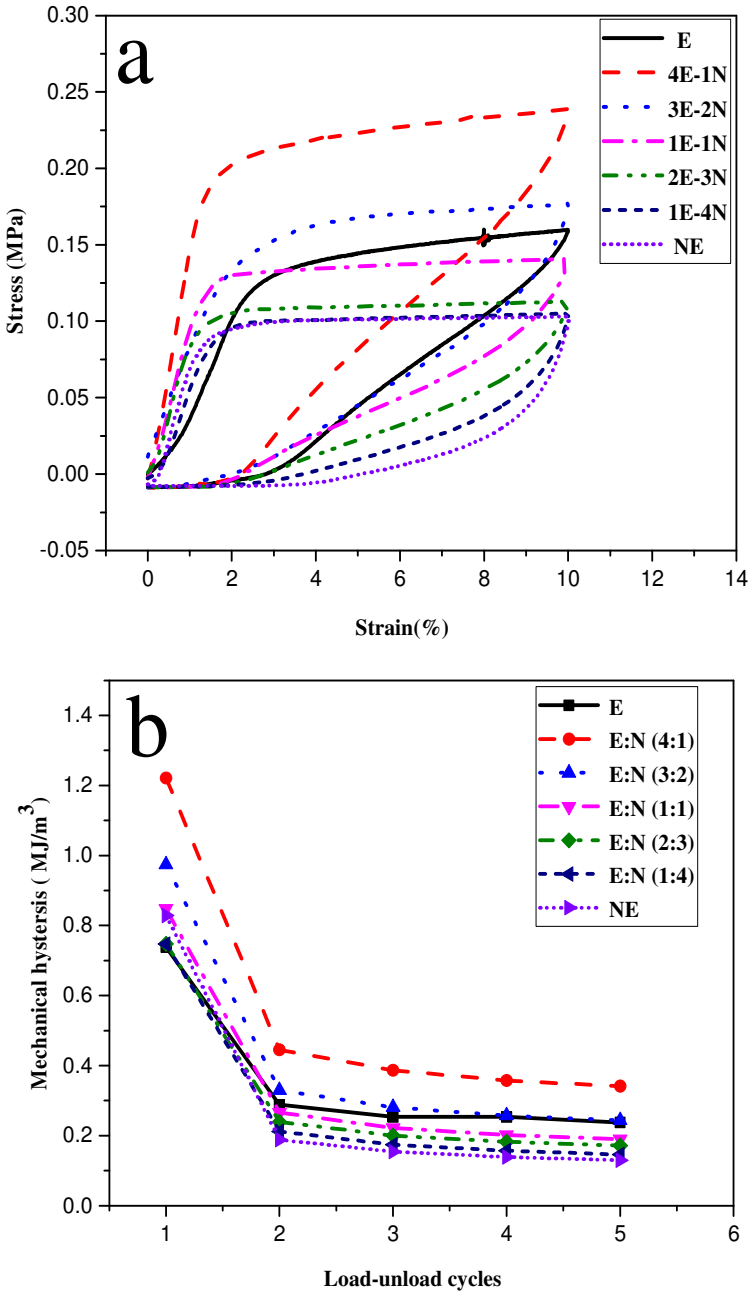


Fig.4.22: (a) Comparison of load-unload curves for elastic SBS, nonelastic SBS-blended elastic SBS (4:1, 3:2, 1:1, 2:3 and 1:4) and nonelastic SBS at 10 % strain (b) Mechanical hysteresis-load-unload cycles curve.

4.4. Conclusion

SBS fibrous scaffolds have been fabricated through simple and non-expensive technique such as electrospinning. Nonelastic SBS is blended to elastic SBS with 17 wt% which led to uniform fibrous scaffolds with ratios (5:0, 4:1, 3:2, 1:1, 2:3, 1:4 and 0:5). Uniform fibrous structures have been obtained with 17 wt % and the average fibre diameter of SBS scaffolds is 2 µm. The chemical composition of SBS remained stable after blending. Chemical composition, crystallography and thermal properties of the SBS blend is similar to pure elastic and nonelastic SBS fibrous scaffolds. The SBS fibrous scaffolds have an average pore size of 7.62217±0.0788 µm and it is permeable to air. Mechanical properties of fibrous scaffolds could be tuned by blending elastic SBS to nonelastic SBS with the highest strain % of 916.23±0.84% achieved in elastic SBS.

Chapter.5

Endothelialisation of SBS fibrous scaffolds

5.1. Overview

Many studies have been attempted using electrospun nanofibres as tissue engineering scaffolds. In most of these trials, synthetic polymers such as poly (caprolactone) (PCL), polyurethane, poly (L-lactide) (PLA) and poly (glycolide) (PGA) were widely used [32-46]. On the other hand, natural polymers such as collagen, silk protein, elastinmimic peptide and fibrinogen have also been used [46-53]. Synthetic polymers have good mechanical properties. However, they lack cell-recognition signals, which can be found in natural polymers. But, natural polymers lack good mechanical properties. Therefore, Blending natural polymer with synthetic polymers or blending synthetic polymers or blending natural polymers could be a solution for this problem [54-62].

In this chapter, endothelialization of SBS fibrous scaffolds is performed. ECs attachment, ECs proliferation and cellular morphology are studied. To the best of our knowledge, this is, the first time, the interaction of ECs with SBS fibrous scaffolds has been investigated.

5.2. Experimental procedures

SBS fibrous scaffolds were in vitro tested using ECs. ECs attachment, proliferation and morphology were studied by MTS assay, SEM and confocal microscopy. Detailed description of materials and methods is found in chapter three.

5.3. Results and discussion

5.3.1. Surface wettability of SBS fibrous scaffolds after air plasma treatment.

SBS fibrous scaffolds are naturally hydrophobic with high contact angle as shown in table.5.1. Therefore, it is treated by air plasma to make it wettable to help the cells to easily attach to its surface and infiltrate the porous structure of SBS scaffolds. The usefulness of air plasma treatment is confirmed by WCA measurements. Table.5.1 shows the contact angles of SBS fibrous scaffolds before and after air plasma treatment for 5 min as well as after aging the fibrous scaffolds for 24h. Although the fibrous scaffolds showed high contact angles before air plasma treatment, they became highly wettable after treatment with contact angle of 0 °. The drop was fully absorbed by the fibrous scaffolds when tested after 3 sec. However, the fibrous scaffolds tended to return to their hydrophobic nature after 24 h. therefore, the in vitro cell culture has been carried out before 24 h.

The difference in wettability of the SBS fibrous scaffolds before and after the air plasma treatment can be explained as the following. Surface roughness affects the water contact angle of a solid surface in such a way: if a material is hydrophobic, the water cannot penetrate the fibrous structure of the material and can be considered as adsorbing on a semi-solid surface or semi-air plane surface. This will lead to a high contact angles. Conversely, after air plasma treatment, the water can adsorb and penetrate the fibrous structure of a hydrophobic material forming partly solid and liquid surface. This will lead to a low contact angles [130]. It is clear that SBS fibrous scaffolds obtained a big improvement after air plasma treatment from hydrophobic to hydrophilic which help cells to attach and penetrate their fibrous structures.

Table.5.1: Water contact angles of SBS fibrous scaffolds measured within 3 sec and aged for 24h.

Samples	WCA/degree			
	Without plasma treatment	After 3 sec with 5 min treatment	Greater than 3 sec with 5 min treatment	Aged for 24 h
E	116.686±0.04759	35.08733 ± 19.04796	0 ± 0	70.93167±21.0781
E:N (4:1)	86.72394±0.07905	64.258±13.53817	0 ± 0	92.951±5.264528
E:N (3:2)	90.37433±0.03401	0	0 ± 0	83.26667±9.111834
E:N (1:1)	116.2344±8.551081	100.34± 15.86014	0 ± 0	107.0577±4.075522
E:N (2:3)	101.7515±0.04852	71.858±22.95146	0 ± 0	101.303±3.872871
E:N (1:4)	105.3901±0.06422	0	0 ± 0	96.61767±3.302049
NE	114.4733±0.07417	101.2655±16.23588	0 ± 0	112.2903±6.300823

5.3.2. ECs Cytotoxicity evaluation of SBS fibrous scaffolds.

The relation between SBS fibrous scaffolds and ECs cells has been investigated in terms of biocompatibility, especially because, to the best of our knowledge, ECs in vitro cell culture for SBS fibrous scaffolds has not been carried out

before. However, in vitro studies of SBS scaffolds with other cells have been conducted with contradictory results: some denote a strong cytotoxic effect [131] and others not [132]. Cytotoxicity study is considered as a basic step to evaluate the biocompatibility of a material for tissue engineering applications and point out the suitable material for successful in vitro study. To determine the influence of SBS fibrous scaffolds extract medium on the metabolic activity of ECs, MTS assay was performed after culturing for 3 days in extract media of 3h, 3days and 7days (fig.5.1 to fig.5.3) and the morphology of ECs after toxicity study was studied by optical microscope (fig.5.4 to fig.9). According to ISO standard 10993-5, the material is considered cytotoxic if there is a reduction of cell viability by 30% [120]. It is obvious from MTS assay study that SBS fibrous scaffolds showed cell numbers comparable to the positive control and there was no difference in cell number between the extract media of various time points. This indicates that SBS fibrous scaffolds are not cytotoxic to ECs.

The morphologies of ECs cultured for 3h in the extract media of 3h, 3days and 7days of SBS fibrous scaffolds and incubated for 3 days in the extract media of 3h, 3days and 7days of SBS fibrous scaffolds are shown in fig.5.4 to fig.5.9. These images showed that when ECs are in contact with the extract media of SBS fibrous scaffolds, they presented an abundant growth comparable to the positive control. These results of ECs morphologies agrees with MTS study demonstrating that SBS fibrous scaffolds can be used for biomedical applications and tissue engineered blood vessel in particular, because ECs consist the primary layer of native blood vessels. Therefore, cytotoxicity study paved the way for further investigation of in vitro cell culture study.

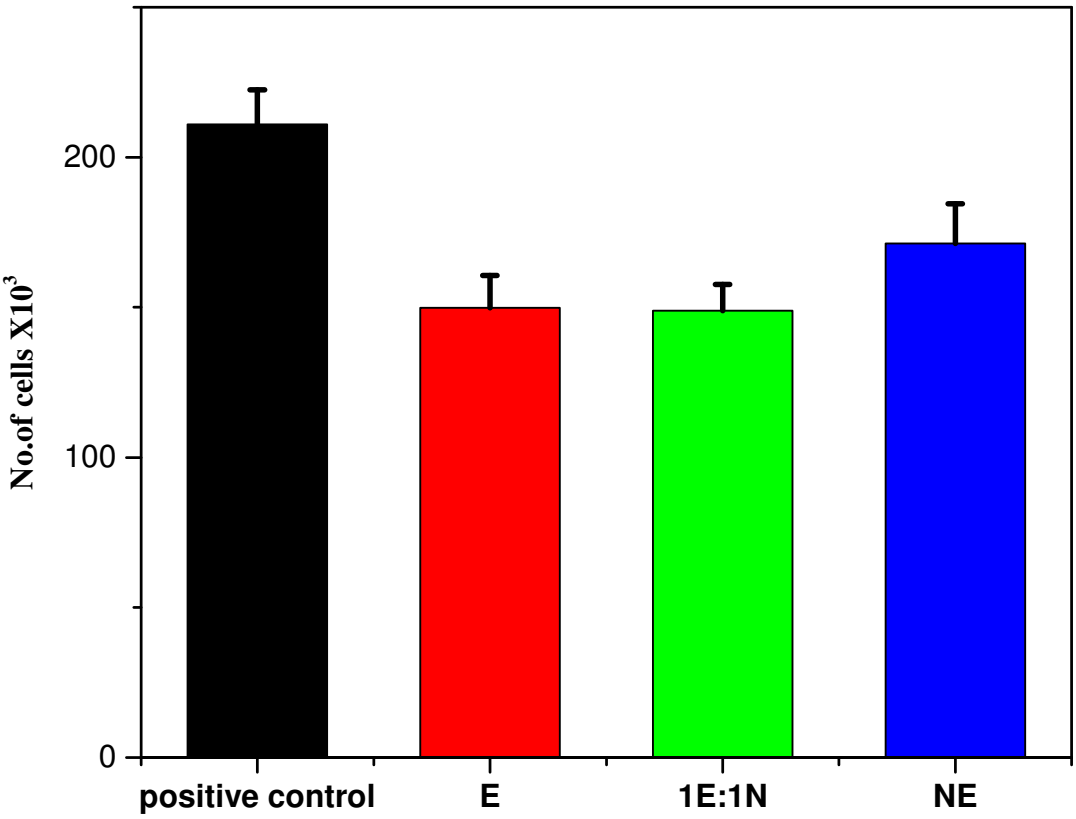


Fig.5.1: MTS assay of ECs cytotoxicity assay in contact with the extract media of E, E: N (1:1) and NE fibrous scaffolds of 3h up to 3days.

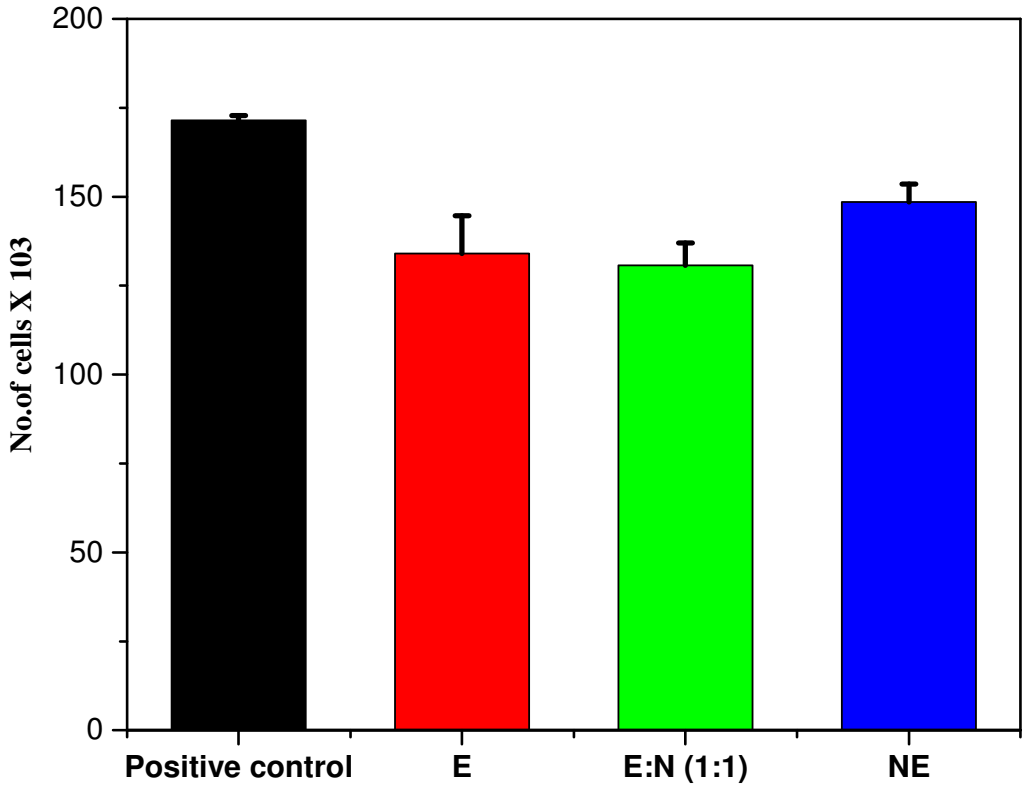


Fig.5.2: MTS assay of ECs cytotoxicity assay in contact with the extract media of E, E: N (1:1) and NE fibrous scaffolds of 3h up to 3days.

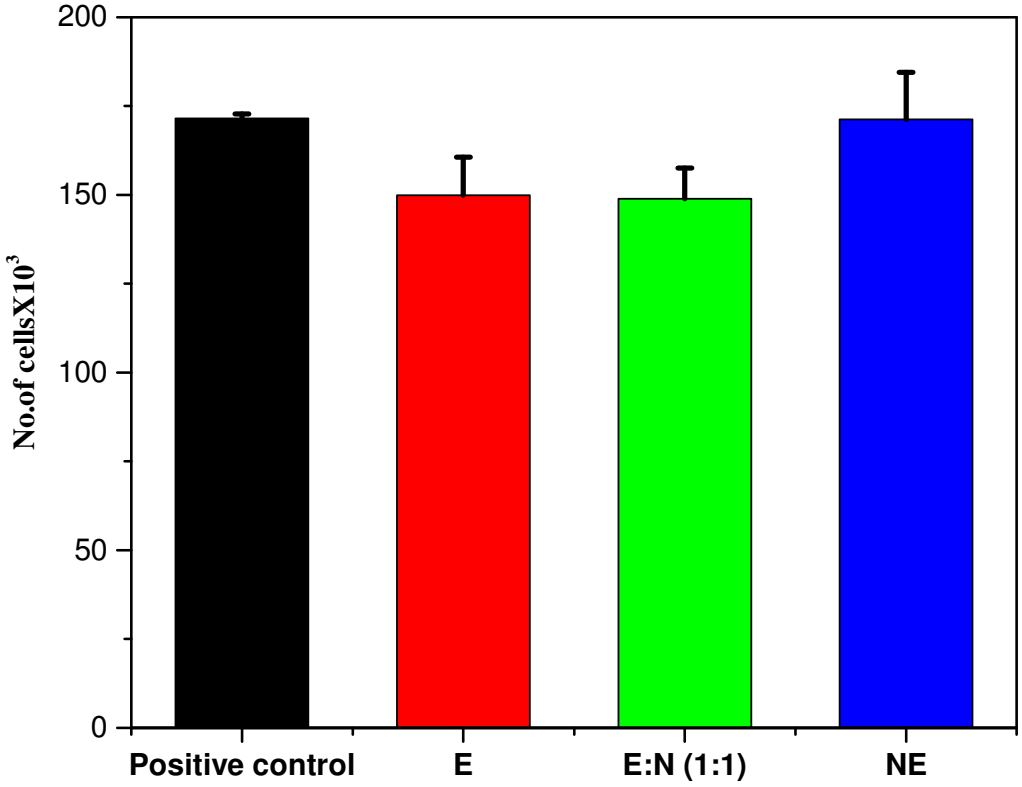


Fig.5.3: MTS assay of ECs cytotoxicity assay in contact with the extract media of E, E: N (1:1) and NE fibrous scaffolds of 3h up to 3days.

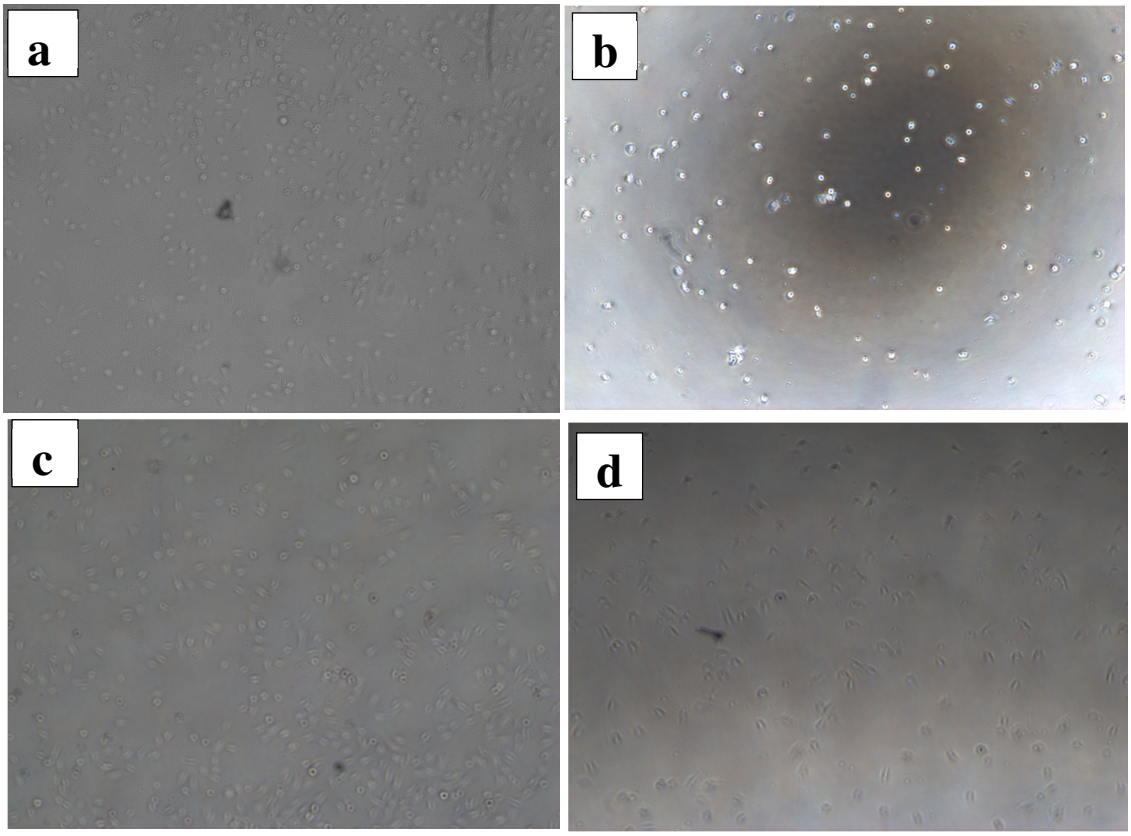


Fig.5.4: Images of ECs cultured for 3h in the 3h'extract media of these scaffolds: (a) positive control, (b) E, (C) E: N (1:1) and (d) NE.

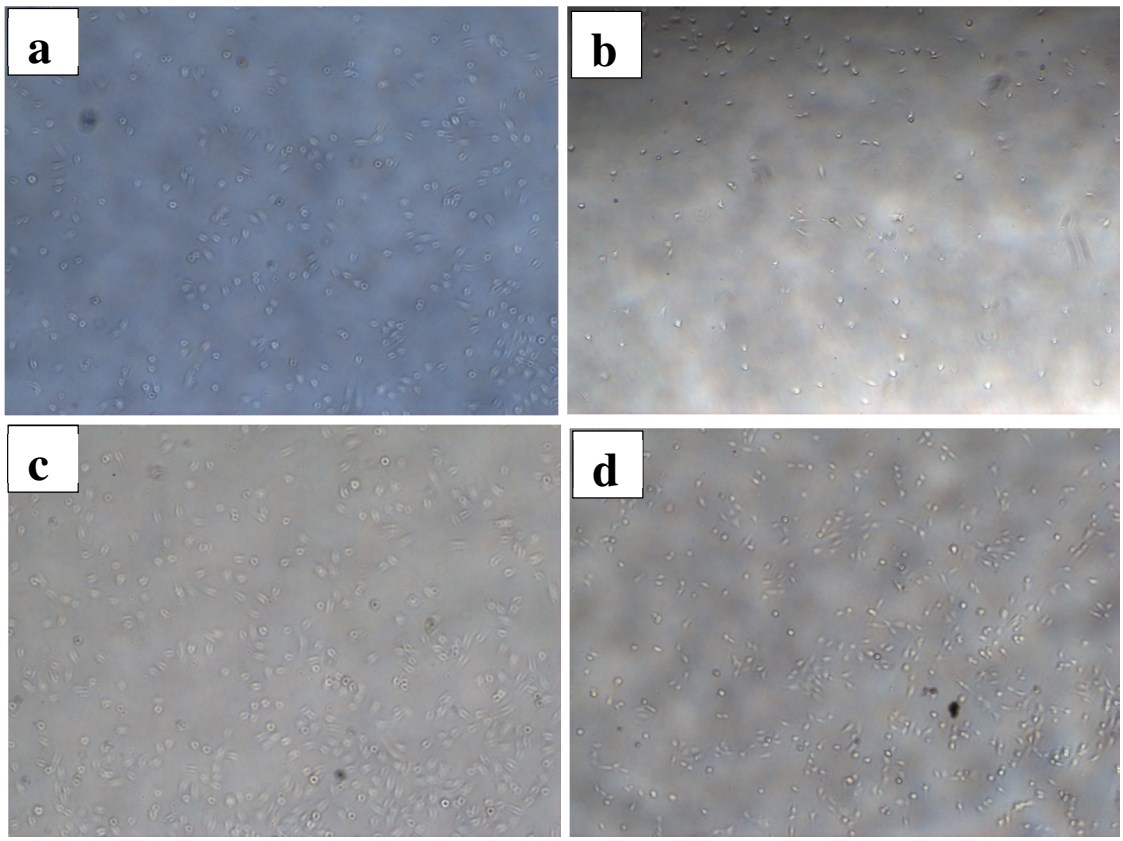


Fig.5.5: Images of ECs cultured for 3h in the 3days'extract media of these scaffolds: (a) positive control, (b) E, (C) E: N (1:1) and (d) NE.

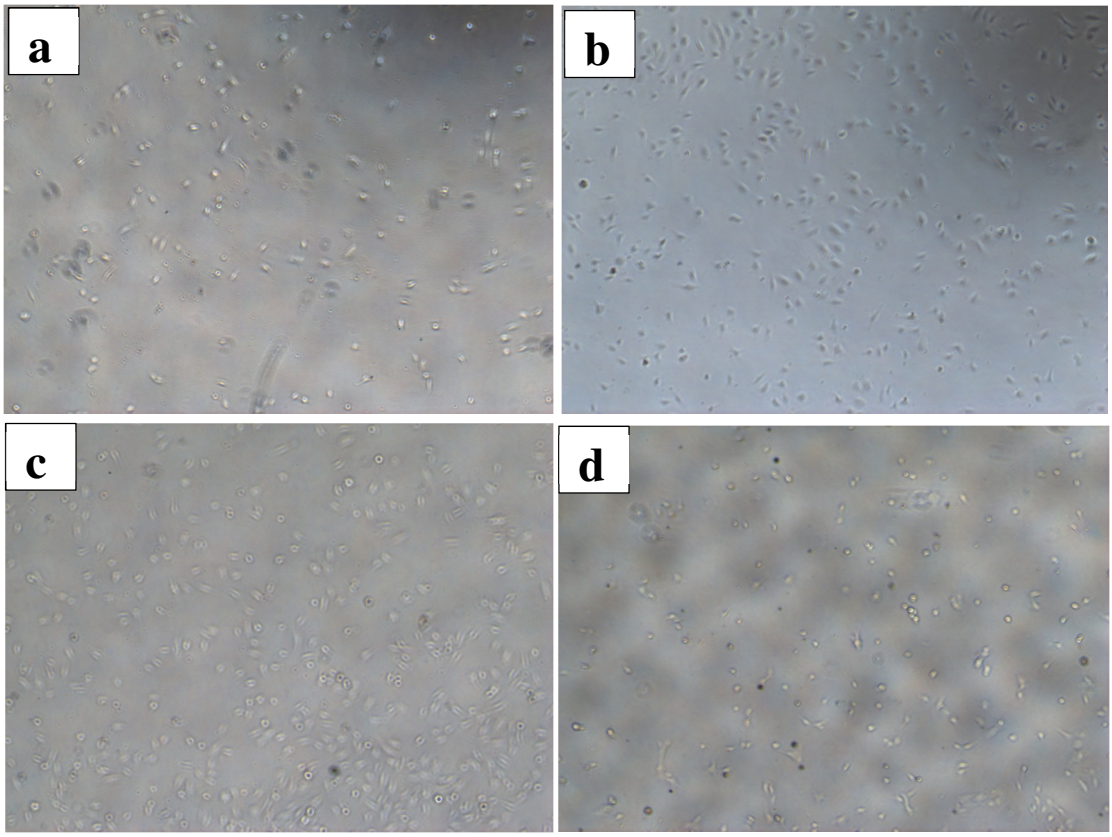


Fig.5.6: Images of ECs cultured for 3h in the 7days'extract media of these scaffolds: (a) positive control, (b) E, (C) E: N (1:1) and (d) NE.

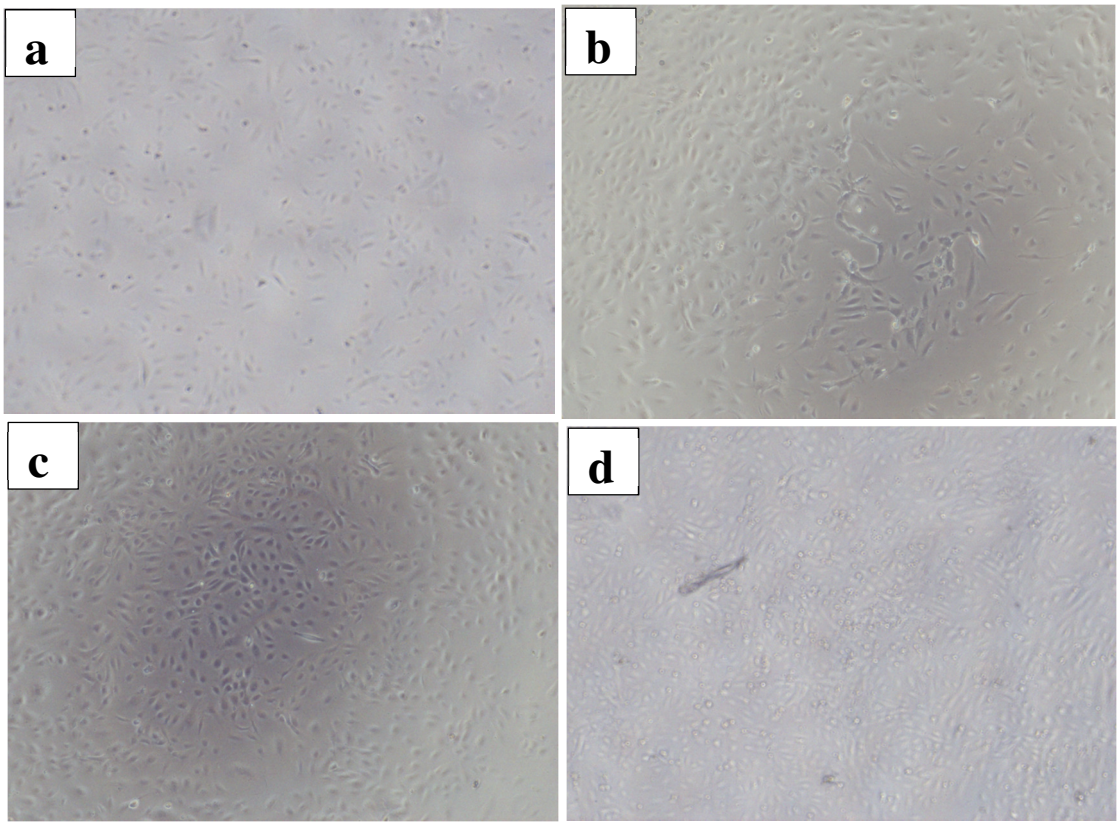


Fig.5.7: Images of ECs cultured for 3days in the 3h'extract media of these scaffolds: (a) positive control, (b) E, (C) E: N (1:1) and (d) NE.

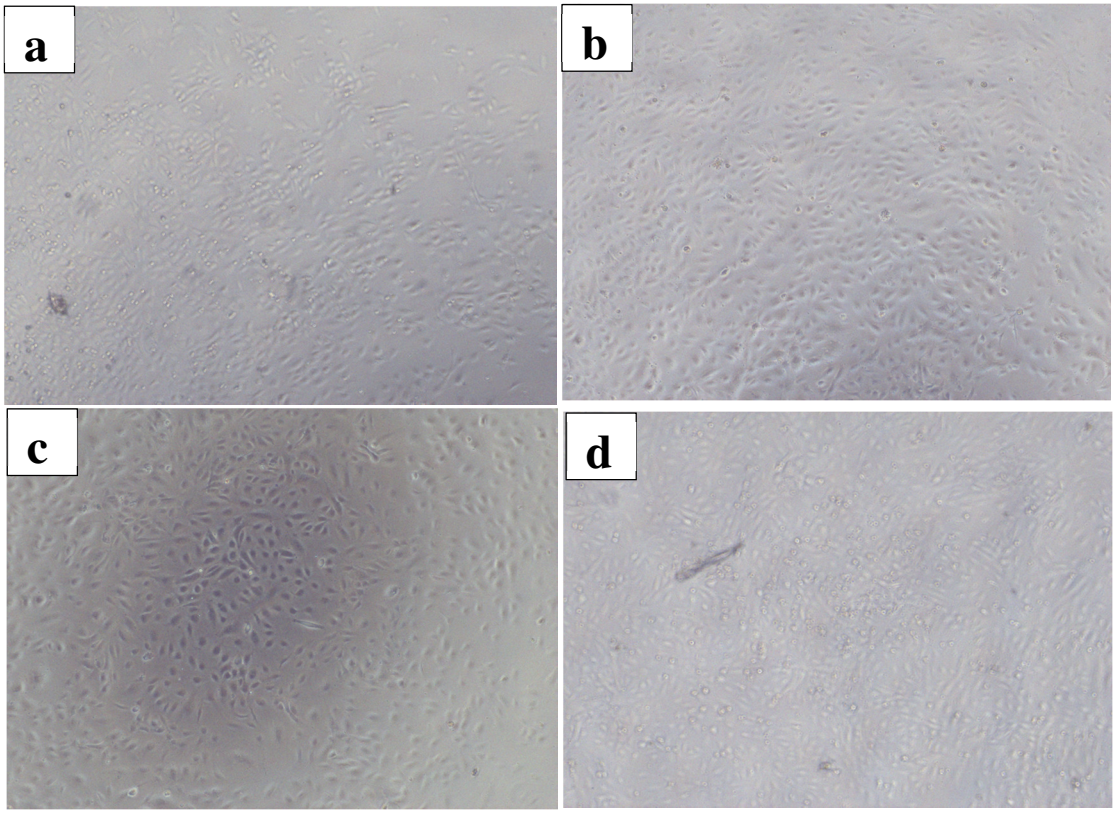


Fig.5.8: Images of ECs cultured for 3days in the 3days'extract media of these scaffolds: (a) positive control, (b) E, (C) E: N (1:1) and (d) NE.

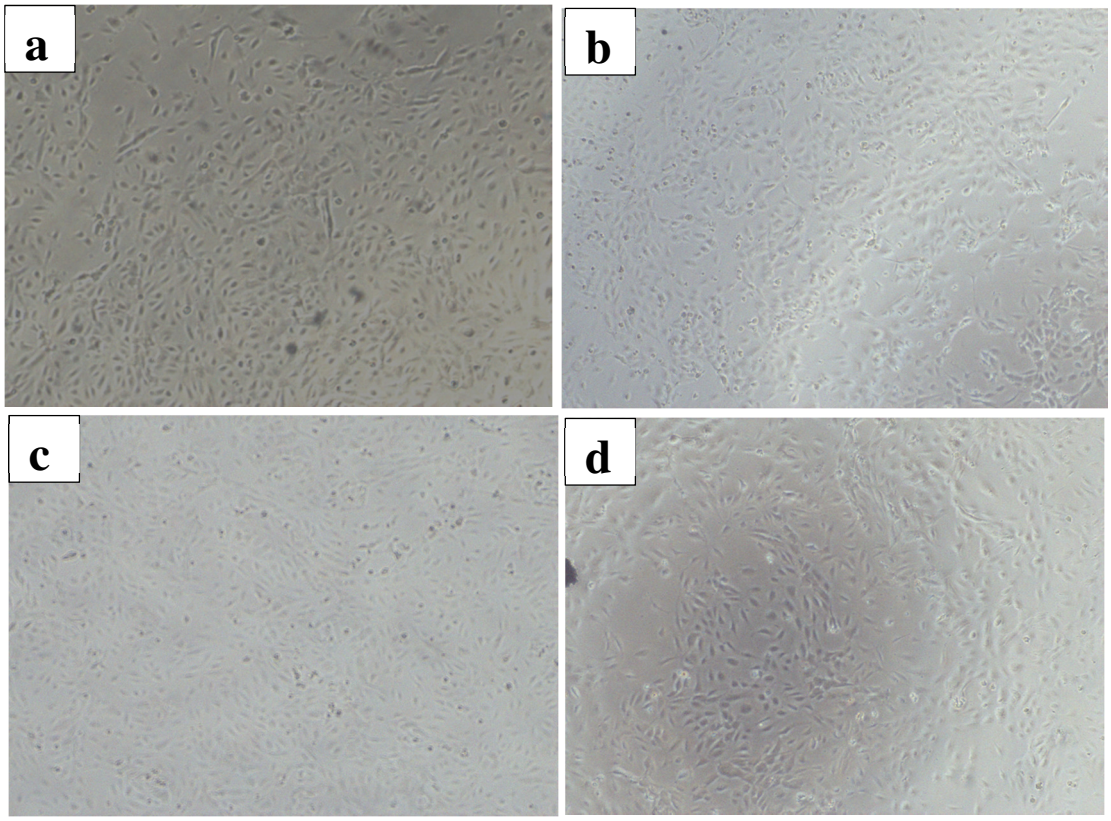


Fig.5.9: Images of ECs cultured for 3 days in the 7days'extract media of these scaffolds: (a) positive control, (b) E, (C) E: N (1:1) and (d) NE.

5.3.3. MTS assay of ECs cultured for 7 days on SBS fibrous scaffolds with unchanged and changed media.

In order to point out whether it is necessary to change the culture media for ECs when they are in vitro cultured or not. ECs were cultured for 7 days in two modes one with unchanged media and the other is changing the media every 2 days. MTS assay was carried out for both of them as shown in fig.5.10 and fig.5.11. On the basis of MTS results, the morphologies of ECs were investigated. From MTS study, it is clear that ECs growth is inhibited when media remained unchanged for 7 days due to the fact that cell number decreased from the original seeding number that is 20000 (fig.5.10). However, ECs number increased above 20000 cells when the media is changed as shown in fig.5.11. Fig.5.12 showed that ECs became round which means that the cells were unhealthy and not viable. This is in agreement with the results obtained from MTS assay (fig.5.10). Therefore, the media is changed every two days in in vitro cell culture study.

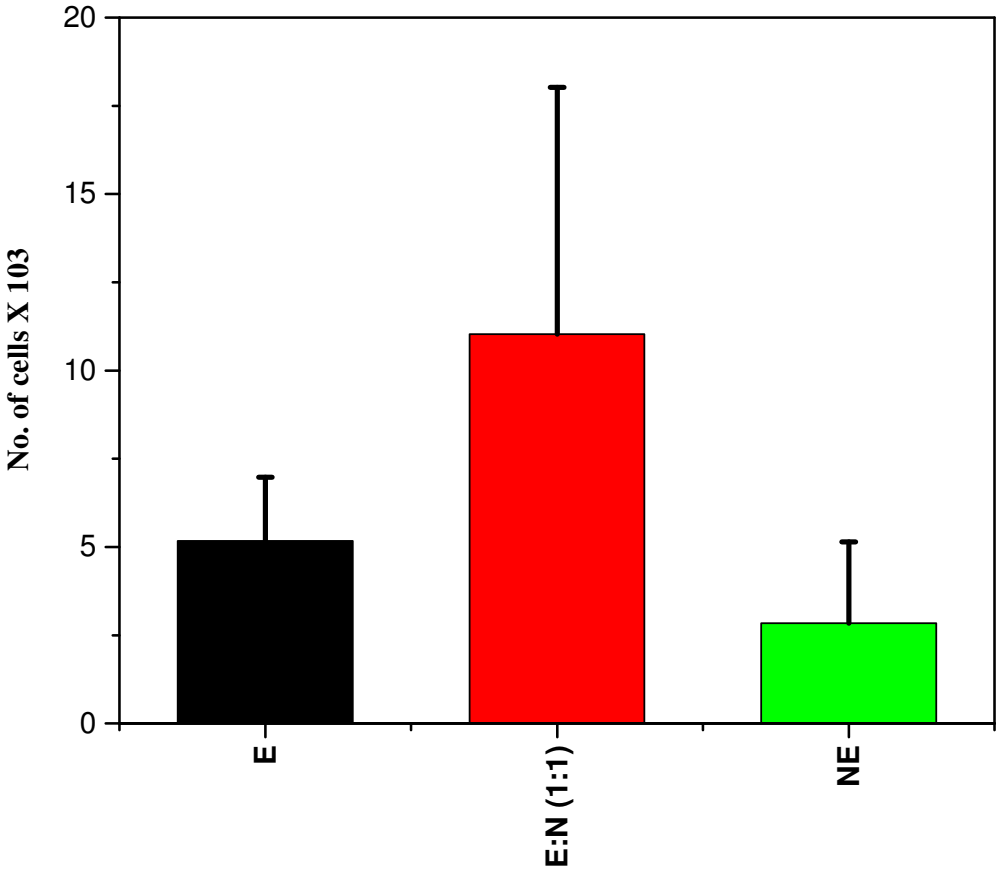


Fig.5.10: MTS assay of ECs cultured on SBS fibrous scaffolds for 7 days with unchanged media.

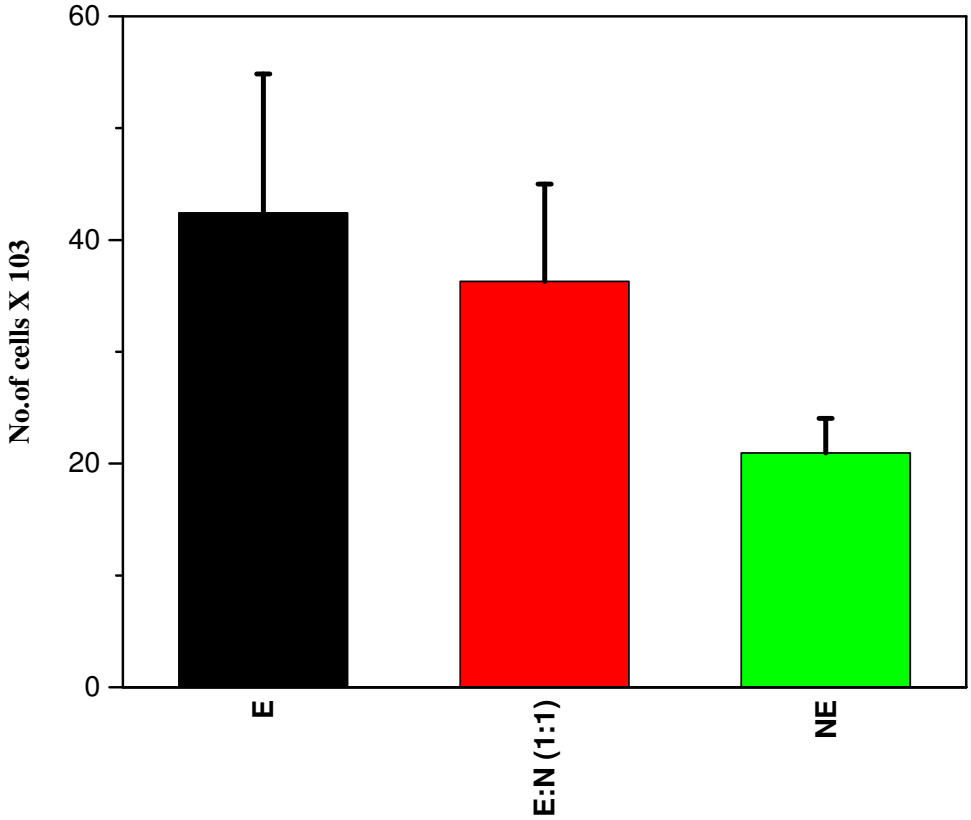


Fig.5.11: MTS assay of ECs cultured on SBS fibrous scaffolds for 7 days with changed media.

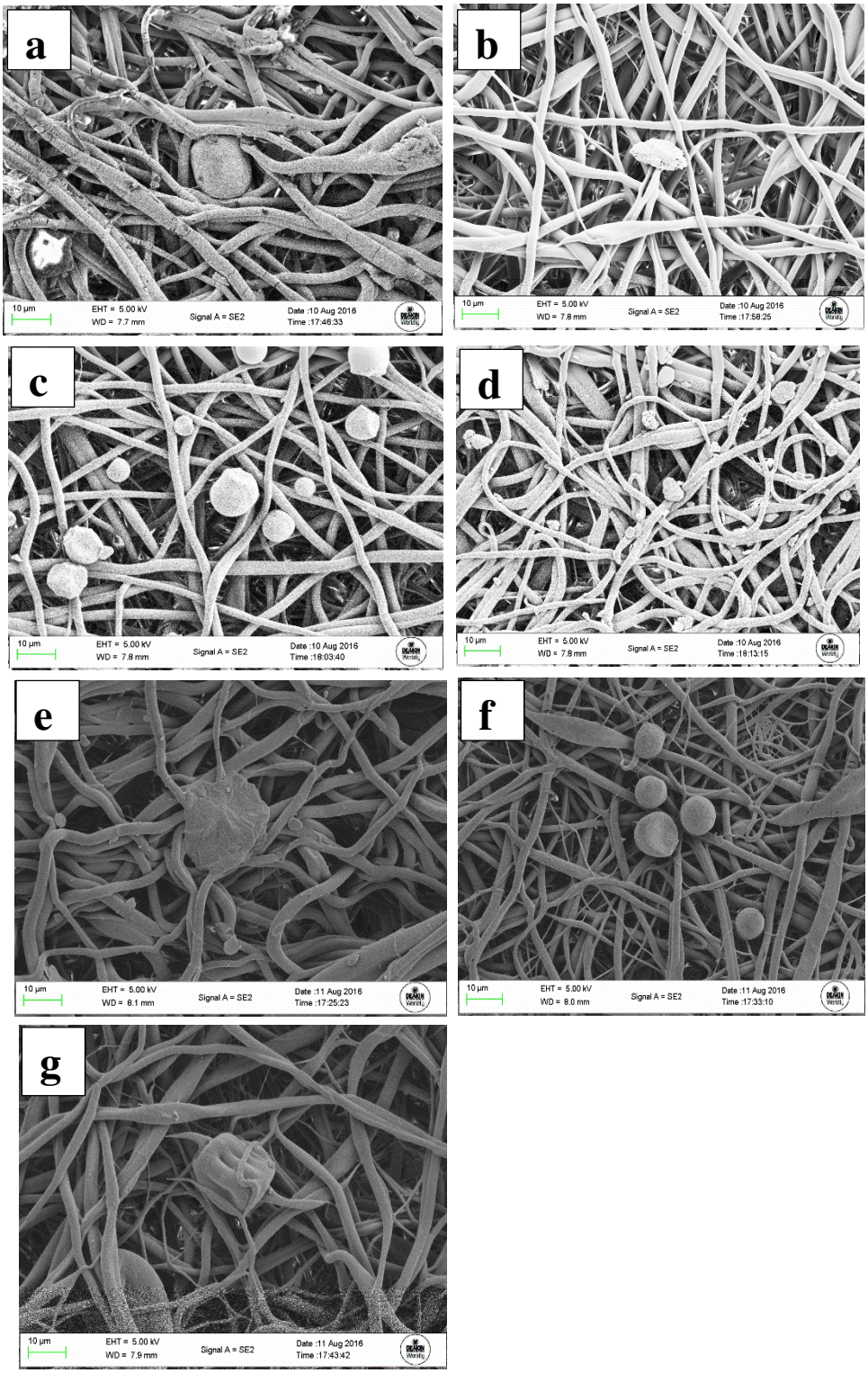


Fig.5.12: SEM images of ECs cultured on SBS fibrous scaffolds for 7days with unchanged media: (a) E, (b) E: N (4:1), (c) E: N (3:2), (d) E: N (1:1), (e) E: N (2:3), (f) E: N (1:4) and (g) NE.

5.3.4. ECs behaviour characterization

5.3.4.1. ECs attachment

5.3.4.1.1. MTS assay of ECs cultured on SBS fibrous scaffolds.

For ECs attachment study, HUVECs were seeded on elastic SBS, nonelastic SBS blended elastic SBS, nonelastic SBS fibrous scaffolds at the density of $10x10^3$ cells/cm^2 (70% confluent). After 3h of ECs seeding, unattached cells were washed out by PBS and living cells were quantified by MTS assay. Attachment of ECs to elastic SBS, nonelastic SBS blended elastic SBS, nonelastic SBS fibrous scaffolds is shown in fig.5.13. It is clear from MTS assay study that ECs are able to attach to the surface of the whole fibrous scaffolds in 3h without significant difference ($p > 0.05$).

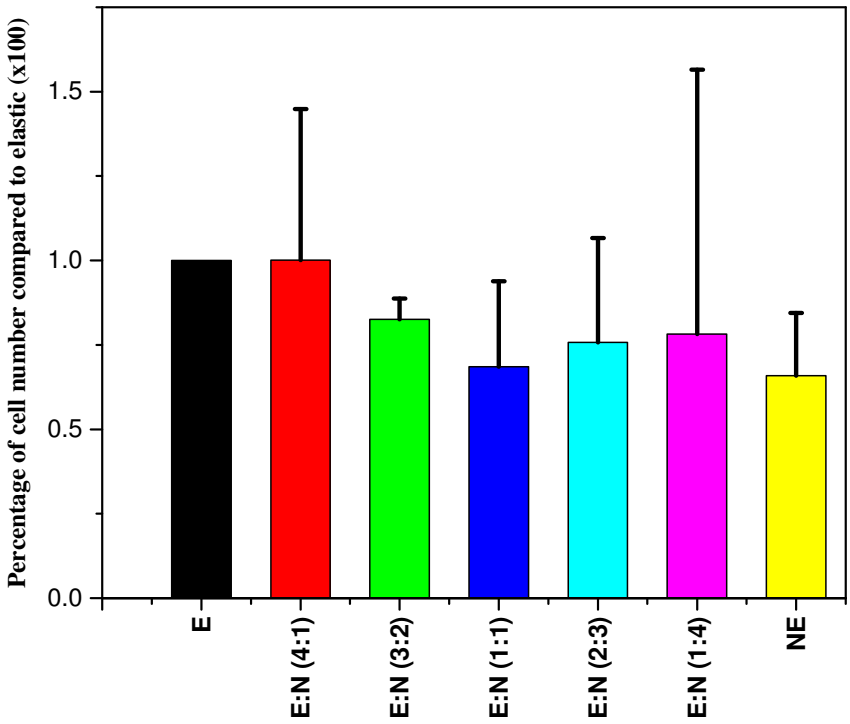


Fig.5.13: MTS assay of ECs cultured on SBS fibrous scaffolds for 3h.

5.3.4.1.2. Morphology of ECs attached on SBS fibrous scaffolds.

The morphologies of ECs attached to the surface of elastic SBS, nonelastic SBS blended elastic SBS, nonelastic SBS fibrous scaffolds were studied by SEM and confocal microscope (LSCM) as shown in fig.5.14 and fig.5.15. It is obvious that ECs maintained a spreading polygonal shape that is similar to normal ECs morphology. It is also observed that ECs intercommoned well with the surface of SBS fibrous scaffolds regardless of their elasticity and pseudopods of ECs were oriented along SBS fibrous scaffolds. Further, ECs showed a typical shape of motility, i.e.: broad, flat lamella in the direction of migration and ending in a narrow ruffling lamellipodium [133]. Therefore, SBS fibrous scaffolds supports ECs to attach and maintain its normal shape in 3h of in vitro culture.

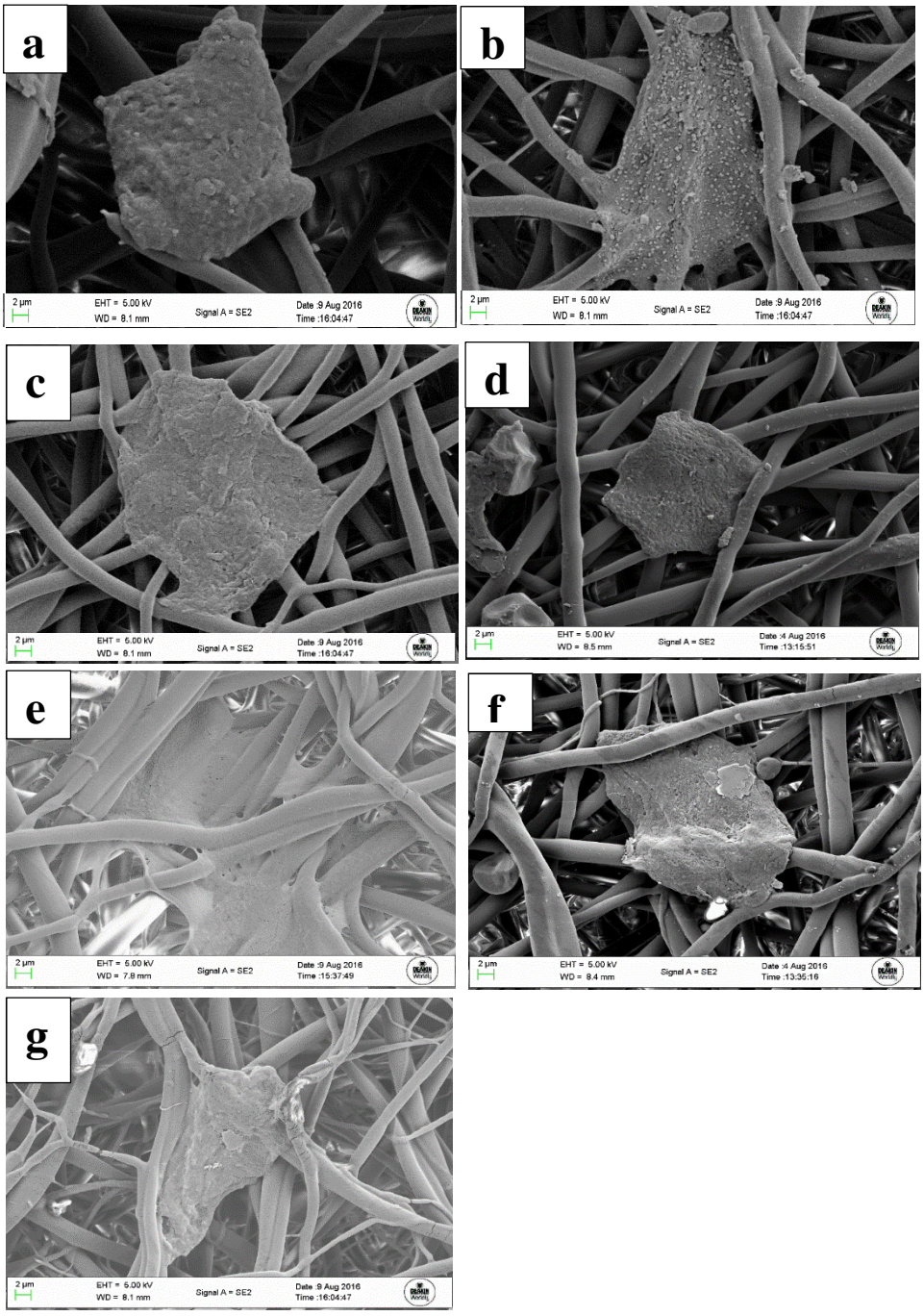


Fig.5.14: SEM images of ECs cultured on SBS fibrous scaffolds for 3h (a) E, (b) E:N (4:1), (c) E:N (3:2), (d) E:N (1:1), (e) E:N (2:3), (f) E:N (1:4) and (g) NE.

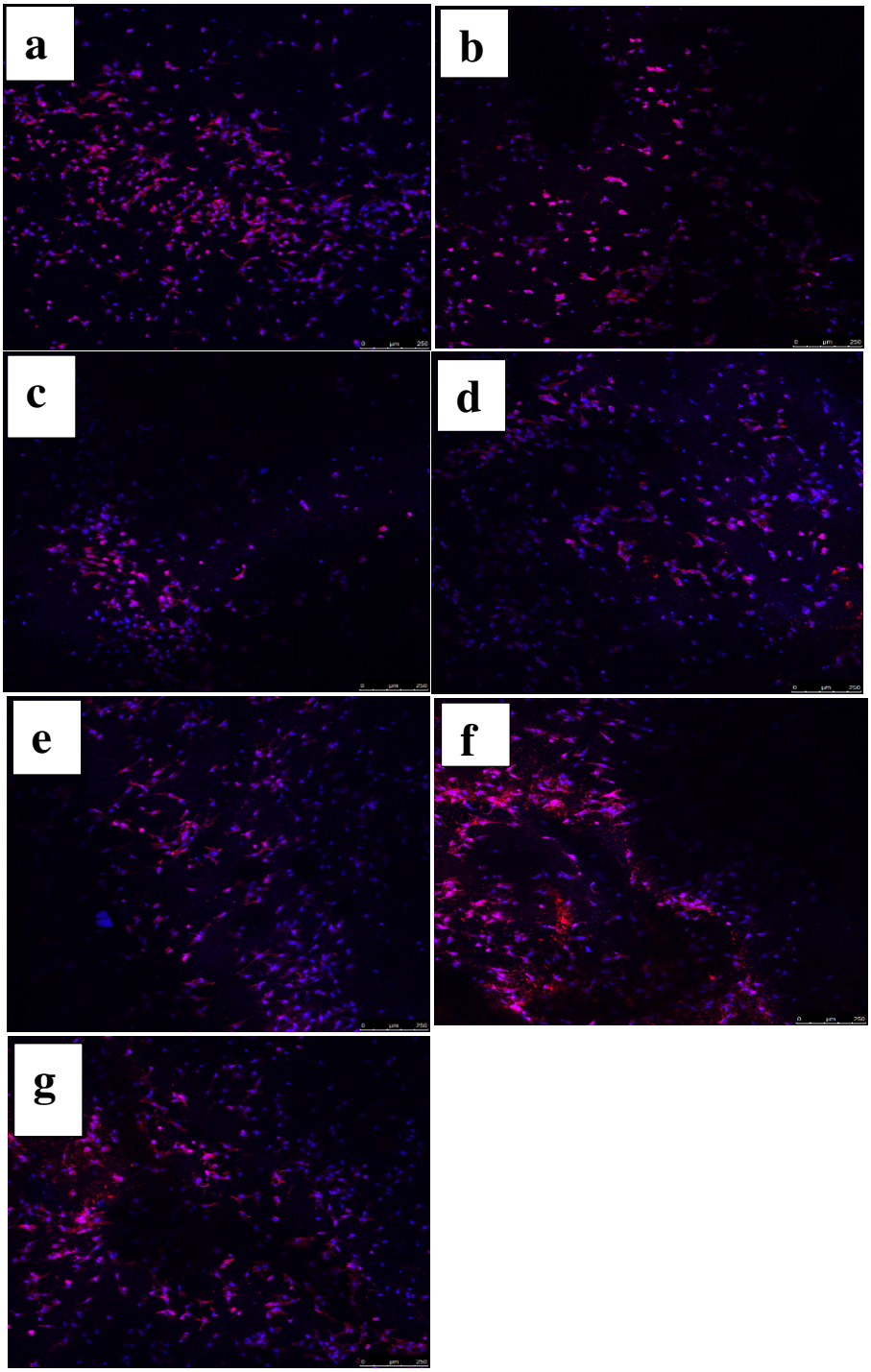


Fig.5.15: LSCM images of ECs cultured on SBS scaffolds for 3 h: (a) E, (b) E: N (4:1), (c) E: N (3:2), (d) E: N (1:1), (e) E: N (2:3), (f) E: N (1:4) and (g) NE.

5.3.4.2. ECs proliferation

5.3.4.2.1. MTS assay of ECs cultured on SBS fibrous scaffolds

For cellular proliferation study, HUVECs were seeded on elastic SBS, nonelastic SBS blended elastic SBS, nonelastic SBS at a density of $10X10^3$ cells/cm^2 (70 % confluence). After culturing for 3 and 7 days, unattached cells were washed out by PBS and viable cells were quantified by MTS assay. Fig.5.16 and fig.5.17 show the viability of HUVECs cultured on elastic SBS, nonelastic SBS blended elastic SBS with different ratios (E:N (4:1), E:N (3:2), E:N (1:1), E:N (2:3), E:N (1:4)) and nonelastic SBS on days 3 and 7 after seeding. ECs viability increased on SBS fibrous scaffolds from day 3 to day 7 indicating that SBS fibrous scaffolds are able to support ECs not only in terms of adhesion but also in terms of proliferation.

Perhaps, it is crucial to adjust weight ratios of elastic SBS to nonelastic SBS for optimal cell growth. From fig.5.17, it can be seen that nonelastic SBS-to-elastic SBS weight ratio of 1:1 and 2:3 imply better ECs viability than pure nonelastic SBS and elastic SBS. This indicates that ECs growth may be supported by certain elasticity of SBS fibrous scaffolds that can be achieved at weight ratio of 1:1 and 2:3.

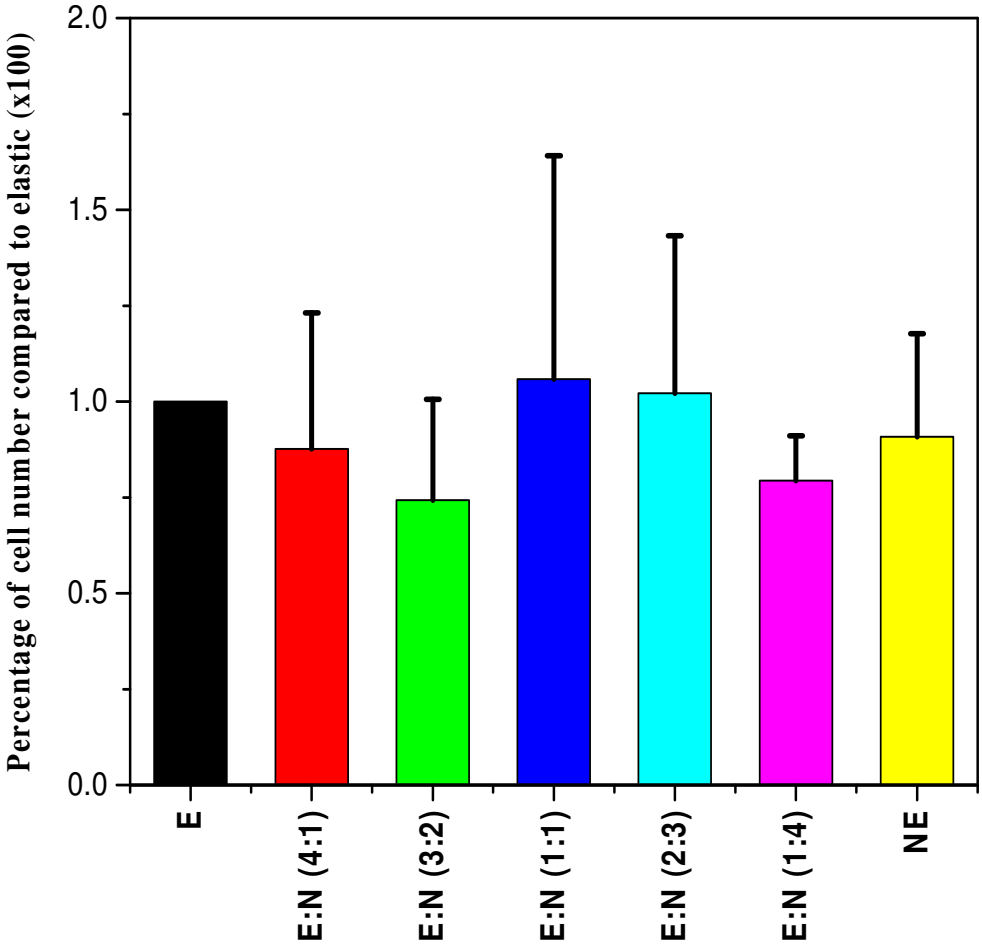


Fig.5.16: MTS assay of ECs cultured on SBS fibrous scaffolds for 3days.

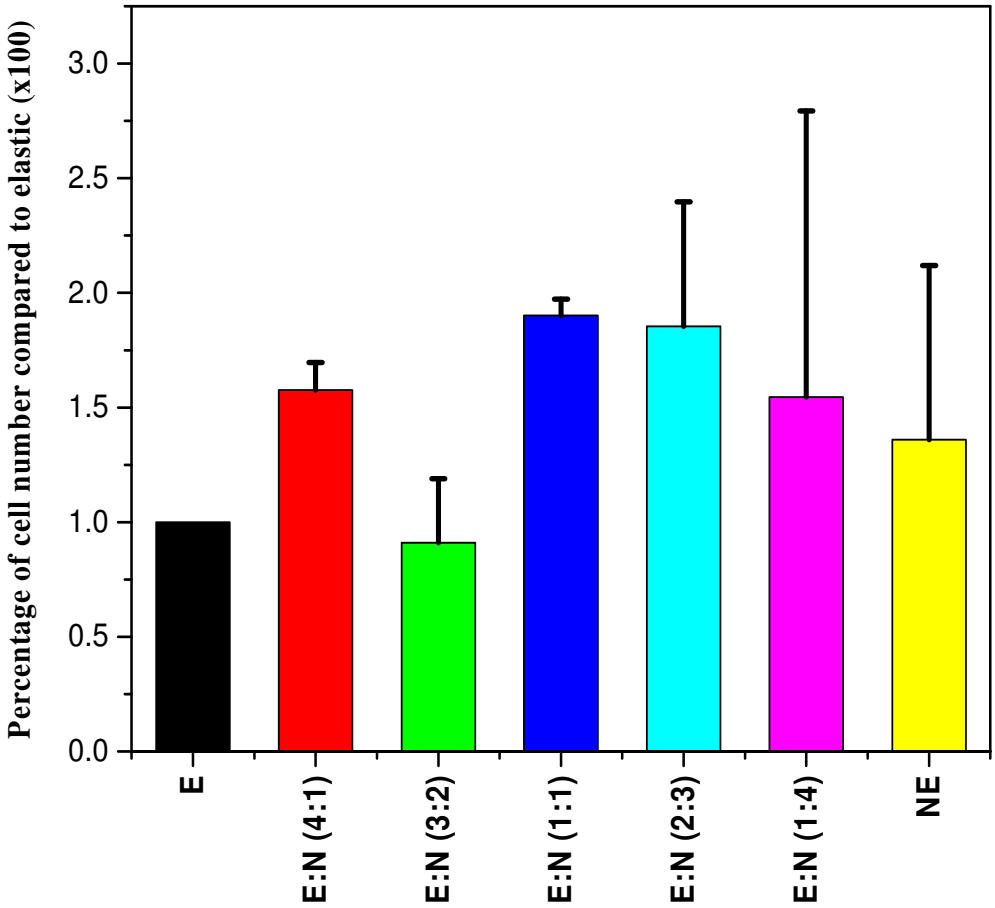


Fig.5.17: MTS assay of ECs cultured on SBS fibrous scaffolds for 7 days.

5.3.4.2.2. Morphology of ECs cultured on SBS fibrous scaffolds.

HUVECs were seeded on elastic SBS, nonelastic SBS blended elastic SBS, nonelastic SBS at a density of $10X10^3$ cells/cm^2 (70 % confluence) and observed on day 3 and 7 under SEM and confocal microscope (LSCM) (fig.5.18, fig.5.19 and fig.5.20, fig.5.21). An increase in the number of ECs can be clearly seen from day 3 to day 7. The majority of the surface of SBS fibrous scaffolds is covered by ECs at day 7. ECs showed phenotypic spreading on both day 3 and 7. It is also observed that, ECs interconnected well with the fibrous scaffolds at day 3 and 7 regardless of their elasticity, oriented along the fibrous structure and maintained a typical shape of motility [133].

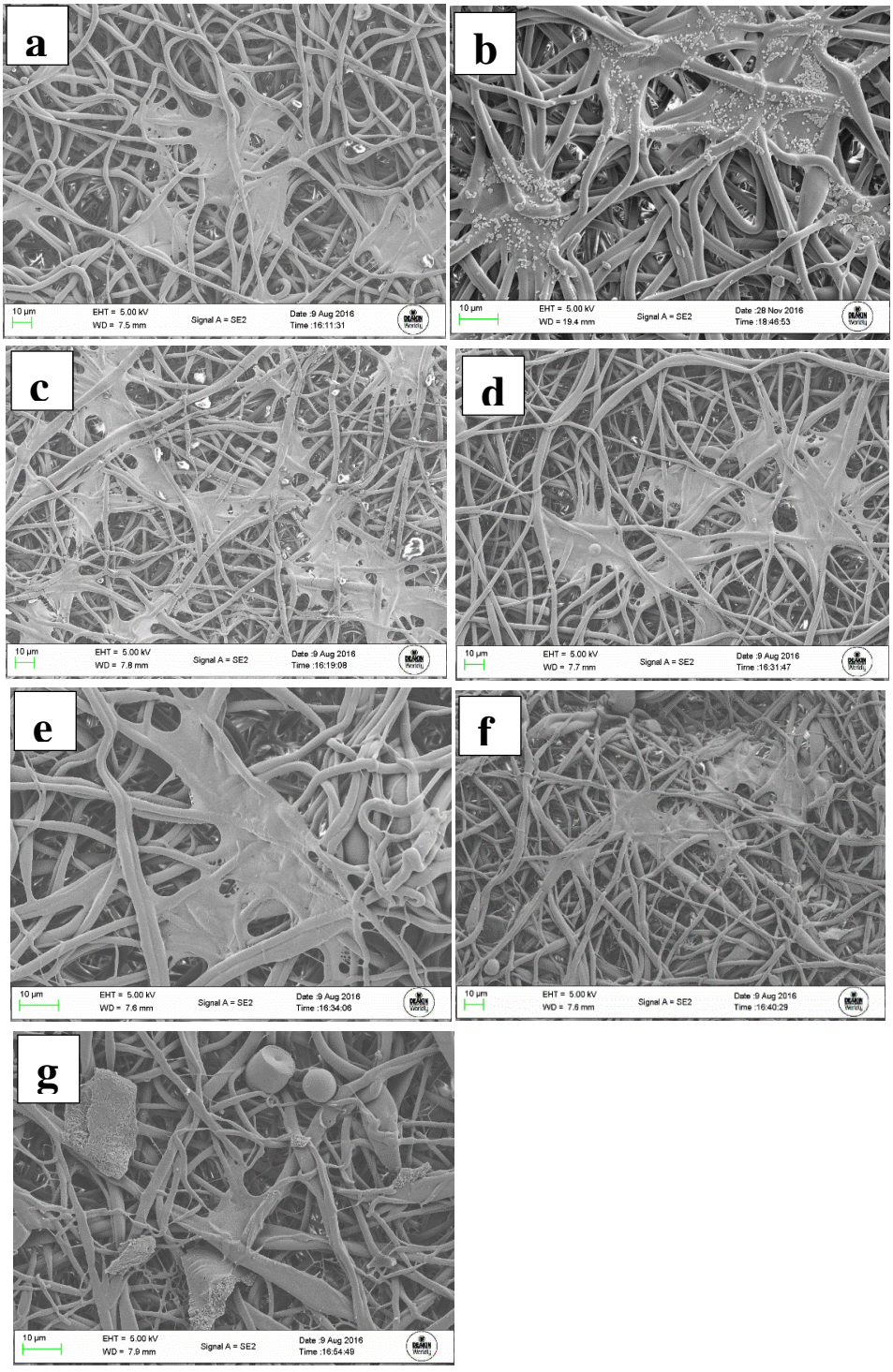


Fig.5.18: SEM images of ECs cultured on SBS fibrous scaffolds for 3days (a) E, (b) E: N (4:1), (c) E: N (3:2), (d) E: N (1:1), (e) E: N (2:3), (f) E: N (1:4) and (g) NE.

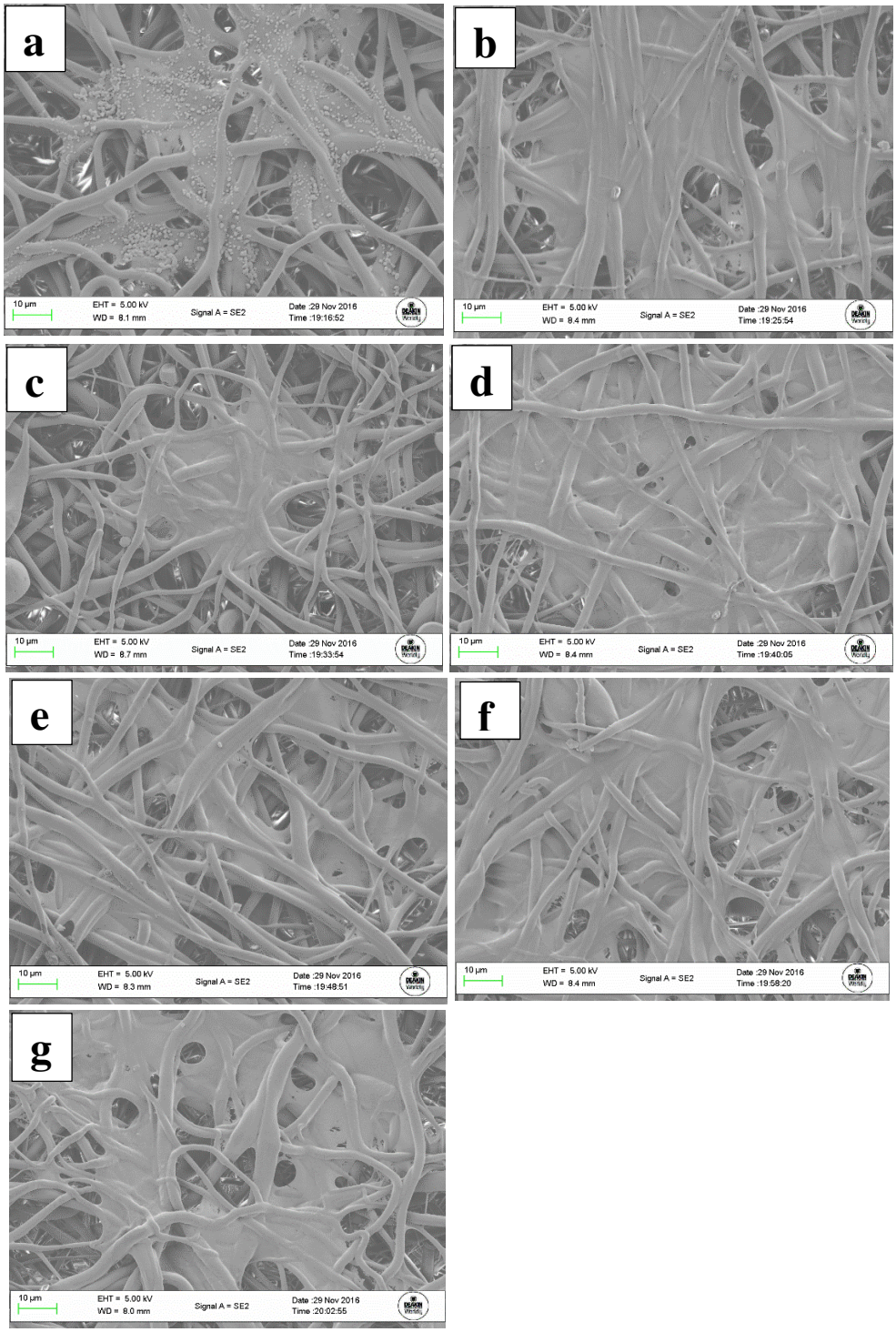


Fig.5.19: SEM images of ECs cultured on SBS fibrous scaffolds for 7days (a) E, (b) E: N (4:1), (c) E: N (3:2), (d) E: N (1:1), (e) E: N (2:3), (f) E: N (1:4) and (g) NE.

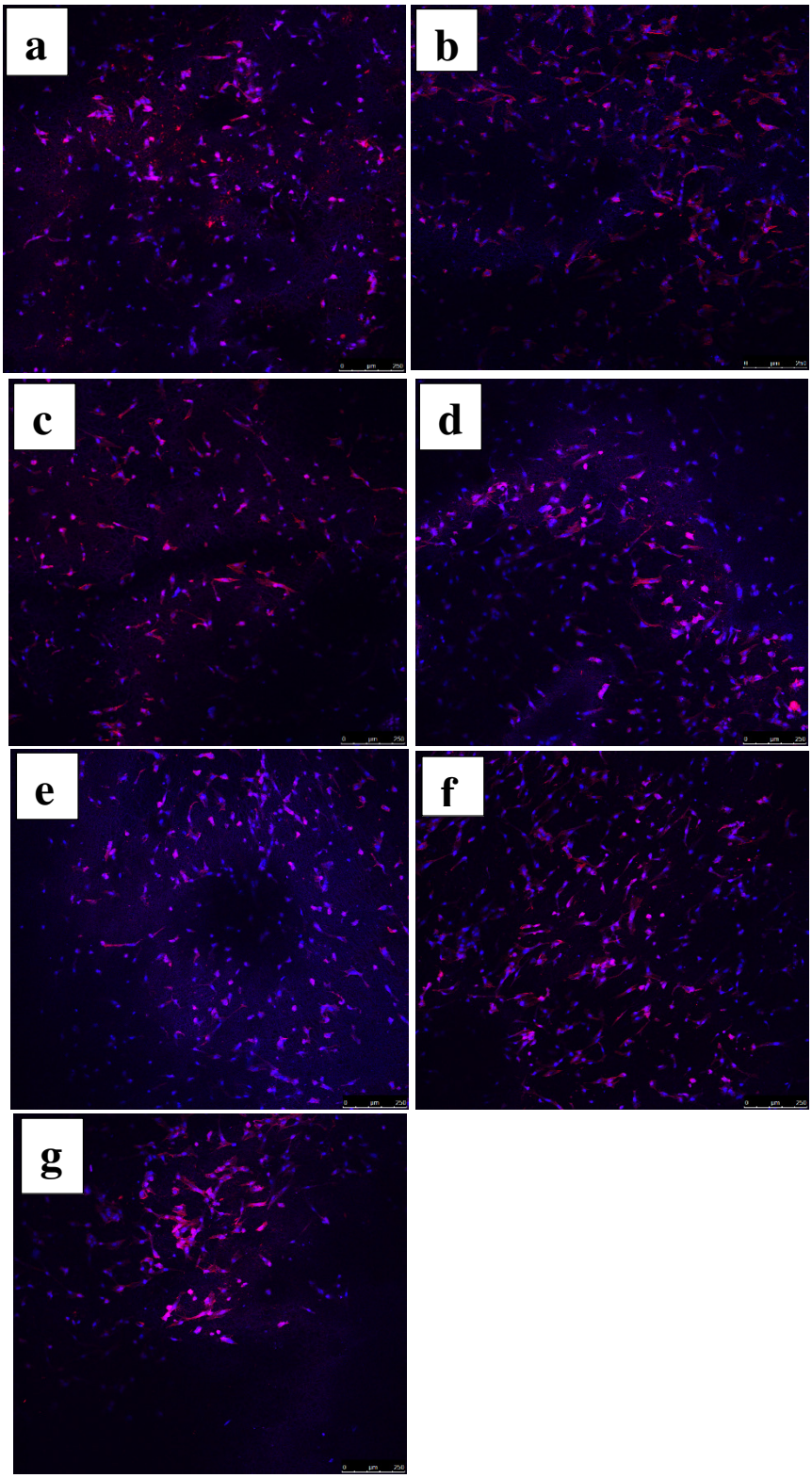


Fig.5.21: LSCM images of ECS cultured on SBS scaffolds for 3 days: (a) E, (b) E: N (4:1), (c) E: N (3:2), (d) E: N (1:1), (e) E: N (2:3), (f) E: N (1:4) and (g) NE.

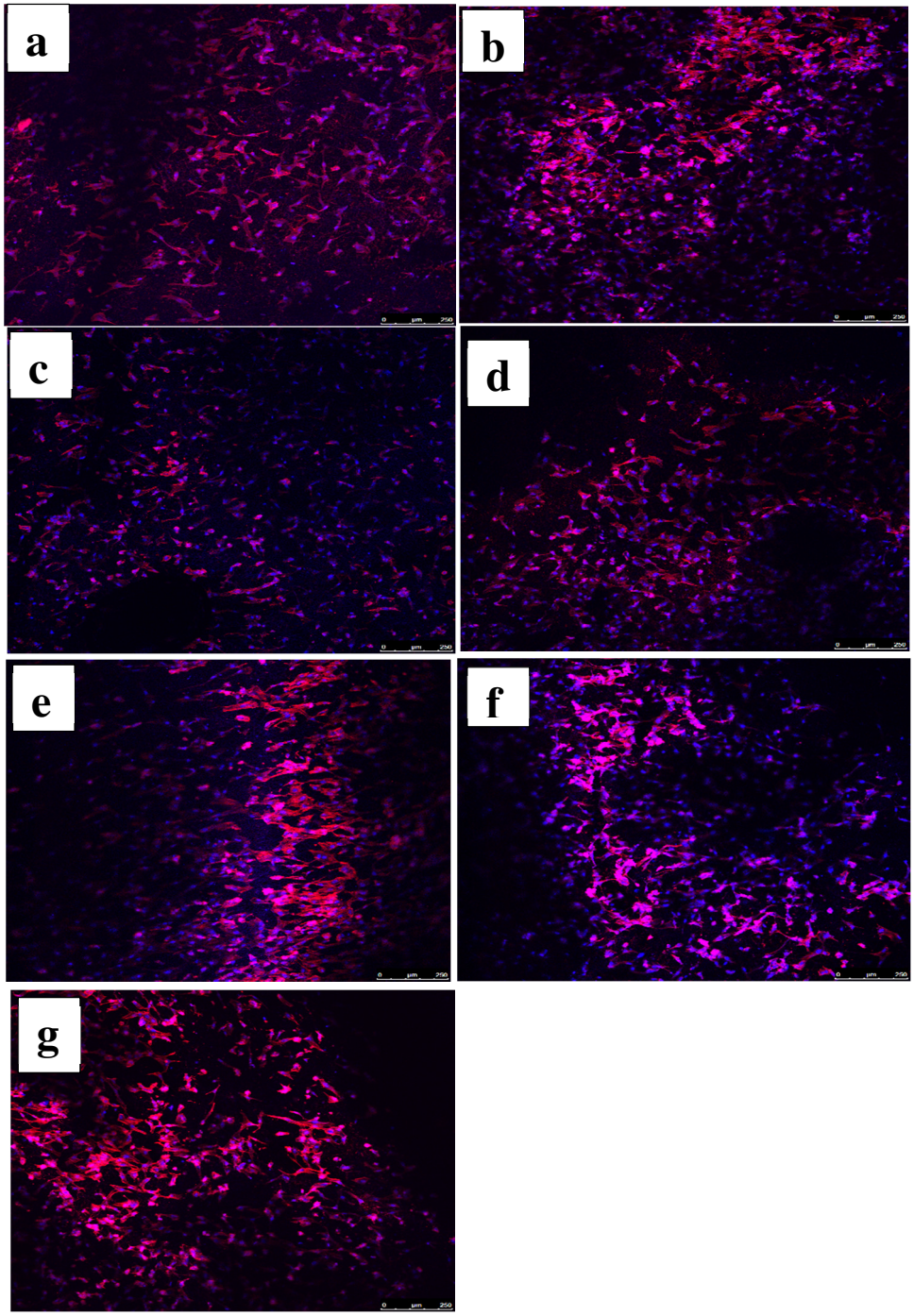


Fig.5.21: LSCM images of ECS cultured on SBS scaffolds for 7 days: (a) E, (b) E: N (4:1), (c) E: N (3:2), (d) E: N (1:1), (e) E: N (2:3), (f) E: N (1:4) and (g) NE.

5.4. Conclusion

Air plasma treatment showed beneficial role to improve the surface wettability of SBS fibrous scaffolds converting the hydrophobic nature of SBS fibrous scaffolds to hydrophilic. The hydrophilicity of the surfaces helped ECs to attach and proliferate on SBS fibrous Scaffolds. Prior to carrying out in vitro cell culture, cytotoxicity study is essential to ascertain whether SBS fibrous scaffolds have toxic influence on ECs or not since endothelialisation of SBS fibrous scaffolds has not been studied before. SBS fibrous scaffolds showed no cytotoxic effect on ECs and comparable cell viability to the positive control. ECs in vitro cell culture on SBS fibrous scaffolds over 7 days showed attachment of cells at 3h which led to an increase in ECs growth. Controlling the elasticity of SBS fibrous scaffolds though blending nonelastic SBS to elastic SBS at weight ratios of 1:1 and 2:3 led to enhanced cell growth from day 3 to day 7 and appeared to produce one of the highest cell numbers compared to elastic SBS.

Chapter.6

Conclusion and future work

The scope of this research contains fabrication of fibrous scaffolds from elastic SBS, nonelastic blended elastic SBS at different ratios (E: N (4:1), E: N (3:2), E: N (1:1), E: N (2:3) and E: N (1:4)), nonelastic SBS and constructing tissue engineered ECM by hybridizing the scaffolds and endothelial cells (ECs).

6.1. Main conclusion

Fabrication of fibrous sacffolds of elastic SBS, nonelastic SBS blended elastic SBS at different ratios (E: N (4:1), E: N (3:2), E: N (1:1), E: N (2:3) and E: N (1:4)), and nonelastic SBS was successfully achieved. SBS fibrous scaffolds maintained uniform fibrous structure at 17 wt%. The obtained averaged diameter of SBS fibrous scaffolds is 2 µm. Chemical composition, crystallography and thermal properties of the SBS blend is similar to pure elastic and nonelastic SBS fibrous scaffolds. The SBS fibrous scaffolds have an average pore size of 8±0.01 µm and are permeable to air. It was possible to tune the mechanical properties of SBS polymer through blending elastic SBS to nonelastic SBS while keeping the same chemical structure of unblended SBS.

SBS fibrous scaffolds maintained hydrophobic nature and it is essential to be treated by air plasma to become hydrophilic. The hydrophilicity of the surfaces helped ECs to attach and proliferate on SBS fibrous Scaffolds. SBS fibrous scaffolds demonstrated no cytotoxic effect on ECs and the viability of ECs on SBS fibrous scaffolds is comparable to positive control. ECs were able to attach and

proliferate on SBS fibrous scaffolds regardless of their elasticity when cell cultured is carried out for 3 h, 3days and 7 days. The typical polygonal morphology of ECs were maintained when cultured on SBS fibrous scaffolds and they are oriented along the fibrous scaffolds. Blending nonelastic SBS to elastic SBS at weight ratio of 1:1 and 2:3 demonstrated better cell viability compared to elastic SBS. SBS fibrous scaffolds demonstrated a new cell surface structure that can support ECs adhesion and spread and it paves the way for various tissue engineering application.

6.2. Limitations

What is left in this study is that some experiment designs are not constructed to optimal level. Firstly, only one cell type, i.e. human umbilical vein endothelial cells (HUVECs) has been investigated. Other cell types like fibroblast and smooth muscle cells (SMCs) should be tried as they compose the middle and outer layer of native blood vessels structure. Secondly, the effect of tensile strain of SBS fibrous scaffolds on ECs attachment and proliferation should be studied to examine and compare the cellular interaction of ECs and the various SBS scaffolds under static and dynamic conditions. Thirdly, implanting SBS tubular fibrous scaffolds in an animal model should be investigated to demonstrate the integration of the artificial blood vessels with the native blood vessels.

6.3. Future work

Fig.6.1 shows the image of fabricated small diameter blood vessel of SBS that has diameter of 5 mm. Fig c confirmed that the tubular structure of SBS maintains fibrous structure. Tubular form of SBS has been successfully electrospun and isolated from the mandrel. To the best of our knowledge, that is the first time that SBS polymer electrospun in tubular fibrous scaffolds. In the future, the tubular form

of SBS fibrous sacffolds will be tested in a bioreactor to mimic the contraction and dilation of native blood vessel. Further, the effect of cyclic strain of SBS fibrous scaffolds on the viability of ECs will be investigated. An animal trial of tubular SBS fibrous scaffolds will be tried to point out the integration of SBS fibrous scaffolds with the living native blood vessels.

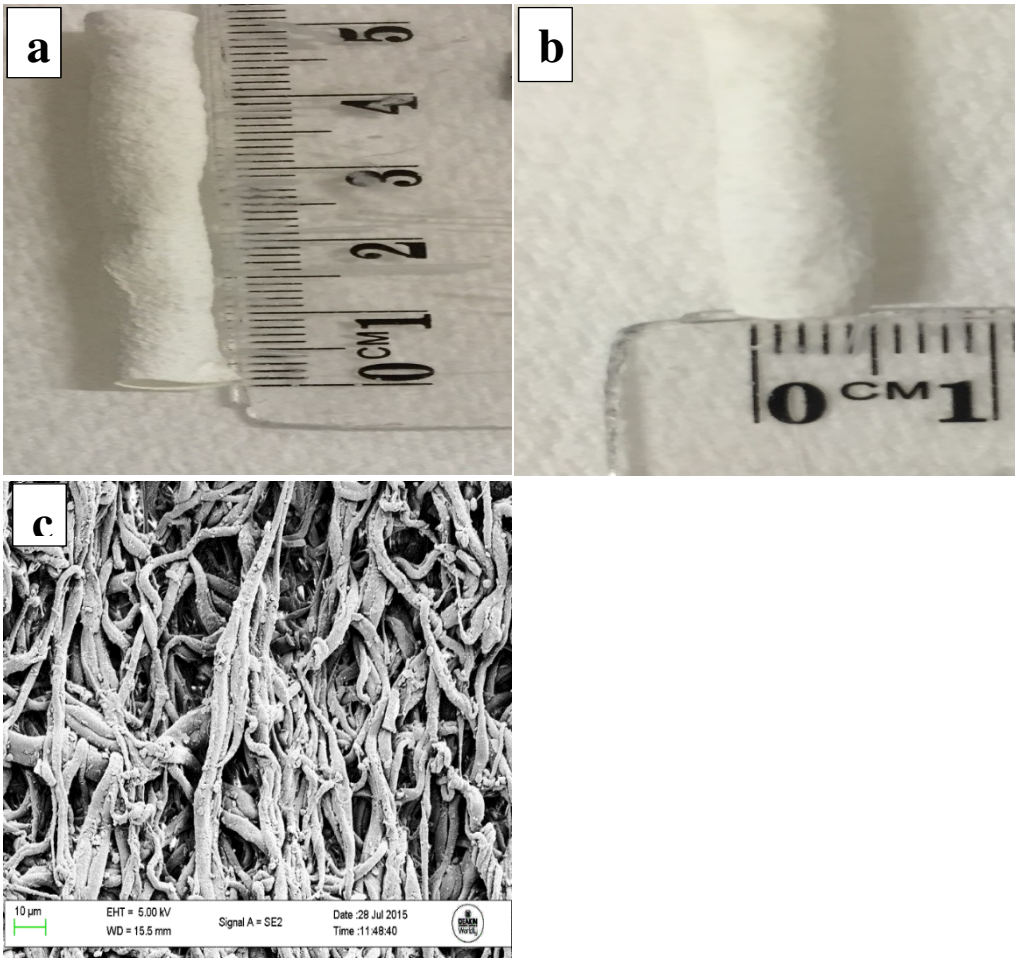


Fig.6.1: (a, b) Macroscopic images of SBS tubular fibrous scaffolds (5 mm diameter) fabricated with 17 wt%, (c) SEM image of the fibrous structure of SBS tube

References

[1] J.P.Stegemann, S.N.Kaszuba and S.L.Rowe. Review: advances in vascular tissue Engineering using protein-based biomaterials. Tissue Eng 13 (2007)2601-2613.

[2] Z.Gong and E.Niklason. Blood vessels engineered from human cells. Trends Cardiovasc Med16 (2006)153-156.

[3] M.B. Browning, D. Dempsey, V. Guiza, S. Becerra, J. Rivera, B. Russell, M. Hook c, F. Clubb, M. Miller, T. Fossum, J.F. Dong, A.L. Bergeron, M. Hahn and E. Cosgriff Hernandez. Multilayer vascular grafts based on collagen-mimetic proteins. Acta Biomaterialia 8 (2012) 1010-1021.

[4] S.L Dahl, A.P.Kypson, J.H.Lawson, J.L Blum, J.T.Strader, Y.Li, R.J.Manson, W.E.Tente, L.DiBernardo, M.T.Hensley, R. Carter, T. P. Williams, H. L. Prichard, M. S. Dey, K. Begelman and L. E. Niklason. Readily Available Tissue-Engineered Vascular Grafts. Sci.Transl. Med.3 (2011) 68ra9.

[5] M.E.De Bakey, G.L. Jordan, J.P.Abbott, B.Halpert and R.M. ONeal.The fate of Dacron vascular grafts. Arch Surg 89 (1964) 757-782

[6] R.D.Sayers, S.Rapti, M. Berce and J.H Miller.Long-term results of femorotibial bypass with vein or polytetrafluoroethylene. Br J Surg 85 (1998)934-938.

[7] Y.Z .Zhang, C.T Lim, Z.M .Huang and S. Ramakrishna. Recent development of polymer nano fibres for biomedical and biotechnological applications. J Mater Sci Mater Med 16 (2005)933-46.

[8] Z.M. Huang, Y.Z.Zhang, M.Kotaki and S.Ramakrishna. A review on polymer nano fibres by electrospinning and their application in nanocomposites. Compos Sci Technol 63 (2003) 2223-2253.

References

[9] R.chen, Y.Morsi, S.Patel, Q.KE and X.Mo.A novel approach via combination of electrospinning and FDM for tri-leaflet heart valve scaffold fabrication. Front.Mater. Sci. China 3(2009)359-366.

[10] A. Owida, R.Chen, S. Patel, Y. Morsi and X. Mo.Artery vessel fabrication using the combined fused deposition modeling and electrospinning techniques.Rapid Prototyping Journal 17(2011) 37- 44.

[11] P.Klinkert, P.N.Post, P.J.Breslau and J.H.van Bocke.Saphenous vein versus PTFE for above knee femoropopliteal bypass. A review of the literature. Eur J Vasc Endovasc Surg 27 (2004)357-362.

[12] C.P.Barnes, S.A.Sell, E.D.Boland, D.G.Simpson and G.L.Bowlin. Nano fibre technology: designing the next generation of tissue engineering scaffolds. Adv Drug Deliv Rev 59(2007)1413-1433.

[13] A.Ndreu, L. Nikkola, H.Ylikauppila, N.Ashammakhi and V.Hasirci. Electrospun biodegradable nanofibrous mats for tissue engineering. Nanomedicine 3(2008)45-60.

[14]S.L.Stupp, V.LeBonheur, K.Walker, L.S.Li, K.E.Huggins, M.Keser and A.Amstutz. Supramolecular materials: Self-organized nanostructures. Science 276 (1997) 384-389.

[15]http://www.mheducation.ca/school/learningcentres/file.php/9780070915800/olc2/dl/843355/BIO11-CH12v2.pdf.

[16]A. Ratcliffe.Tissue engineering of vascular grafts. Matrix Biol 19(2000)353-357.

[17] J.P.Stegemann, S.N. Kaszuba and S.L.Rowe. Review: advances in vascular tissue engineering using protein-based biomaterials. Tissue Eng 13(2007)2601-2613.

[18]D.L.Donovan, S.P.Schmidt, S.P.Townshend, G.O.Njus and W.V.Sharp. Material and structural characterization of human saphenous vein. J Vasc Surg 12(1990) 531- 537.

[19] M.Stekelenburg, M.C.M. Rutten, L.H.E.H.Snoeckx and F.P.T.Baaijens. Dynamic straining combined with fibrin gel cell seeding improves strength of tissue engineered small diameter vascular grafts. Tissue Eng Part A 15 (2009)1081-1089.

[20]L.Soletti, Y.Hong, J.Guan, J.J.Stankus, M.S. El-Kurdi and D.A. Wagner. A Bilayered elastomeric scaffold for tissue engineering of small diameter vascular grafts. Acta Biomater 6 (2009)110-22.

[21]H.Yamada. Mechanical properties of circulatory organs and tissues. In: Evans FG, editor. Strength of biological materials. New York: Robert E. Krieger (1970)106-137.

[22]Y.C. Fung. Blood flow in arteries. New York: Springer Verlag (1984).

[23] N.L.Heureux, N. Dusserre, G.Konig, B.Victor, P. Keire, T.N. Wight, Ni.A.F. Chronos, A.E. Kyles, C.R. Gregory, G.Hoyt, R.C.Robbins and T.N. McAllister. Human tissue engineered blood vessels for adult arterial revascularization. Nat Med 12 (2006)361-365.

[24] R.E.Shadwick. Mechanical design in arteries. J Exp Biol 202 (1999) 3305-3313.

[25] C.P.Barnes, S.A.Sell, E.D. Boland, D.G.Simpson and G.L.Bowlin.Nano fibre technology: designing the next generation of tissue engineering scaffolds. Adv Drug Deliv Rev 59(2007)1413-1433.

[26] S.Agarwal, J.H.Wendorff and A. Greiner. Use of electrospinning technique for biomedical applications. Polymer 49(2008)5603-5621.

[27] N.Bhardwaj and S.C.Kundu. Electrospinning: a fascinating fibre fabrication technique. Biotechnol Adv 28 (2010) 325-347.

[28] T.J.Sill and H.A.Von Recum. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials 29 (2008)1989-2006.

[29] D.H.Reneker and I.Chun.Nanometre diameter fibres of polymer, produced by electrospinning. Nanotechnology 7 (1996) 216-223.

[30] C.Meechaisue, R.Dubin, P.Supaphol, V.P.Hoven and J.Kohn. Electrospun mat of tyrosine derived polycarbonate fibres for potential use as tissue scaffolding material. J Biomater Sci Polym Ed 2006; 17:1039-56.

[31] U.Boudriot, R.Dersch, A.Greiner and J.H.Wendorff .Electrospinning approaches toward scaffold engineering - a brief overview. Artif Organs 30 (2006)785-792.

[32] S.D.Valence, J.C. Tille, D.Mugnai, W.Mrowczynski, R.Gurny, M. Moller and B. H. Walpoth. Long term performance of polycaprolactone vascular grafts in a rat abdominal aorta replacement model Biomaterials 33 (2012) 38-47.

[33] B. Nottelet, E. Pektok,D. Mandracchia, J.C. Tille,B. Walpoth, R. Gurny and M. Moller. Factorial design optimization and in vivo feasibility of poly (e-caprolactone)-micro- and nano fibre-based small diameter vascular grafts. J Biomed Mater Res A. 15 (2009) 865-75.

[34] J.J.Hu, W.C.Chao, P.Y.Lee and C.H.Huang. Construction and characterization of an electrospun tubular scaffold for small-diameter tissue-engineered vascular grafts: A scaffold membrane approach. Journal of the mechanical behaviour of biomedical materials 13 (2012) 140-155.

[35] C.M. Vaz, S. van Tuijl, C.V.C. Bouten and F.P.T. Baaijens. Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique Acta Biomaterialia 1 (2005) 575-582.

[36] C. H.Mun, Y.Jung, S.H. Kim, H. C. Kim and S. H. Kim. Effects of Pulsatile Bioreactor Culture on Vascular Smooth Muscle Cells Seeded on Electrospun Poly (lactide-co-ecaprolactone) Scaffold. Artificial Organs 37(2013) E168-E178.

[37] H.Inoguchi, I.K.Kwon, E. Inoue, K.Takamizawa, Y.Maehara and T.Matsuda. Mechanical responses of a compliant electrospun poly (L-lactide-co-e-caprolactone) small-diameter vascular graft Biomaterials 27 (2006)1470-1478.

[38] C. H. Mun, Y.Jung, S.H.Kim, S.H.Lee, H.C.Kim, K.Kwon, and S.H. Kim. Nano fibres Three-Dimensional Electrospun Poly (Lactide-Co e-Caprolactone) for Small-Diameter Vascular Grafts. Tissue engineering: Part A18 (2012)1608-1616.

[39] W. He, Z.Hu, A. Xu, R. Liu, H.Yin, J.Wang and S.Wang. The Preparation and Performance of a New Polyurethane Vascular Prosthesis. Cell Biochem Biophys 66 (2013)855-866.

[40] J.P. Theron , J.H. Knoetze , R.D. Sanderson , R. Hunter , K. Mequanint , T. Franz ,P.Zilla and D. Bezuidenhout. Modification, crosslinking and reactive electrospinning of a thermoplastic medical polyurethane for vascular graft applications. Acta Biomaterialia 6 (2010) 2434-2447.

[41] S.Baudis, S.C. Ligon, K. Seidler, G.Weige, C. Grasl, H. Bergmeister, H.Schima and R. Liska. Hard-Block Degradable ThermoplasticUrethane-Elastomers for Electrospun Vascular Prostheses. Journal of polymer science part A: polymer chemistry 50 (2012) 1272-1280.

[42]L. Soletti, Y. Hong, J. Guan, J.J. Stankus , M.S. El-Kurdi ,W. R. Wagner and D. A. Vorp .A bilayered elastomeric scaffold for tissue engineering of small diameter vascular grafts. Acta Biomaterialia 6 (2010) 110-122.

References

[43]P. Uttayarat, A.Perets, M. Li, P.Pimton, S.J. Stachelek, I.Alferiev, R. J. Composto, R. J. Levy and P. I. Lelkes. Micropatterning of three-dimensional electrospun polyurethane vascular grafts. Acta Biomaterialia 6 (2010) 4229-4237.

[44] B. L. Dargaville, C. Vaquette, F. Rasoul, J.J. C.White, J. H. Campbell and A.K. Whittaker .Electrospinning and crosslinking of low-molecular-weight poly (trimethylene carbonate-co-L-lactide) as an elastomeric scaffold for vascular engineering. Acta Biomaterialia 9 (2013) 6885-6897.

[45] O.Mazalevska, M. H.Struszczyk and I. Krucinska. Design of Vascular Prostheses by Melt Electrospinning-Structural Characterizations. J.Appl.Polym.Sci (2013) 779-792.

[46] L. Soffer , X. Wang, X. Zhang, J. Kluge, L.Dorfmann, D. L. Kaplan and G.Leisk. Silk-based electrospun tubular scaffolds for tissue-engineered vascular grafts. J. Biomater. Sci. Polymer 19 (2008) 653-664.

[47] J. Zhou, C.Cao and X.Ma. A novel three-dimensional tubular scaffold prepared from silk fibroin by electrospinning. International journal of biological macromolecules 45 (2009) 504-510.

[48] Z. Wang , Y. Cui , J. Wang , X.Yang , Y. Wu , K.Wang , X. Gao , D.Li , Y. Li , X.L. Zheng, Y. Zhu, D. Kong and Q.Zhao. The effect of thick fibres and large pores of electrospun poly (ε-caprolactone) vascular grafts on macrophage polarization and arterial regeneration. Biomaterials 35 (2014) 5700-5710.

[49] A. A. Salifu, B. D. Nury and C. Lekakou. Electrospinning of Nanocomposite Fibrillar Tubular and Flat Scaffolds with Controlled fibre Orientation. Annals of Biomedical Engineering 39 (2011) 2510-2520.

[50] D. Lamprou, P. Zhdan, F. Labeed and C. Lekakou. Gelatine and gelatine/elastin nanocomposites for vascular grafts: processing and characterisation. J. Biomater. Appl. 26 (2011) 209-226.

[51] M. A .Zulliger, A. Rachev, and N. Stergiopoulos. A constitutive formulation of arterial mechanics including vascular smooth muscle tone. Am. J. Physiol. Heart Circ. Physiol. 287 (2004) H1335-H1343.

[52] G.H.Holzapfel, G. Sommer, C. T. Gasser and P. Regitnig. Determination of layer specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling. Am. J. Physiol. Heart Circ. Physiol. 289 (2005) H2048-H2058.

[53] K. A. McKenna , M.T. Hinds , R. C. Sarao , P.C. Wu, C. L. Maslen ,R. W. Glanville , D. Babcock and K. W. Gregory. Mechanical property characterization of electrospun recombinant human tropoelastin for vascular graft biomaterials. Acta Biomaterialia 8 (2012) 225-233.

[54] W.He, Z.Ma, W. E. Teo, Y. X.Dong, P. A. Robless, T. C. Lim and S. Ramakrishna. Tubular nano fibre scaffolds for tissue engineered small-diameter vascular grafts. J Biomed Mater Res A. 90 (2009)205-216.

[55] T.H. Nguyen, A. R. Padalhin, H. S. Seo and B.T.Lee. A hybrid electrospun PU/PCL scaffold satisfied the requirements of blood vessel prosthesis in terms of mechanical properties, pore size, and biocompatibility. Journal of Biomaterials Science 24 (2013)1692-1706.

[56] K.K.Sankaran, U. M.Krishnan and Swaminathan Sethuraman. Axially aligned 3D nanofibrous grafts of PLA-PCL for small diameter cardiovascular Applications. Journal of Biomaterials Science 25 (2014) 1791-1812.

[57] B.Marelli, M.Achilli, A.Alessandrino, G. Freddi, M.C.Tanzi, S. Fare and D. Mantovani. Collagen-Reinforced Electrospun Silk Fibroin Tubular Construct as Small Calibre Vascular Graft. Macromol. Biosci. 12 (2012) 1566-1574.

[58]W.Fu, Z. Liu1, B.Feng, R.Hu, X.He, H.Wang, M.Yin, H.Haung, H.Zhang and W.Wang. Electrospun gelatin/PCL and collagen/PLCL scaffolds for vascular tissue engineering. International Journal of Nanomedicine 9 (2014) 2335-2344.

[59] S.A.Sell, M.J.McClure, C.P.Barnes, D.C.Knapp, B.H.Walpoth, D.G.Simpson and G.L.Bowlin. Electrospun polydioxanone-elastin blends: potential for bioresorbable vascular grafts. Biomed. Mater. 1 (2006) 72-80.

[60] S. J.Lee, J. J. Yoo, G. J. Lim, A. Atala and J.Stitzel. In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application. Journal of Biomedical Materials Research Part A 83 (2007) 999-1008.

[61] S. J.Lee, J. J. Yoo, G. J. Lim, A. Atala and J.Stitzel. In vitro evaluation of electrospun nano fibre scaffolds for vascular graft application. Journal of Biomedical Materials Research Part A 83 (2007) 999-1008.

[62] S.Wang, Y.Zhang, H.Wang, G.Yin and Z. Dong. Fabrication and Properties of the Electrospun Polylactide/Silk Fibroin-Gelatin Composite Tubular Scaffold. Biomacromolecules 10 (2009) 2240-2244.

[63] S.Wang, Y.Zhang, G.Yin, H.Wang and Z.Dong. Electrospun Polylactide/Silk Fibroin- Gelatin CompositeTubular Scaffolds for Small-Diameter Tissue Engineering Blood Vessels. Journal of Applied Polymer Science 113 (2009) 2675-2682.

[64] P. Uttayarat, A.Perets, M. Li, P.Pimton, S.J. Stachelek, I.Alferiev, R. J. Composto, R.J. Levy and P. I. Lelkes. Micropatterning of three-dimensional electrospun polyurethane Vascular grafts. Acta Biomaterialia 6 (2010) 4229-4237.

[65] C.Huang, R.Chen, Q. Ke, Y. Morsi, K. Zhang and X.Mo. Electrospun collagen-chitosan-TPU nanofibrous scaffolds for tissue engineered tubular grafts. Colloids and Surfaces B: Biointerfaces 82 (2011) 307-315.

[66] A.Yin,K.Zhang, M. J. McClure,C.Huang, J. Wu,J. Fang, X.Mo,G.L. Bowlin, S. S. A. Deyab and M. E.Newehy. Electrospinning collagen/chitosan/poly (L-lactic acid-co-e- caprolactone) to form a vascular graft: Mechanical and biological characterization. Journal of biomedical materials research 101A (2013) 1292-1301.

[67] Y.Hong, S.H.Ye, A.Nieponice, L. Soletti, D.A. Vorp and W. R. Wagner .A small diameter, fibrous vascular conduit generated from a poly (esterurethane) urea and phospholipid polymer blend. Biomaterials 30 (2009) 2457-2467.

[68] M.Centola, A.Rainer, C. Spadaccio, S. D.Porcellinis, J .A .Genovese and M. Trombetta. Combining electrospinning and fused deposition modeling for the fabrication of a hybrid vascular graft. Biofabrication 2 (2010) 014102.

[69] C. D. Gaudio, L.Fioravanzo, M. Folin, F.Marchi, E. Ercolani and A. Bianco. Journal of biomedical materials research B: applied biomaterials 100 B (2012) 1883- 1898.

[70] M.Zhang, K.Wang, Z.Wang, B.Xing, Q.Zhao and D. Kong. Small-diameter tissue engineered vascular graft made of electrospun PCL/lecithin blend. J MaterSci: Mater Med 23 (2012)2639-2648.

[71] P.C.Georges and P.A.Janmey. Cell type-specific response to growth on soft materials. J Appl Physiol 98 (2005) 1547-1553.

[72] H. Inoguchi, T. Tanaka, Y. Maehara and T. Matsud. The effect of gradually graded shear stress on the morphological integrity of a huvec-seeded compliant small-diameter vascular graft Biomaterials 28 (2007) 486-495.

[73] Y. M. Ju, J. S.Choi, A. Atala, J.J. Yoo, S. J. Lee. Bilayered scaffold for engineering cellularized blood vessels. Biomaterials 31(2010) 4313-4321.

[74] S.J. Lee, J.Liu, S.H.Oh, S.Soker, A. Atala and J. J. Yoo. Development of a composite vascular scaffolding system that withstands physiological vascular conditions Biomaterials 29 (2008) 2891-2898.

[75] B.W. Tillman, S.K. Yazdani, S. J.Lee, R. L. Geary, A.Atala and J. J. Yoo. The in vivo stability of electrospun polycaprolactone-collagen scaffolds in vascular reconstruction, Biomaterials 30 (2009) 583-588.

[76] H.Zhang, X. Jia, F. Han, J.Zhao, Y.Zhao, Y. Fan, X.Yuan. Dual-delivery of VEGF and PDGF by double-layered electrospun membranes for blood vessel regeneration. Biomaterials 34 (2013) 2202-2212.

[77] H. Wu, J. Fan, C.C.Chu and J. Wu. Electrospinning of small diameter 3-D nanofibrous tubular scaffolds with controllable nano fibre orientations for vascular grafts. J Mater Sci: Mater Med 21 (2010) 3207-3215.

[78]P.Xiang, M. Li, C. Y. Zhang, D.L.Chen and Z.H.Zhou. Cytocompatibility of electrospun nano fibre tubular scaffolds for small diameter tissue engineering blood vessels. International Journal of Biological Macromolecules 49 (2011) 281- 288.

[79]J.Zhao, H.Qiu, D.L.Chen, W.X.Zhang, D.C.Zhang and M.Li. Development of nanofibrous scaffolds for vascular tissue engineering. International Journal of Biological Macromolecules 56 (2013) 106-113.

[80] D.Srinath, S. Lin, D.K. Knigh, A.S. Rizkalla and K.Mequanint. Fibrous biodegradable L-alanine-based scaffolds for vascular tissue engineering. J Tissue Eng Regen Med 8 (2014) 578-588.

[81] Y.Wang, H.Shi, J. Qiao, Y.Tian, M. Wu, W. Zhang, Y. Lin, Z.Niu and Y. Huang. Electrospun Tubular Scaffold with Circumferentially Aligned Nano fibres for Regulating Smooth Muscle Cell Growth. ACS Appl. Mater. Interfaces 6 (2014) 2958- 2962.

[82] S.Rayatpisheh, D.E. Heath, A.Shakouri, P.O. Rujitanaroj, S.Y.Chew and M. B. C. Park. Combining cell sheet technology and electrospun scaffolding for engineered tubular, aligned, and contractile blood vessels. Biomaterials 35 (2014) 2713-2719.

[83]J.Zhang, H.Qi, H.Wang, P. Hu, L.Ou, S. Guo,J. Li, Y. Che, Y. Yu and D.g Kong. Engineering of Vascular Grafts with Genetically Modified Bone Marrow Mesenchymal Stem Cells on Poly (Propylene Carbonate) Graft. Artif Organs 30(2006)898-905.

[84] W.Zheng , Z.Wang, L. Song, Q.Zhao , J.Zhang , D. Li , S.Wang ,J. Han , X.L.Zheng , Z.Yang and D. Kong. Endothelialization and patency of RGD-functionalized vascular grafts in a rabbit carotid artery model. Biomaterials 33 (2012) 2880-2891.

[85] D. Mugnai, J.C.Tille, W.Mrowczynski, S.D.Valence, X. Montet, M. Moller and B. H.Walpoth. Experimental noninferiority trial of synthetic small-caliber biodegradable versus stable vascular grafts. The Journal of Thoracic and Cardiovascular Surgery c (2013) 400-407.

[86] L. Soletti, A. Nieponice, Y. Hong, S.H.Ye, J. J. Stankus,W. R. Wagner and D.A. Vorp. In vivo performance of a phospholipid-coated biodegradable elastomeric graft for small-diameter vascular applications. Journal of biomedical materials research A 96 (2011) 436-448.

[87] H.Bergmeister, C.Grasl, I.Walter, R.Plasenzotti, M.Stoiber, C. Schreiber, U. Losert, G. Weige and H. Schima. Electrospun Small-Diameter Polyurethane Vascular Grafts: Ingrowth and Differentiation of Vascular Specific Host Cells. Artificial Organs36 (2011)54-61.

[88] H. Bergmeister, C. Schreiber, C. Gras, I. Walter, R. Plasenzotti, M. Stoiber, D. Bernhard and H. Schima. Healing characteristics of electrospun polyurethane grafts with various Porosities. Acta Biomaterialia 9 (2013) 6032-6040.

[89] C. K. Hashi, N.Derugin, R. R. R. Janairo, R. Lee, D.Schultz, J. Lotz and S. Li. Antithrombogenic Modification of Small-Diameter Microfibrous Vascular. Grafts Arterioscler Thromb Vasc Biol 30 (2010)1621-1627.

[90] F.Kuwabara, Y.Narita, A.Y.Ogata, K. Kanie, R. Kato, M.Satake, H. Kaneko, H.Oshima, A. Usui and Yuichi Ueda. Novel Small-Caliber Vascular Grafts with Trimeric Peptide for Acceleration of Endothelialization. Ann Thorac Surg 93 (2012) 156-163.

[91] H.Wang, Y. Feng, H.Zhao, R.Xiao and J. Guo. Biomimetic hemocompatible nanofibrous scaffolds as potential small-diameter blood vessels by bilayering electrospun technique. Advanced Materials Research 306 (2011) 1627-1630.

[92] L.Ye, X.Wu, H.Y. Duan, X. Geng, B. Chen, Y.Q. Gu, A.Y. Zhang, J.Zhang and Z.G. Feng. The in vitro and in vivo biocompatibility evaluation of heparin-poly (caprolactone) conjugate for vascular tissue engineering scaffolds. Journal of biomedical materials research 100A (2012) 3251-3258

[93] R.R. R. Janairo, J.J. D. Henry, B. L.P. Lee, C. K. Hashi, N.Derugin, R.Lee and S. Li. Heparin-Modified Small-Diameter NanofibrousVascular Grafts. IEEE Transaction on nanobiosciences 11 (2012) 22-27.

[94] C.Huang, S.Wang, L.Qiu, Q.Ke, W.Zhai and X.Mo .Heparin Loading and Pre-endothelialization in Enhancing the Patency Rate of Electrospun Small-Diameter Vascular Grafts in a Canine Model. ACS Appl. Mater. Interfaces 5(2013) 2220-2226.

[95] L.U.Guang, C.U.I.S.jun, G.Xue, Y.Lin, C. Bing, F. Z.Guo, Z. Jian and L.Zhi. Design and preparation of polyurethane-collagen/heparin conjugated polycaprolactone double-layer bionic small-diameter vascular graft and its preliminary animal tests. Chin Med J
126 (2013)1310-1316.

[96] Y.Yao, J.Wang , Y.Cui, R.Xu , Z. Wang, J. Zhang , K.Wang ,Y. Li ,Q.Zhao and D. Kong .Effect of sustained heparin release from PCL/chitosan hybrid small-diameter vascular grafts on anti-thrombogenic property and endothelialization. Biomaterialia 10 (2014) 2739-2749.

[97] C. Del Gaudio , E. Ercolani , P. Galloni , F. Santilli , S. Baiguera , L. Polizzi and A. Bianco. Aspirin-loaded electrospun poly (ε-caprolactone) tubular scaffolds: potential small-diameter vascular grafts for thrombosis prevention. J Mater Sci Mater Med 24 (2013) 523-532.

[98] P. Punnakitikashem, D. Truong, J. U. Menon, K. T. Nguyen and Y.Hong. Electrospun biodegradable elastic polyurethane scaffolds with dipyridamole release for small diameter vascular grafts. Acta Biomaterialia 10 (2014) 4.

[99] O'Brien FJ. Biomaterials & scaffolds for tissue engineering. Mater Today 14 (2011) 88- 95.

[100] B.P. Chan and K.W. Leong. Scaffolding in tissue engineering: general approaches and tissue specific considerations. Eur Spine J 17 (2008) S467-79.

[101] T.F. Otero, J.G .Martinez, .J .Arias-Pardilla. Biomimetic electrochemistry from conducting polymers. A review: artificial muscles, smart membranes, smart drug delivery and computer/neuron interfaces. Electrochim Acta 84 (2012) 112-128.

[102] S .Rathbone, P .Furrer, J .Lübben, M .Zinn, S. Cartmell. Biocompatibility of polyhydroxyalkanoate as a potential material for ligament and tendon scaffold material. J Biomed Mater Res-Part A 93 (2010)1391-403.

[103] R .Tzoneva, C .Weckwerth, B .Seifert, M. Behl, M. Heuchel and I Tsoneva. In vitro evaluation of elastic multiblock co-polymers as a scaffold material for reconstruction of blood vessels. J Biomater Sci Polym Ed 22 (2011) 2205-26.

[104] International Conference on Thermoplastic Elastomers RTL. TPE 2004: the seventh international conference on new opportunities for thermoplastic elastomers: Brussels, Belgium, 15-16 September 2004. Shrewsbury (UK): Rapra Technology Ltd.; 2004.

[105] S .Agarwal, J.H. Wendorff and A .Greiner. Use of electrospinning technique for biomedical applications. Polymer 49 (2008) 5603-21.

[106] H. Zhou, H.Wang, H. Niu and T. Lin. Electrospun Fibrous Membranes with Super- large- strain Electric Superhydrophobicity. Sci. Rep. 5 (2015) 1-9.

[107] S .Hölzer, M .Ganß, K .Schneider, K. Knoll and R .Weidisch. Deformation mechanisms in lamellar S-S/B-S triblock copolymers. Eur Polymer J 49 (2013) 261-9.

[108] S. Heijden, K.Bru ycker, R. Simal, F. Prez and K. Clerck. Use of Triazolinedione Click Chemistry for Tuning the Mechanical. Macromolecules 48 (2015) 6474-6481.

[109] P.Bittmann, P. Dittes and W. Muller. Artificial body for a prosthesis.1990. Patent US4932964 A.

[110] B.D. Ratner and Biomaterials Science. An Introduction to Materials in Medicine, third ed., Elsevier, Amsterdam, 2013, ISBN: 9780123746269.

[111] E. Diler , M. Schwarz , R .Nickels, M.D.Menger, C. Beisswenger, C. Meier and T. Tschernig. Influence of external calcium and thapsigargin on the uptake of polystyrene beads by the macrophage-like cell lines U937 and MH-S. BMC.Pharmacol. Toxicol. 15 (2014) 16.

[112] V. Nikolic, S.Velickovic, D. Antonociv and A.Popovic. Biodegradation of starch-graft-polystyrene and starch-graft-poly (methacrylic acid) copolymers in model river water. J. Serb. Chem. Soc. 78 (2013) 1425-1441.

[113] N.Shimpi, M. Borane, S. Mishra1 and M. Kadam. Biodegradation of polystyrene (PS)-poly(lactic acid) (PLA) nanocomposites using Pseudomonas aeruginosa, Macromol. Res. 20 (2012) 181-187.

[114] V.R. Sastri, in: V.R. Sastri (Ed.), Plastics in Medical Devices: Properties, Requirements, and Applications, Elsevier/William Andrew, Amsterdam, 2010, ISBN: 978-0-8155- 2027-6, pp. 108-117.

[115] J.M. Yang, J. Yang and H.T. Huang. Chitosan/polyanion surface modification of styrene- butadiene-styrene block copolymer membrane for wound dressing. Mater. Sci. Eng. C 34 (2014) 140-148.

[116] E.Biazar, M. Heidari, A. Asefnezhad and N.Montazeri. The relationship between cellular adhesion and surface roughness in polystyrene modified by microwave plasma radiation. Int. J. Nanomed. 6 (2011) 631- 639.

[117] S. Bagheri-Khoulenjani and H. Mirzadeh. Polystyrene surface modification using excimer laser and radio-frequency plasma: blood compatibility evaluations. Prog. Biomater. 1 (2012)1-8.

[118] B. Motealleh , P. Zahedi , I. Rezaeian , M. Moghimi , A.H. Abdolghaffari ,M.A. Zarandi . Morphology, drug release, antibacterial, cell proliferation, and histology studies of chamomile-loaded wound dressing mats based on electrospun nano fibrous poly(ε-caprolactone)/polystyrene blends. J. Biomed. Mater. Res. Part B Appl. Biomater. 102 (2013) 977-987.

[119] T. Nitanan , P. Akkaramongkolporn , T. Rojanarata ,T. Ngawhirunpat , P. Opanasopit .Neomycin-loaded poly (styrene sulfonic acid-co-maleic acid) (PSSA-MA)/polyvinyl alcohol (PVA) ion exchange nanofibers for wound dressing materials. Int. J. Pharm. 448 (2013) 71-78.

[120] S. Ribeiro , P. Costa , C. Ribeiro , V. Sencadas , G. Botelho and S. Lanceros-Méndez. Electrospun styrene-butadiene-styrene elastomer copolymers for tissue engineering applications: Effect of butadiene/styrene ratio, block structure, hydrogenation and carbon nanotube loading on physical properties and cytotoxicity Composites: Part B 67 (2014) 30-38.

[121] J. M. Yang and S. C. Tsai. Biocompatibility of epoxidized styrene–butadiene–styrene block. Copolymer membrane. Materials Science and Engineering C 30 (2010) 1151-1156.

[122] T.Sasaki, M. Tanaka and T. Takahashi. Structure of freeze-dried atactic polystyrene from dilute solutions. Polymer 39 (1998) 3853-3857.

[123] A. F .Halasa, G. D. Wathen, W. L. Hsu, B. A. Matrana, J. M. Massie. Relationship between interchain spacing of amorphous polymers and blend miscibility as determined by wide-angle X-ray scattering. J Appl. Polym Sci 43(1991) 183-190.

[124] R. Murugan and S. Ramakrishna. Nano-featured scaffolds for tissue engineering: a review of spinning methodologies.Tissue Engineering 12 (2006) 435-447.

[125]S.B. Munteanu and C. Vasile. Thermal and thermo-oxidative behaviour of butadienestyrene copolymer with different architectures. Polymer Degradation and Stability 89 (2005) 501-512.

[126]M.D. Romero-Sánchez, M.M. Pastor-Blas, J.M. Martín-Martinez. Environmental friendly surface treatments of styrene-butadiene-styrene rubber: Alternative to the solvent-based halogenation treatment. International Journal of Adhesion and Adhesives 25 (2005) 19-29.

[127] M.D .Romero-Sánchez, M.M .Pastor-Blas, J.M. Martín-Martínez and M.J. Walzak. Addition of ozone in the UV radiation treatment of a synthetic styrene-butadiene-styrene (SBS) rubber. International Journal of Adhesion and Adhesives 25 (2005) 358-370.

[128] R. Machado ,A. da Costa , V. Sencadas , C. Garcia-Arévalo ,C.M. Costa , J. Padrão , A. Gomes ,S. Lanceros-Méndez , J.C. Rodríguez-Cabello and M. Casal M. Electrospun silk-elastin-like fibre mats for tissue engineering applications. Biomed Mater 8 (2013) 065009.

[129] G. Ayoub, F. Zaïri, M. Naït-Abdelaziz, J.M. Gloaguen. Modeling the low-cycle fatigue behavior of visco-hyperelastic elastomeric materials using a new network alteration theory: application to styrene-butadiene rubber. J Mech Phys Solids 59 (2011) 473-495.

[130] Z.W. Ma, M. Kotaki, R. Inai and S. Ramakrishna. Potential of nanofiber matrix as tissue-engineering scaffolds. Tissue Engineering 11 (2005) 101-109.

[131] G .Jia , H .Wang, L .Yan, X .Wang, R .Pei, T .Yan. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. Environ Sci Technol 39 (2005)1378-83.

[132] A. Kroustalli, A.E .Zisimopoulou, S. Koch, L. Rongen and D. Deligianni, S. Diamantouros . Carbon nanotubes reinforced chitosan films: mechanical properties and cell response of a novel biomaterial for cardiovascular tissue engineering. JMater Sci -Mater Med 24 (2013)2889-96.

[133] T. Wittmann and C.M. Waterman-Storer. Cell motility: can RhoGTPases and microtubules point the way? . J Cell Sci 114 (2001) 3795-3803.

Optimizing Assistive Technologies for Aging Populations

Yosry S. Morsi
Swinburne University of Technology, Australia

Anupam Shukla
ABV – Indian Institute of Information Technology and Management Gwalior, India

Chandra Prakash Rathore
Oracle India Private Limited, India

A volume in the Advances in Medical Technologies and Clinical Practice (AMTCP) Book Series

Medical Information Science Reference
An Imprint of IGI Global

Detailed Table of Contents

Section 3
Elderly Healthcare

The section presents novel and cost effective disease diagnosis and health monitoring techniques for elderly healthcare.

Several techniques like detecting cell, filling gaps, dilating gaps, removing border, smoothing the objects, finding structures & extracting large objects have been used. Finally finding the granulometry of tissues in an Image without explicitly segmenting (detecting) each object. Compared to existing multiscale enhancement approaches, images processed with this method appear more familiar to radiologists and naturally close to the original mammogram.

Barring any cancer prevention breakthroughs, the expansion of the aged population will likely increase number of older individuals diagnosed for cancer in the coming decades. Dimensions of the cancer burden and its devastating manner of challenge ahead are inferred in the context of with aging populations to underscore the possible increase that demographic factors may have on the magnitude of the cancer problem for older persons in the future years. Presently the detection procedure is very time consuming and not accurate, in this respect there is a need of more accurate, fast and efficient method through computing technologies. The present research work incorporates quantum computing with clustering algorithm i.e. Shor's algorithm of quantum computing with hierarchical clustering technique. Here adaptation of Shor's algorithm helps to increase accuracy, and hierarchical clustering technique helps to detect the stages of cancer.

About the Contributors

Yosry S. Morsi obtained his MSc, PhD and DIC from Imperial College/University College, London and carried out his initial academic training as a Research Fellow in UCL UK. He has held both industry positions and a range of senior academic positions involving lecturing, research, research supervision, research leadership and industry collaboration. He has also been a member of numerous senior academic committees and responsible for managing his own research group, as well as contributing to the management of his faculty. He is currently leading Bio-Mechanical Engineering and Tissue Engineering labs, and has over 200 publications with an H index of 20. Prof Yos Morsi's area of expertise is in tissue engineering of heart valves, and mathematical modelling and processing. The research into tissue engineering of heart valves in Australia is deemed a niche area of research due to the high complexity of simulating a natural heart valve. The research conducted by this group is ultimately aimed at the creation of living heart valves as replacement entities rather than using the current option of artificial heart valves. Biocompatible and biodegradable materials will be used as scaffolds to reduce the risk of host rejection. Thus far, a polyurethane tri-leaflet heart valve scaffold has been created and the design is constantly refined to achieve the optimal haemodynamic characteristics so as to simulate natural heart valves. Work of the BTE group has been nationally and internationally recognised in the areas of modelling mechanics, biomechanics and soft tissue engineering of heart valves. He has over 150 publications with H Index of 14 and is Member of the Editorial Boards of three international journals.

Anupam Shukla is an Associate Professor in the Information and Communication Technology Department of Indian Institute of Information Technology and Management, Gwalior, India. He has 21 years of teaching experience. His research interest includes Artificial Intelligence, Soft Computing, Biometrics, Bio-Medical Engineering, Bioinformatics Robotics, Animation and Signal processing. He has published around 100 papers in various national and international journals/conferences, authored nine book chapters and a book from CRC press. He is the editor and reviewer for reputed international journals/books/conferences; and also member of program and technical committees at international conferences. He received Young Scientist Award from Madhya Pradesh Government and Gold Medal from Jadavpur University in his post-graduation.

Chandra Prakash Rathore was awarded with a B.E. (Information Technology) in 2005 by Pt. Ravishankar Shukla University, India and M.Tech (Software Engineering) in 2007 by ABV- Indian Institute of Information Technology and Management Gwalior, India. He is currently pursuing Ph.D. (Information Technology Engineering) from Dr. C. V. Raman University, India. He started his career as Assistant Systems Engineer in 2007 at Tata Consultancy Services Limited, India. In 2011, he joined Oracle India

Private Limited, India and currently posted as Senior Member of Technical Staff and responsible for oracle performance tuning. He is Oracle Certified Associate (OCA), Oracle Certified Professional (OCP) and Oracle Certified Expert (OCE). His area of interests include oracle database administration, development and performance engineering, data modeling, data mining, biomedical expert systems design and development, soft computing tools, speech recognition and image recognition.

* * *

Ivor D. Addo is an Adjunct Assistant Professor (IT/Management) and a PhD student at Marquette University, USA. He is also a member of ACM and IEEE. Ivor's research interests are focused on Architectural Design, Affective Computing, as well as Privacy and Security issues in IoT and Sociable Robotic applications. Ivor has a number of published peer-reviewed journal papers, conference papers and book chapters.

Sheikh Iqbal Ahamed is a professor and director of Ubicomp Lab in the department of Math., Stat., and Computer Science at Marquette University, USA. He is also a faculty member of Medical college of Wisconsin, USA. He is a senior member of the IEEE Computer Society and ACM. He completed his Ph.D. in Computer Science from Arizona State University, USA in 2003. His research interests include mHealth, security and privacy in pervasive computing and middleware for ubiquitous/pervasive computing. He has published 100+ peer reviewed journals, conferences and workshop papers including nine best paper/posters. He serves regularly on international conference program committees in software engineering and pervasive computing such as COMPSAC 13, COMPSAC 12, PERCOM 08, and SAC 08. He is the Guest Editor of Elsevier Computer Communications Journal.

Md. Shamsul Arefin has a PhD on developing the next generation ventricular assist device (VAD) system. He specializes in the hemodynamics and structural changes of the human left ventricle (LV) using the fluid structure interaction (FSI), VADs and the diseased LV conditions (Dilated Cardiomyopathy). He completed his PhD from Swinburne University of Technology (SUT), Australia and B.SC from Daffodil International University (DIU), Bangladesh on Electronics & Telecommunication Engineering (ETE). He was a Lecturer for a year in DIU before moving to Australia. Currently, he is a sessional staff in Swinburne University of Technology. His research mainly focuses on the wireless VAD system, diseases LV model and the overall cardiac system. He is a member of Institute of Electrical & Electronics Engineers (IEEE). He lives in Melbourne, Australia.

Nasser K. Awad obtained his B.Sc.degree in Chemistry from Faculty of science at Sohag University, Egypt in 2004. Further, he joined the National Research Centre, Egypt where he pursued his master of science in collaboration with faculty of science at Helwan University, Egypt. Then, he held the position of assistant researcher at National Research Centre, Egypt in 2010. He has published couple of papers on corrosion of Cu and Cu alloys as well as solar fuel production. Currently, he is a PhD student at Swinburne University of Technology, Australia working on Electrospun nanofibers for small-diameter blood vessels.

Subhasis Bhaumik has completed his M.E & Ph.D. in Production Engineering from Jadavpur University, Kolkata, India. Currently he is serving as Professor and Head, Department of Aerospace Engineering & Applied Mechanics and Coordinator, School of Mechatronics & Robotics at Indian Institute of Engineering Science & Technology, Shibpur, Howrah (formerly BESU Shibpur), India. His areas of interest are Mechatronics, Robotics, Industrial Automation, Fluid Power System and Control, CAD/CAM. He is connected to several reputed organizations like Association of Machines & Mechanism, Rehabilitation Council of India, Robotics Society of India and AICTE. He has completed several industrial consultancy and R& D projects sponsored by several reputed Govt. agencies & companies such as L& T, AICTE, DST, SERB, NIOH, SEED etc. He has guided 3 PhD scholars and presently he has 5 PhD scholars working under his supervision. He has published more than 50 research papers in various national and international conferences and journals.

Neera Bhansali is Executive Director, Biomedical Informatics at Integrated Biostatistics and Data management Center, Florida International University, Miami, Florida. In her previous roles she was Head of Informatics at CTNeT, The Statewide Clinical Trials Network of Texas and Director of Data Quality and Standards, at H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida. She received her Masters and PhD in Business from Royal Melbourne Institute of Technology, Melbourne, Australia. She is an expert in the areas of data strategy, data governance and data warehousing. Over past 20 years, she has facilitated transformations and provided strategic direction to organizations in manufacturing, airlines, consulting, media, finance and healthcare industries in Asia, Australia and North America. Her expertise extends to areas of data semantics, data fusion, data integrity and data quality. She has authored/edited books, 'Strategic Data Warehousing: Alignment to Business' and 'Data Governance: Creating Value from Information Assets' published by CRC Press in 2009 and 2013 respectively.

Manuele Bonaccorsi graduated in Biomedical Engineering as cum laude in 2010 at the University of Pisa, Italy. He has been attending PhD courses on biorobotics at the BioRobotics Institute of Sant'Anna School of Advanced Studies in Pontedera, Italy after graduating. His main research activities are Ambient Assisted Living and indoor localization of people using wireless sensor networks to provide user position awareness to robotic platforms.

Paolo Bongioanni, MD, PhD, is Neurologist, Senior Consultant and Head of the *Geriatric Neurorehabilitation Service* in the *Neurological Rehabilitation Operative Unit*, *Neuroscience Department*, *Azienda Ospedaliero-Universitaria Pisana*. Lecturer at the University of Pisa in "*Rehabilitation of Neuromuscular Disease Patients*", "*Fundamentals of Geriatric Rehabilitation*", and "*Cognitive Rehabilitation*" (Degree Course for Physiotherapists); and in "*Neuropsychological Rehabilitation*", "*Geriatric Neurorehabilitation*", and "*Occupational Rehabilitation*" (Post-graduate School of Physical Medicine and Rehabilitation). Research interests: clinical and experimental neurorehabilitation, neuropsychology, neuropsychopharmacology and neuroimmunology. Author/co-author of more than 50 papers in national/international peer-reviewed journals. Founder and President of *NeuroCare onlus*. Coordinator of the *Neurodegenerative Disease Care & Research Multidisciplinary Group*. Member of the *European College of NeuroPsychoPharmacology*.

Filippo Cavallo, MScEE, PhD in Bioengineering, is Assistant Professor at BioRobotics Institute, Sant'Anna School of Advanced Studies, Pisa, Italy, focusing on cloud and social robotics, ambient assisted living, wireless and wearable sensor systems, biomedical processing, acceptability and AAL roadmapping. He participated in National and European projects and currently is project manager of Robot-Era, AALIANCE2 and Parkinson Project. He was visiting researcher at the the EndoCAS Center of Excellence, Pisa; at the Takanishi Lab, Waseda University, Tokyo; at Tecnalia Recerch Center, Spain. He was granted from the International Symposium of Robotics Research Committee as Fellowship Winner for best PhD thesis in Robotics; from the Regional POR FSE 2007-2013 for a 3-years Research position at The BioRobotics Institute; from the ACCESS-IT 2009 for the Good Practice Label in Alzheimer Project; from the Well-Tech Award for Quality of Life with the Robot-Era Project. He is author of various papers on conferences and ICI journals.

Chih-Hung Chang received his BE, MS, and Ph.D degrees in Computer Science from Feng Chia University in 1995 and 1997, 2004 respectively. Currently, he is an associate professor at Hsiuping University of Science and Technology, Taiwan. His research interests include Software Engineering, Software Architecture, Software Requirement Engineering, Object-Oriented Technology, Software Development of Embedded System and Cloud Service. He has published more than 100 papers in various international journals and conferences. He served as program chair for TSA 25014, local arrangement chair for CompSAC 2015, ICS 2014, and SAC 2011, and co-chair for SAC 2011 APGCC Track, WESQA'10, and SQHE'13.

Setu Kumar Chaturvedi has around 14 years of research experience in Data Mining, Text and Web Mining, Data Analytics, Business Intelligence, Quantum Computing, Cloud Computing, Bioinformatics, Information Retrieval and Grid Computing. He has completed Ph.D. in Information Technology from Madhaya Pradesh State Technological University Rajeev Gandhi Prodhoyoki Vishwavidyalaya (RGPV), Bhopal (M.P.), India in 2012. He completed his M.Tech. in Software Systems in 2007 from Samrat Ashok Technological Institute Vidisha affiliated by RGPV, Bhopal (M.P.), India. He has also done MCA in 2004 from GJU Hissar University, Hariyana, India; MIT (Master of Information Technology) in 2002 and Bachelor of Science (Computer Science) in 2000 from Dr. Hari Singh Gour Central University, Sagar (M.P.), India. He has published 46 research papers in various National / International Journals and Conferences. He has guided 40 candidates for M. Tech dissertations. He has membership of IEEE, CSI, ACM's (CSTA), IAENG and IACSIT.

Cheng-Chung Chu is Director of Software Engineering and Technologies Center of Tunghai University, a professor of the Department of Computer Science, He had served as Dean of Engineering College at Tunghai University, Taiwan. From 2008 to 2011, Dean of Research and Development office at Tunghai University from 2004 to 2007, Taiwan. From 1994 to 1998, he was an associate professor at the Department of Information Engineering and Computer Science at Feng Chia University. He was a research scientist at the Software Technology Center of the Lockheed Missiles and Space Company, Inc., where he received special contribution awards in both 1992 and 1993 and a PIP award in 1993. In 1992, he was also a visiting scholar at Stanford University. He is serving as the associate editor for Journal of Software Maintenance and Evolution (JSME), International Journal of Advancements in Computing Technology (IJACT) and Journal of Systems and Software (JSS). His current research interests include software engineering, embedded systems, and E-learning. He received his MS and PhD degrees from

Northwestern University in Evanston Illinois, in 1987 and 1989, respectively, both in computer science. He has edited several books and published over 100 referred papers and book chapters, as well as participating in many international activities, including organizing international conferences, serving as steering committee for COMPSAC, APSEC and the program committee of more than 100 international conferences.

Sara Comai is an associate professor at the Department of Electronics Information and Bioengineering of Polytechnic University of Milan, Italy since March 2007. She received her Ph.D. in Computer Engineering and Automation from Polytechnic University of Milan, Italy in 2000. Her recent research interests include extensions of smart home solutions based on wireless sensor-actuator network for monitoring users' behavior, design of interfaces for people with special needs, methods and tools for the development of haptic-based applications and gestural interaction, the study of presentation issues of Web interfaces, the specification, design and automatic generation of complex Web applications. She is author of more than 100 international papers published in journals and conference proceedings.

Anjali Deshpande is graduated in Science from Devi Ahilya University, India in 1992. She received her Master of Science Degree in Electronics from School of Electronics, Devi Ahilya University, India in 1994. She got Master of Philosophy in Electronics in year 2007. In year 2008, she joined the doctoral program in Electronics at School of Studies in Electronics, Pt. Ravishankar Shukla University, India. While pursuing her research on Correlation between speech features and functioning of human heart, she worked with various educational institutions. She has presented her research on speech signal processing in national and international conferences, workshops. She has published her research in various international and national journals. She has supervised dissertations in the field of signal processing, blood pressure monitoring and ECG signal analysis, embedded systems etc.

Unai Díaz-Orueta is a Psychology Ph.D. graduated at Deusto University, Spain (2006). Since 2000, he worked as a clinical psychologist in a variety of settings, including Crownsville Hospital Center, Maryland (USA, 2000-2001), Bermeo Psychiatric Hospital (Spain, 2001-2002), La Loma Geriatric Residence (Spain, 2003-2005), and Zutitu Ltd (2005-2006). In 2006, he finished his doctoral dissertation "Effects of psychological intervention in cognitive decline of residentialized elderly people". He performed several workshops in maintenance of cognitive functions, wellbeing, and laugh-therapy for older people at IPACE Ltd, Vitoria (Spain, 2007-2008). From 2008-2012, he worked as a research psychologist at Fundación INGEMA, in several projects related to aging and physical disability. Since May 2012, he has been working as a researcher at the R+D+i Department of Nesplora S.L. (Donostia-San Sebastian, Spain), and since May 2014, as a Collaborator Professor for the Neuropsychology and Education Master's Degree of the UNIR -International University of La Rioja (Spain).

Mingchui Dong graduated from EE Dpt. of Tsinghua Univ., Beijing in 1970. Took advanced study at Rome Univ. of Italy in 1979~1981. Joined R&D of National 863/CIMS Project in 1988~1998. The project won USA "1994' SME University LEAD Award", 1st Prize of S&T Progress Award of National Educational Committee, 2nd Prize of National S&T Progress Award of China. As chief designer, he completed the overall design of 6 CIMS engineering projects. Since 1998 he was employed by Univ. of Macau and INESC-Macau, later became the Executive Director of INESC-Macau. He headed and joined successfully the R&D of 51 projects financially supported by International/National Foundations

or Universities, including EUREKA project "Inocompanies" and "SIGORDE" as well as "Machine Auto Translation", "e-Home Healthcare", etc. He had published more than 200 refereed journal/conference papers, supervised 1 post-doctoral fellow, 12 PhD and 35 master students. Recently his research direction focuses to "AI" and "*e*-Home Healthcare".

Dario Esposito received the Master Degree in Electronical Engineering at Federico II University of Naples (Italy) in 2011. Since September 2011 he has been assistant researcher at the BioRobotics Institute of Sant'Anna School of Advanced Studies. His professional expertise concerns the design, development, prototype and engineering of hardware and software solutions related to the implementation of technological ICT systems. His fields of research are focused on ambient assisted living, assistive robotics, wearable devices based on sensor systems and wireless sensor networking. He is author of articles and patents concerning the fields of AAL and wearable devices for application in Parkinson's disease management.

Raffaele Esposito was awarded a M.Sc. in Biomedical Engineering from School of Engineering at University of Pisa, Italy in 2009. To date, he is a research assistant at the BioRobotics Institute of Sant'Anna School of Advanced Studies, Pisa, Italy. His main research interests are in the fields of usability, acceptability and dependability of Ambient Assisted Living (AAL) and Service Robotics solution to prevent, support and enhance the quality of life of senior citizens.

Laura Fiorini is a PhD Fellow at BioRobotics Institute of Sant'Anna School of Advanced Studies, Pisa, Italy. She was awarded M.Sc. in Biomedical Engineering (with honours) from the School of Engineering at University of Pisa, Italy in 2012. In her thesis she designed and implemented a wireless endoscopic capsule with vision system for lower gastrointestinal bleeding. Her PhD Fellowship, funded by Telecom Italia, is focused on the design of Cloud Robotic solution for Ambient Assisted Living (AAL) applications. Her main research interests are in the fields of Ambient Assisted Living (AAL) and Service Robotics to prevent, support and enhance the quality of life of senior citizens.

Md. Osman Gani was born in Feni, Bangladesh, in 1986. He received his B.Sc. degree in Computer Science and Engineering from the Military Institute of Science and Technology (MIST), Dhaka, Bangladesh, in 2008, and his M.Sc. degree in Computational Sciences from Marquette University, USA, in 2013. He is currently pursuing his Ph.D. in Computational Sciences at Marquette University. In 2009, he joined the Department of Computer Science and Engineering, MIST, as a Lecturer and served there for two and a half years. His current research interests include pervasive computing, machine learning, and indoor localization.

Milan Jain is an M.Tech. Research Scholar in Department of Computer Science and Engineering at Technocrats Institute of Technology, Bhopal (M.P.), India affiliated by Rajiv Gandhi Proudyogiki Vishwavidyalaya (RGPV), Bhopal (M.P.), India. She completed her Bachelor of Engineering from RITS, Bhopal (M.P.), India affiliated by RGPV, Bhopal (M.P.), India in 2011. Her interested research areas are Quantum Computing, Cloud Computing, and Data Mining.

Sebin Jung is a Design Responsible Engineer and Coach of the Design for Six Sigma for the Interior Engineering Division of the General Motors Korea. She designs and develops automotive interior systems and components, and effectively identifies and communicates conditions that affect overall vehicle performance and appearance. Sebin received her Bachelor of Business Administration in Design and Management with honours at Parsons the New School for Design, New York and a Masters in Integrated Product Design at Brunel University, London. She currently holds the PhD Electrical Engineering and Electronics at Brunel University, focusing on human need and a design research approach that integrates a human-centred design investigation with the technological or systems dimensions enabled by design thinking.

Philip Kinsella graduated with a BEng (Honours) in mechanical engineering in 2012 and is currently pursuing a multidisciplinary PhD in engineering and design at Swinburne University of Technology (Melbourne, Australia). His experience as a mechanical engineer is working with 3D design software focusing on the design and construction of systems for transportation of highly sensitive goods across difficult terrain. Philip's current research focuses on the customization of devices from 3D scan data, particularly relating to the complexities in obtaining and automatically processing 3D scans of the human anatomy with the ultimate goal of automating design of medical devices from 3D scan data.

Prasanna Kumar Lenka received his Ph.D. in Engineering (Biomedical) from School of Bioscience & Engineering, Jadavpur University, Kolkata, India in 2008 and M. Tech (Information Technology) from Bengal Engineering College & Science University, Howrah, India in 2003. He has been working as an Assistant Professor since 2003 at NIOH (Under Department of Disability Affairs, Ministry of Social Justice & Empowerment, Govt. of India), Kolkata, looking after the department of Rehab Engineering, R&D and IT Services as an additional Charges. He has more than 10 years' experience in the field of Rehabilitation Engineering. He is a life time member of Registered Member of Rehab Council of India, Govt. of India, Indian Society of Ergonomics, Indian Society of Biomedical Engineering, Indian Society of Prosthetic & Orthotic, Indian Society of Biomechanics and Institution of Engineers.

Raffaele Limosani is a PhD Student in biorobotics at the BioRobotics Institute of Sant'Anna School of Advanced Studies, Pisa, Italy. His main interest is robotic navigation, in particular in indoor area shared with humans.

JiaLi Ma received her M.Sc. degree in Electrical and Computer Engineering from University of Macau (UM), Macau, in 2014 and B.Sc. degree in Electronics and Information Engineering from Tianjin University, Tianjin, China in 2011. During her Master studies, she also participated in five provincial-level research projects, including three Macau Science and Technology Development Fund supported and two UM granted projects. She has authored and co-authored over 10 referred papers in scientific journals and conference proceedings. She was the recipient of Scholarship in UM (2011-2013) and the 3rd Prize of Macau Technological Invention Award (2014). Her current research interests include construction of embedded-link *e*-health systems, bio-information management, and biomedical signals compression.

AKM Jahangir Alam Majumder is a Doctoral candidate in department of Math, Statistics and Computer Science at Marquette University, Milwaukee Wisconsin. His PhD research explores smartphone- and smart shoe-based fall prevention system. In this system, smartphones are integrated with two powerful sensors -accelerometer and gyroscopes- with pressure sensor embedded shoes to identify abnormalities in walking patterns. He received his Master's in Computational Sciences in 2013 from the same university. He also received Masters in Electrical and Electronic Engineering (EEE) in 2008 from Bangladesh University of Engineering and Technology (BUET) of Bangladesh. Before coming to Marquette, he worked as an Assistant professor at Ahsanullah University of Science and Technology (AUST) of Bangladesh. He is the recipient of the Richard W. Jobling Fellowship Award from Marquette University for an outstanding PhD student in 2014 and 2013.

Soumya Kanti Manna received his M. Tech in Mechatronics from Indian Institute of Engineering Science & Technology, Shibpur, India in 2013. In 2013 he joined as an Assistant Professor for the College of Engineering & Management, Kolaghat, India and is currently working in the same position. He is also working as a consultant of a company named Shilpa polymer. Besides he has completed several industrial R & D projects funded by different agencies like Shilpa Polymer, ESI hospital Kolkata. His research interests include Robotics, Rehabilitation Engineering, and Mechatronics. He has published seven research papers in several reputed journals, conferences. He is the editor of a book entitled "Biomedical Engineering & Assistive Devices" (2010, Kolaghat).

Alessandro Manzi received the Laurea degree (M.S.) in Computer Science from University of Pisa, Italy in 2007. From 2007 to 2013, he worked as Research Assistant in the BioRobotics Institute of the Sant'Anna School of Advanced Studies (SSSA) and now he is a PhD student. He was involved in several European Projects like DustBot, HydroNet and currently in Robot-Era. His research interests include computer vision, path-planning in dynamical environments and point cloud processing of depth sensors.

Carlo Maremmani graduated in Medicine and Surgery with first class honours at the University of Pisa. He completed specialist postgraduate study in Neurology at the Department of Clinical Neurology in Pisa in 1990, with distinction. In 1994 he completed PhD in Neurological and Neurosensory studies again with distinction. Since 1993 Dr. Maremmani has worked as a neurologist in the Italian National Health Service (INHS) and currently works in the Operative Unit of Neurology in ASL1 of Massa and Carrara. He deals with Parkinson's and motor diseases and Cephalalgia wards. He coordinates the Electromyographic Service and he is assigned for the confirmation of brain death. He is co-author of more than 40 scientific publications on national and international peer-reviewed journals. He collaborates directly with a wide number of hospitals, clinics and scientific research organizations.

Charlie Ranscombe is a researcher and lecturer in Industrial design and Product design engineering at Swinburne University of Technology (Melbourne Australia). His experience is both as a practicing Industrial designer in the UK and China as well as in academic design research at the University of Bath, UK. Following the completion of his PhD (2012) researching design, product styling and branding, Charlie's primary research interest is in product appearance and user perception of products. His latest research focuses on exploring computational measures for product appearance and there use in improving decision making in product design practice as well as design research in aesthetics.

Erika Rovini received Master Science Degree in Biomedical Engineering in 2011 by University of Pisa (Italy). From 2011 to 2014, she was Assistant Researcher with the Sant'Anna School of Advanced Studies, Pisa, Italy. She is currently pursuing the PhD degree in BioRobotics among the same institute about the study and validation of novel ICT-based diagnostic and therapeutic approaches in Parkinson's disease for sustainable healthcare. Her research interests include data analysis, pattern recognition, biomedical signal processing, experimental protocols, wearable sensors, acceptability and usability criteria and ambient assisted living. She is author of articles and patents concerning the fields of AAL and wearable devices for application in Parkinson's disease management.

Hassan Saidinejad is currently a PhD student of Polytechnic University of Milan. He received his MSc in Computer Engineering from the Department of Electronics, Information and Bioengineering of Polytechnic University of Milan (Italy) in 2012 and his BSc in Hardware Engineering from Sharif University of Technology (Iran) in 2008. He is currently a member of Assistive Technology Group (ATG) of Polytechnic University of Milan with the main focus on user interface and interaction especially for smart environments and aiming at improvement of the quality of life and independent ageing of the elderly. Furthermore, he works on making sense and insight of (person + context) data through data analysis and visualization techniques as a tool for helping independent ageing.

Fabio Salice is an associate professor in the Department of Electronics, Information and Bioengineering (DEIB) of the Polytechnic University of Milan, Italy. Currently, his research interests are mainly focused on ICT Assistive Technologies for fragile people (in particular for elderly and low cognitive disabilities); other research topics are design and synthesis methodologies of dependable systems, fault identification and fault injection methodologies and criteria, pervasive systems design and method including issues concerning low power, power management and energy harvesting. In 2010, Fabio founded the interdisciplinary ATGroup – in Como Campus – structuring a set of collaborations with both the third sector associations and private and public organization (ASL, hospitals, residences, etc.). Furthermore, in 2012, Fabio founded with So.La.Re. (Social Lario Rete) the CRAiS Center (Centro Risorse per le Autonomie e l'Inclusione Sociale); CRAiS aims at supporting individuals, families and contexts in fragile condition and social vulnerability.

Rajalakshmi Santhakumar received her B. Tech in Biotechnology from Anna University, Chennai, India in 2010. She recently graduated with M.S. (By Research) in Biotechnology from Indian Institute of Technology Madras (IIT M), Chennai, India and is currently working as Scientist at Vantage Research, Chennai, India. Her areas of interest include stem cell research, cardiac regeneration and molecular biology.

Jahnavi Sarvepalli graduated with B.S Physician Assistant from Institute of Cardiovascular Diseases, Chennai, India and M.Tech in Nanomedical Sciences from Amrita Institute of Medical Sciences, Kochi, India. She has work experience as first assistant in Cardiovascular Surgery and is currently pursuing Ph.D. in Biomedical Devices from Indian Institute of Technology Madras (IIT M), Chennai, India in collaboration with Sree Chitra Tirunal institute of Medical Sciences and Technology (SCTIMST), Trivandrum, India. Her areas of interest include heart valve tissue engineering and nanotechnology.

Booma Devi Sekar received her Ph.D. and Master degree in Electrical and Electronics Engineering from University of Macau, Macau, in 2014 and 2007 respectively. She received her Bachelor degree in Electrical and Electronics Engineering from AKCE, Madurai Kamaraj University, India. Currently she is positioned as a Post-doctoral Fellow in Faculty of Science and Technology, University of Macau, Macau. Her research work focuses on the development of *e*-home healthcare system for cardiovascular disease diagnosis for aging population. Through her research, she has explored numerous key technologies, including biomedical engineering, bioinformatics, information theory, machine learning, intelligent technology, biological signal processing, etc. Her research work has resulted in the publication of 19 high level research papers, participation in 3 invited seminars and workshop presentation, external research collaborations from reputed Universities & hospitals and has also won multi-year research grants.

Tripty Singh is Assistant Professor in Department of Computer Science at Amrita School of Engineering, Bangalore, India. She has obtained her Ph.D. in 2013 from R.G.P.V. University, India under guidance of Dr. Sarita Singh Bhadauria, Dr. Arun Wadhwani and Dr. Sulochana Wadhwani. She did her M.Tech from IIITM, Gwalior, India in 2004. She has also done internship at Indian Institute of Technology, Kharagpur, India. She did her B.E. from S.G.S.I.T.S., Indore, India in 1999. She has 12 years of teaching experience. She has more than 30 International publications in different conferences and journals. She has served as program chair and program co-chair for IEEE International Conferences. She is reviewer of many reputed journals. She has served as TPC in more than 50 Internationals conferences. She is serving as Editor or Editorial board member for more than 10 journals. She has organized International/National Conferences/Symposiums. She is well known for her contributions in Digital Image Processing (Medical), Artificial Intelligence, Computer and Information Sciences, Distributed Systems, Ad-Hoc Networks, Network Security and Cloud Computing.

Paul Stoddart graduated with BSc (Honours) in physics and PhD in laser spectroscopy from the University of the Witwatersrand, South Africa. After working on industry-focused surface science and microanalysis problems in a national lab for three years, he joined Swinburne University of Technology in 2001. He is currently Director of the ARC Training Centre in Biodevices and Professor of Biomedical Engineering at Swinburne. His research interests include applied optics, biophotonics and medical devices, with projects in the areas of fibre optic sensors, Raman spectroscopy and infrared neural stimulation.

Chandana P. Tamma is an Adjunct Assistant Professor in the Electrical and Computer Engineering department and a Research Scientist at Ubicomp Lab (Department of Mathematics, Statistics and Computer Science) at Marquette University. His research interests include biomedical imaging, instrumentation and mHealth solutions for patient engagement and disease management.

Kavita Thakur received her B.E. (Electrical Engineering) in 1989, M.E. (Electronics & Telecommunication Engineering - Control and Guidance) in 1991, Doctoral degree in Electrical Engineering in 1999 at Pt. Ravishankar Shukla University, India and is presently working as Professor in School of Studies in Electronics, Pt. Ravishankar Shukla University, India. She has published more than 110 research papers in various National / International Journals and Conferences of repute. Her areas of interest are Speech and Image signal processing. She is member of many professional bodies viz. ISTE, senior member IEEE, ASI and APSIPA. She has published 02 books with ISBN 978-3-8465-4338-2 and ISBN 978-3-659-17007-2, LAP LAMBERT Academic Publishing GmbH & Co, Germany. She is recipient of Rashtriya Gaurav Award for year 2010-2011 & 2014 from IIFS, New Delhi, India and Best citizen award for the year 2011 from IPS, New Delhi, India.

Mong-Te Wang is a Research Assistant in Software Engineering & Technology Center of Tunghai University, Taiwan (2004-2014). He majored in Computer Science, and graduated with a Master's degree at Tunghai University in May, 1998. Besides human-computer interfaces, system simulation, bioinformatics and Stat., Mong-Te Wang's research interests include object-oriented technology, software re-engineering, requirement modeling of embedded system and the development of mHealth applications.

Drew Marie Williams is a PhD student working towards a degree in Computational Science at Marquette University, located in Milwaukee, Wisconsin. She received her Bachelor of Science in Computer Science from the University of Chicago in 2012, and her Master of Science in Computing from Marquette University in 2014. She currently works at Marquette University's Ubicomp Lab as a research assistant, working directly in a number of funded projects. She also acts as manager of the lab. Her research interests include human-computer interaction, accessibility, and usable computing technology. She also has research interests in wearable computing, the Internet of Things, and security/privacy issues.

Fabio Veronese received his master's degree in Biomedical Engineering in April 2011, with a thesis concerning the treatment chain of Three-dimensional Multimodal Magnetic Resonance Images to identify cancer in the "head and neck" district. Extending this thesis work he published also an article at EMBC 2012, presenting a novel quantitative technique for cancerous tissues recognition. After the degree, since June 2011, he worked for Polytechnic University of Milan as Temporary Research Consultant. Under leadership of Matteo Matteucci he implemented a prototype of P300-based BCI (Brain Computer Interface) for ALS patients, in collaboration with Infosolution SPA. Since November 2012 he has been a PhD fellow in Information Technology under the supervision of Fabio Salice. He is a member of the ATG, dealing with humans' localization, dependability, data organization and human behavior analysis. His specialties and interest areas are in complex, multidimensional and biological signals processing and analysis, stream reasoning and mobile applications.

Rama Shanker Verma is currently associated with Department of Biotechnology, Indian Institute of Technology Madras (IIT M), Chennai, India. He has done Ph.D. from Jawaharlal Nehru University, New Delhi, India. He was a staff scientist at the National Institute of Health, Bethesda, MD and as an Assistant Professor at the Department of Medicine at University of Pennsylvania, Philadelphia, USA. His research interest involves translational regenerative medicine, tissue engineering, immunotoxins, and developing MAb against receptors.

Arun Kumar Wadhwani is a Professor (presently Professor & Head, Department of Electrical Engineering) at Madhav Institute of Technology & Science (MITS), Gwalior, India. He has obtained his Ph.D. in Application of DSP in Biomedical Engineering from Indian Institute of Technology Roorkee, India in 2003, M.E. Electrical (Measurement & Instrumentation) from University of Roorkee, India in 1993 and B.E. Electrical from Bhopal University, India in 1987. He has total 26 years of teaching experience. He has published more than 125 research papers in various International / National journals/conferences. 07 Ph.D. thesis are awarded under his supervision and 10 are under progress. He has guided number of M.E. dissertations and U.G. projects. His research areas of interest are Application of Artificial Neural Network, Fuzzy Logic and Wavelets in field of Biomedical Engineering. He is supervising number of research projects sponsored by various funding agencies such as AICTE, UGC etc. of approximately 5.7 Million INR. He has organized 05 national conferences and 02 staff development programmes sponsored by AICTE. He is a life time member of professional bodies like ISTE, IETE and IE(I). He is well known for his contributions in Bio-medical Engineering, Measurement & Instrumentation and Signal Processing. He is also working as Controller Examination since last 9 years at MITS, Gwalior.

Sulochana Wadhwani is an Associate Professor at Madhav Institute of Technology & Science (MITS), Gwalior, India. He has obtained her Ph.D. in Application of DSP in condition monitoring of Electrical Machines from Indian Institute of Technology Roorkee, India in 2007, M.E. Electrical (Control System) from Government Engineering College (GEC), Jabalpur, India in 1994 and B.E. Electrical from GEC Jabalpur in 1991. She has total 18 years of teaching & research experience. She has published more than 80 research papers in various International / National journals/conferences. 03 Ph.D. thesis are awarded in her supervision and 4 are under progress. She has guided number of M.E. dissertations and U.G. projects. Her research areas of interest are Application of Artificial Neural Network, Fuzzy Logic and Wavelets in the field of Biomedical Engineering, Condition Monitoring of Electrical Machines & Signal Processing. She is supervising number of research projects sponsored by various funding agencies such as AICTE, UGC etc. She has organized 04 national conferences and 02 staff development programmes sponsored by AICTE. She is a life time member of professional bodies like ISTE, IETE and IE(I). She is well known for her contributions in Bio-medical Engineering, Condition Monitoring of Electrical Machines, Measurement & Instrumentation and Signal Processing.

Arun Shrihari Zadgaonkar awarded with B.E. (Electrical Engineering) degree in 1965 by Pt. Ravishankar Shukla University, India. In year 1978, he was awarded with M.E. degree by Nagpur University, India and Doctor of Literature in 1996. He started his career as Lecturer in 1966, at Govt. Engineering College, Raipur, India. Subsequently he was promoted as Reader in 1986 and Professor in 1996. He got posted as Joint Director, Technical Education, Govt. of Chhattisgarh, India in 2004; as Principal, Govt. Engineering College (NIT), Raipur, India in 2005; as Director, Rungta College of Engineering and Technology, Bhilai, India in 2006. He is founder and Ex - Vice Chancellor of Dr. C.V. Raman University, India. He has published more than 400 research papers in various National / International Journals and Conferences of repute. He has got one Indian patent to his credit. He has guided fifteen Engineering Ph.D scholars and authored three technical books as well.

Chapter 12
Ventricular Assist Device and Its Necessity for Elderly Population

Md. Shamsul Arefin
Swinburne University of Technology, Australia

Chandra Prakash Rathore
Oracle India Private Limited, India

Nasser K. Awad
Swinburne University of Technology, Australia

Anupam Shukla
ABV-Indian Institute of Information Technology and Management Gwalior, India

Yosry S. Morsi
Swinburne University of Technology, Australia

ABSTRACT

Ventricular Assist Device (VAD) is considered to be the part and parcel to those people who have cardiac complications or heart failure especially the aged patients. Although VADs have contributed remarkably for the past few years, yet these devices possess some limitations, mainly the driveline infections. Due to these conditions, researchers are aiming to improve its functionality as well as other necessary/additional features and hence there is a need to develop the 'next generation' wireless VAD system which could be very effective to reduce the risk of this infection. In this chapter, the necessity of the VAD and different kinds of VADs are presented and discussed. These features incorporate hemodynamic states after receiving the VADs, selection of biomaterials for the VAD system, VAD pumps and its classifications. Finally, a brief discussion is also provided based on the recent advancement of the VAD system and the scope for the future research.

INTRODUCTION

Ventricular Assist Devices (VADs) are considered as lifesaving systems. It is well known that a lack of usual blood flow can develop different heart diseases. These diseases indubitably influence the natural operational activities of the heart and a prime factor for causing death. To be precise, left ventricle (LV) of the cardiac system is the most important chamber which helps in the circulation of blood to the entire body. On the other hand, when usual functionalities of left ventricle are hindered because of aging or weakened cardiac muscles then it is vulnerable to various cardiac diseases primary treatments for these

DOI: 10.4018/978-1-4666-9530-6.ch012

diseases are utilization of ventricular assist devices (VADs), which are basically implanted/set-up inside the patients. To date, researches have produced substantial advancements in development of the VADs including sizes and shapes but still various limitations exist which cause various cardiovascular diseases (CVD) and/or infections for the VAD-patients (Arefin, 2015).The cardiovascular system comprises of a pumping-organ, (the heart) blood and blood-vessels which act as a branching network throughout the whole body. The heart is a conically formed pumping organ which serves the total requirement of blood needed by the whole body and it is constructed from the muscle tissues (Bronzino, 2006). Cardiac diseases itself are one of the main factors for human morbidity. Precisely, a significant knowledge is necessary based on the general and diseased hearts to attain suitable results for the general and clinical cardiac analysis (Vadakkumpadan et al., 2010). Utilization of the VADs substantially minimize mortality rate especially for those people who are on the heart transplant waiting record (Garbade, Bittner, Barten, & Mohr, 2011; Moazami, Sun, & Feldman, 2011). Also, people with CVD, weakened heart muscle and/ or elderly people are in need of VAD systems. Although there are various cardiovascular diseases named-valvular heart diseases (Bender, 1992; Morsi, 2011) and coronary artery diseases/coronary heart diseases (Arefin, 2015; Başçiftçi & Incekara, 2011; Channel, 2014), but special attention has been given towards the heart failure diseases and its treatments, especially which are highly suitable for the elderly people.

Cardiovascular Disease (CVD)

In general, Cardiovascular Diseases (CVDs) refer to every possible disorder of the cardiac system (the heart and blood vessel), which is not functioning properly. This disease is responsible for causing impairment to the blood circulation tracks, such as the veins and arteries where the blood is flowing to and from the heart. In statistics of NHMRC (National Health and Medical Research Council) it is mentioned that total expenditure for research of CVD was $439.5 million in year of 2000 to 2007 in Australia (Arefin, 2015; Council, 2014).

Moreover, it is also stated that CVD is the main cause of death in Australia including approximately 45,600 people died in year of 2011. Specifically due to this disease, in every 720 seconds one person dies in Australia (Council, 2014). Subsequently, in the under developed countries 80% of people was found to be affected and ultimately died because of this disease and the percentage is still elevating at an alarming rate. In contrast, in developed countries during years from 1960 to 2010 CVD-deaths for the aging population have been found to decrease by 50%. Moreover, in the most cases CVD related disorders are often checked at advanced stages and hence this disease is perilous. Consequently, because of this specific reason, apposite drug therapies need to be prescribed at the ~~very beginning of the condition~~ so that it can help blocking the advancement of the disease (Emeto, Moxon, Rush, Woodward, & Golledge, 2011). Furthermore, CVD also acts as the main source of mortality for the people with Hemodialysis (HD). It occurs because of blood pressure, unstable lipid metabolism, oxidative stress, micro inflammation, hyperhomocysteinemia, anaemia, secondary hyperparathyroidism and vascular shunt flow (Petrovi, Obrenovi, Trbojevi-Stankovi, Majki-Singh, & Stojimirovi, 2011). Additionally, when high blood pressure isresponsible for the causing the heart disease, it is characterized as the *hypertensive heart disease* (Badii, 2012). Largely, CVDs affect the cardiac system over extended time frame and this often develop much higher risk of heart attacks and strokes. Also, the blocked blood vessel is unable to circulate the blood into the cardiac system and/or in the brain (Online, 2013). Subsequently, various factors can influence the enhancement of the cardiac system, which is termed as the congenital cardiac disease. Moreover, different infections can affect the general functionalities of the heart valve, which

is known as endocarditis. Furthermore, if the natural movement of the cardiac muscle is affected for example, more slowly or rapidly (inflammation process), then it is termed as the cardiomyopathy and myocarditis, correspondingly (Arefin, 2015; Online, 2013).

Heart Failure (HF)

Heart failure is often caused by lack of appropriate physiological functionalities, which leads to bodily imbalance and eventual death of the patient. The cardiac failure mainly takes place because of dysfunction of ventricle functionalities, which are expansion and contraction. Subsequently, heart failure is responsible for causing minimal or lack of blood flow, elevated lung pressure, increases the de-oxygenated blood and ultimately, death results (Baryalai, Wang, AlMalki, & Masoud, 2011; Hunt et al., 2009). Even in Australia, over 380,000 people are at risk of heart attack at any time and every year around 55,000 people face heart attack related complications (Arefin, 2015; Foundation, 2014). There are different types of cardiac failures, such as (Arefin, 2015):

1. **Dilated Cardiomyopathy:** Dilated cardiomyopathy is the primary kind of cardiac failure. Because of this, the ventricles turn out to be very soft and enlarged, causing ventricular dysfunction of the heart. Moreover, due to this deteriorated heart, stroke volume decreases and the blood circulation rate try to elevate the necessary cardiac output (Arefin, 2015; Baryalai, Wang, AlMalki, & Masoud, 2011; Peschar et al., 2004).

Subsequently, the body becomes very weak because of shortage of enriched arterial blood, which isindispensable for imperativeorgans. Alternatively, similar indications can be seen for the dilated cardiomyopathy due to elevated pressure levels in the heart and in the lungs, which is termed as *Congestive Heart Failure* (Arefin, 2015; Hunt et al., 2009).

2. **Hypertrophic Cardiomyopathy:** When ventricles of the heart become very hard and unable to function properly due to thick cardiac muscle, it is recognized as hypertrophic cardiomyopathy. This stiff cardiac muscle can also produce obstruction of the left ventricle, which is same as Aortic Stenosis (Arefin, 2015; Baryalai et al., 2011; Fogoros, 2014a).
3. **Diastolic Dysfunction:** This type of heart failure is also very frequent and it occurs because of asymmetrical thickening of the ventricles of the heart and uncharacteristic ventricle filling during the diastolic phase. Precisely, high blood pressure, hypertrophic cardiomyopathy, coronary artery diseases, obesity etc can originate this disease (Arefin, 2015; Fogoros, 2014b).

Subsequently, coronary heart disease (CHD) is one of the most perilous heart diseases, which is generally characterized when the coronary arteries (deliver blood and oxygen to the cardiac muscle) are impeded by oily materials, such as 'plaque' or 'atheroma'. Plaques gather along the wall inside the arteries which primarily influences the arteries to become thinner and as a result, obstructing the blood circulation inside the arteries. Specifically, this incident is termed as 'atherosclerosis' and it is one of the most critical factors for the coronary diseases (Başçiftçi & Incekara, 2011; Channel, 2014). Reports state that around 1.4 million Aussies primarilyexperience coronary diseases and almost 59 people die every day because of this disease (Arefin, 2015; Foundation, 2014).

Current Treatments of Heart Failure for Elderly Patients and Need for VAD System

Cardiac failure is a life threatening condition but the risk can be minimized with the progression of time. Some of these heart failure diseases can be restricted by staying on thesuggested treatment plans and also by being healthy. Moreover, when the cardiac muscle becomes deteriorated, various treatments are accessible these days which can help lessen the complications and immobilize or slow down the overall circumstances (Center, 2014). Subsequently, quantification of coronary arterial stenosis is found to be very efficient in identifying coronary heart disease (Xu et al., 2011). Also, in order to minimize the cardiovascular mortality, specifically for Hemodialysis (HD) patients the process must hold capability for preliminary identification including much higher-risked patients, permanent measurement dialysis suitability and electrolyte immovability (Petrovi et al., 2011). Moreover, gene therapy could be a potential source of medication especially for the ischemic cardiac disease for people in the imminent days (Lavu, Gundewar, & Lefer, 2011). Furthermore, for the valvular heart diseases, treatment mainly relies on the surrogate or substitution of the mechanical or tissue valves (Morsi, 2011). Consequently, heart transplant is another source of treatments for the cardiovascular patients. In general, it is a surgical process in order to substitute diseased heart of the patient with a healthy new heart from a departed donor. ~~Specifically, this~~ is the ultimate procedure to save a patient's life and ~~this process~~ is only valid when all other medical treatments and other critical operations have unproductive results. However, because of the inadequate donor hearts, patients have to endure an arduous selection process (National Heart, 2012). Although, heart transplant is a life-saving procedure but it still has several snags, such as: children and infants who are getting the treatments of an orthotropic heart transplant holds a much higher risk of mortality during the time of this procedure (Boucek et al., 2008). Practically, it is quite tough to locate a suitable donor and necessity for the transplantation is always in demand. This eternal insufficiency of the donors put a much higher focus on the alternative, which is the implantation/inclusion of left ventricular assist devices (Agarwal & High, 2012). Alternatively, at present, *stem cell* technologies are under research and it is believed that it could be highly effective for the cardiovascular related issues. For instance, it is proven feasible to minimize the overall size of the widened cardiac structure using the stem cell technology but it requires more research work to be established (Arefin, 2015; Mozes, 2011).

Ventricular Assist Devices (VADs)

The VAD is identified as a mechanical pump, which performs as a ventricle and helps circulating the blood to the entire body. From the four chambers of the cardiac structure, the left ventricle is recognized as targeted section for artificial aid due to its considerable responsibility in the stipulation of oxygenated blood to the organs and also vulnerable from different cardiac diseases. During years of 1998 to 2001, left ventricular assist devices were acknowledged as a remedy or bridge to transplantation therapy (BTT). On the other hand, three critical factors are responsible for producing peri-operative complication (Arefin, 2015; Awad et al., 2010; Goldstein, Oz, & Rose, 1998; Rose et al., 2001):

- Native cardiac failure having mediocre organ impairment;
- Long-lasting effects of the implanted devices;
- Surgical actions consisting cardiopulmonary bypass (CPB).

Consequently, the foremost objective of the VAD device is to assist transferring the blood from the lower sections (ventricles) and then to the entire body, comprising imperative organs. The VAD simply replaces the functionalities of the weakened human heart. Moreover, the elementary components of a VAD system incorporate (Arefin, 2015; Institute, 2012):

- A small tube that helps circulating the blood from the heart to the VAD pump;
- An additional tube helps circulating the blood from the pump to the patient's body;
- A power source.

Additionally, this power source is affixed with a control unit and its primary task is to superintend the device's operation procedure. When the device's power is very low and/or if is unable to operate correctly, then this control unit generates cautions or alarms (Arefin, 2015; Institute, 2012).

Latest enhancements in the VAD technologies, especially the LVADs incorporating modifications in selecting the patients and overall management have immensely upgraded the rate of survival (Moazami, 2011). These improved VADs are generally much smaller in size and have advanced options which allow these devices to be a perfect replacement of the left ventricle functionalities and also minimize the mortality rate, who are in the queue for the heart transplantation (Arefin, 2015; Garbade et al., 2011).

To date, different types of VADs are available; some of them can produce pulsatile flow which is similar to the cardiac system and others are able to produce a deliver blood flow. Subsequently, it is practical that with constant blood flow the patient might not be able to feel normal pulse. On the other hand, their body will get the required amount of blood in order to perform properly (Arefin, 2015; Institute, 2012).

In general, two types of VADs can be named as:

- Left ventricular assist device (LVAD),
- Rightventricular assist device (RVAD).

However, if these two VADs are used/operated collectively, then it is identified as the biventricular assist device (BIVAD) (Arefin, 2015; Institute, 2012).

Out of these VADs, LVADs are the most prevalent type ones and its primary function is to drive the blood from the left ventricle to aorta. Consequently, RVAD's primary function is to drive the blood from the right ventricle to the pulmonary artery. Moreover, the non-appearance of an operational, easily implantable RVAD, can drastically reduce long-term treatment for those people who are having a biventricular heart failure (Tanser, 2006). Alternatively, a BIVAD is utilized when both the ventricles of the heart do not function in proper way to fulfil the overall necessities of the body. Generally, when a patient face this ~~sort of situation~~, then another treatment is taken into consideration which is known as total artificial heart (TAH) (Arefin, 2015; Institute, 2012).

Moreover, the VADs encompass two fundamental designs, such as:

1. **Transcutaneous VAD:** Usually, a Transcutaneous VAD includes a pump and power supply, which are positioned on the outer surface of the body. There are tubes which are attached to the pump to the heart by some miniature holes in the abdomen. This kind of VAD is ideal for small-period of time when the operation is taking place or it is over (Institute, 2012).
2. **Implantable VAD:** An Implantable VAD also contains a pump and a power supply. However, the pump is positioned inside the body and the power supply is on the outer surface of the body. A wire

attached to the pump is linked with the power supply by a miniature hole in the abdomen. This kind of VADs are largely operated when the person is imminent for the transplantation of heart or when the person is not suitable for the transplantation, then this VADs are used for the higher period of time (Institute, 2012).

Furthermore, two more types of VADs are accessible depending on the patient's need.

1. **Temporary VADs:** Temporary VADs are very useful when the patient is waiting for the transplantation or any other operation of heart. Temporary VADs generally assist the patient's heart for small-period of time. However, these VADs are highly essential if the patient possesses acute heart diseases namely, heart malfunction, ventricular arrhythmia, cardiogenic shock and so on. Moreover these diseases cannot be healed using any kind of medicines (Institute, 2012).
2. **Enduring VADs:** When the patient has heart malfunction and is in the queue for the transplantation of heart, then enduring VADs might be useful as well. However, if the medications are not enough to prevent the cardiac malfunction, then a VAD can certainly boost a patient's health while the patient is waiting for a giver heart for the transplantation. Moreover, if any person is not qualified for the transplantation of heart, then these enduring VADs are considered to be an excellent therapy. These VADs can enhance the excellence of life and permit lots of daily deeds (Institute, 2012).

Hemodynamic System of a VAD

It is stated that the failure of a VAD system can certainly causes a lot of damage, including the death rate of around 6%. This error can be minimized by implying newer VADs which should certainly take the considerations of the heart movement of the candidate's or in other word, hemodynamic state. Kang and Choi (2011) developed a new pulsatile VAD which removes the blood regurgitation and also includes a small stationary area. It is useful when the pumping force is very low. Timms et al. (2011) reported that adjustment is required for the hemodynamic conditions of the pulmonary system using rotary LVAD into a right heart assist arrangement. It was accomplished by dropping the right-pump rotational speed by 1400 rpm or limiting the right outflow cannula to 22.9 mm^2. Moreover, his team also suggested that, in order to develop an appropriate hemodynamic condition for individual patient, variable banding system should be used with lesser variations in the right pump rotational speed. As a result, this will diminish the risk of thrombus formation in the right sided pump. Geiger et al. (2011) discussed about the whole heart 4D hemodynamics in association with the pulmonary trunk (TP) followed by the detection of the post-operative risks in patients. They also stated that the 4D flow analysis is the utmost method to estimate the vascular morphology and hemodynamics for TGA (Transposition of the Great Arteries) candidates. Moreover, Tay et al. (2011) presented a candidate-specific Cardiovascular-Modelling System (CMS) in order to mimic flow system in left ventricle. As a result, this technique will help physicians to detect the patients before heart failure. This system will go through using hybrid Computational Fluid Dynamics (CFD) simulation and time-resolved Magnetic Resonance Imaging (4-D MRI) and these methods offers amenable study of the flow determination in the ventricles and also their responses. In another study, Tuzun et al. (2011) demonstrated the use of continuous-flow LVAD within a Mock Circulatory System (MCS) containing an interposed valve. As the LVADs are highly common now-a-days, leaflet fusion with resultant aortic regurgitation is getting more frequent. In order to replicate the

hemodynamic features of the LVAD candidates, this MCS is assisted with Jarvik 2000 LVAD. This was located at centre of a piston-pump (servomotor functioned) and driven at 8000-12000 rpm. However, they concluded that the added LVAD flow really affects the impaired aortic valve opening time because of pressure overload reached beyond the aortic valve. This overload can possibly induce in the change of structure, regurgitation and aortic leaflet fusion. Subsequently, Ahn et al. (2011) also reported about the influence of fluid viscosity deviation on hemodynamic energy. This was assessed with the aid of a pulsatile blood pump within a mock system. They stated that when mean pressure increases, then the viscosity increases constantly. On the other hand, the result of an increased viscosity results the flow to decrease. Worku et al. (2011) reported about a device, in order to access the risk after balancing the hemodynamic system assisting with a short term VAD to assume possibility of the survival. This device can be a predictive guide for the physicians as well as family members of the patients primarily in provision period. Moreover, Schampaert et al. (2011) discussed advantages between the 40 cc Intra-Aortic Balloon Pump (IABP) and Impella 2.5 Left Percutaneous (LP), having a speed of 47000 rpm. This evaluation has been done by the circulatory support skills based on the cardiac output, coronary flow, cardiac stroke work and arterial blood pressure. They found that the Impella 2.5 LP is slightly ahead than IABP in terms of the circulatory aid, whereas the pulsatility is better with the IABP. For these two, highest benefit was obtained in terms of hemodynamic condition, when mechanical circulatory support was employed on a simulated situation of deep Cardiogenic Shock (CS). As reported to the study of Impella 2.5 LP, Suradi and Breall (2011) stated effective use for the Impella device for the first time, in terms of hemodynamic aid in a 44-year-old woman having a giant cell myocarditis. This lady appeared with a Cardiogenic Shock (CS) and she was aided hemodynamically using the Impella Recover LP 2.5 LVAD, until a device could be operationally inserted eternally. Table 1 represents the key information obtained from the discussion.

Selection of Biomaterials for VAD System: Titanium (Ti) and Ti-Based Alloys

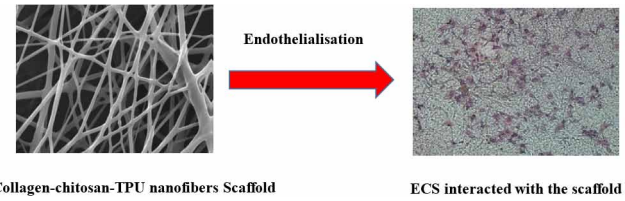
Titanium (Ti) and its alloy have been extensively used as biomaterials for ventricular assisted devices due to its good biomechanical properties such as Young's modulus, fatigue strength, corrosion resistance and biocompatibility. Titanium has a natural attribute analogous to the other valve metals; it creates a top layer of titanium dioxide (TiO_2) which makes it an excellent candidate for biomedical applications. Titanium has been used in biomedical devices either as pure Ti or Ti alloys. Ti6Al4V alloy has been the most common Ti-based alloy for biomedical implantation, with aluminium (Al) constituting 5.5-6.5% of the alloy and vanadium (V) constituting 3.5-4.5%. Al and V have an allergic effect on cells; alternative Ti-based alloys have been sought. For this reason, Ti6Al7Nb and Ti5Al2.5Fe have been developed without vanadium (Niinomi, 2002). ~~Further,~~ Ti15Sn4Nb2Ta0.2Pd and Ti15Zr4Nb2Ta0.2Pd have been developed as Al-free and V-free Ti-based alloys (Okazaki et al., 1995). In terms of mechanical characteristics, β-type Ti alloys have considerably lower Young's modulus than both α-type and α+ β-type Ti-based alloys. Consequently, the focus on fabricating low Young's modulus β-Type Ti alloys has been the main area of research. As a result, Ti13Nb13Zr, Ti12Mo6Zr2Fe, Ti15Mo, Ti15Mo5Zr3Al, Ti35Nb7Zr5Ta and Ti29Nb13Ta4.6Zr (TNTZ) have all been developed (Niinomi, 2003). TiNi alloy (nitinol) has been one of the most notable Ti alloys because of its novel properties such as shape memory effect and super-elasticity (Buehler et al., 1963). The elastic modulus of nitinol is 38-48 MPa, which is much lower than that of standard 316L stainless steel. Nitinol, therefore, has been used in various medical applications, such as orthodontic wires, stents and bone implants. The biocompatibility of nitinol implants

Table 1. Methods and outcomes obtained by the researchers

Author	Technique/Method	Outcome
Kang and Choi (2011)	• Developed a new pulsatile VAD	• Removes blood regurgitation • Contains small stationary area
Timms et al. (2011)	• Suggested to produce a suitable hemodynamic condition	• Necessary adjustments were made for hemodynamic conditions using rotary LVAD
Geiger et al. (2011)	• Reported about the whole heart 4D hemodynamics using pulmonary trunk (TP) • Finding the post-operative threats for the candidates	• Best method so far to verify the vascular morphology and hemodynamics for the Transposition of the Great Arteries (TGA)
Tay et al. (2011)	• Presented a candidate specific Cardiovascular Modelling System (CMS)	• This system will help the doctors to find the patients before heart failure • It uses Computational Fluid Dynamics (CFD) simulation and time-resolved Magnetic Resonance Imaging (4-D MRI)
Tuzun et al. (2011)	• Showed the operation of continuous-flow LVAD within a Mock Circulatory System (MCS)	• Added LVAD flow certainly affects the impaired aortic valve opening time • This overload can possibly provoke in the change of structure/shape, regurgitation and aortic leaflet fusion
Ahn et al. (2011)	• Discussed about the influence of fluid viscosity deviation on hemodynamic energy • Pulsatile blood pump was used within a mock system	• When the mean pressure increases, then the viscosity increases as well • This increased viscosity effects the flow to decrease
Worku et al. (2011)	• Reported about a device to determine the risk after balancing the hemodynamic system	• This device can work as a guide for both the doctors and the family members of the patients primarily at the provision period
Schampaert et al. (2011)	• Compared between the advantages between 40 cc Intra-Aortic Balloon Pump (IABP) and Impella 2.5 Left Percutaneous (LP)	• Impella 2.5 LP is better than IABP in terms of the circulatory aid • IABP has better pulsatality compared to Impella 2.5 LP • Highest benefit was achieved for both devices for hemodynamic condition, when mechanical circulatory support was allowed on a simulated situation of deep Cardiogenic Shock (CS)
Suradi and Breall (2011)	• Stated the effective use for the Impella device in terms of hemodynamic aid • The patient was having a giant cell myocarditis	• This candidate was aided hemodynamically using the Impella Recover LP 2.5 LVAD successfully.

in soft tissues has been confirmed by both in vitro and in vivo investigations (Ryhanen, 1998).Since nitinol contains Ni, the release of Ni ions is expected. However, it has been reported that the concentration of Ni ions released from nitionl is below the critical concentration that induces an allergic effect (Anita et al., 2004). In spite of this, researchers are still attempting to fabricate Ni-free superelastic Ti alloys, which are β-type alloys. Examples of these are TiNbSn, TiMoGa, TiMoGe, TiMoAl, TiTa, TiNb, TiNbAl, TiScMo, TiMoAgSn and TiNbTaZr . However, thrombosis formation is still existed even with Ti and Ti-based alloys (Kudrman et al., 2001). Surface modifications of Ti and Ti-based alloys play a major rule for enhancing its biocompatibility as well as overcoming clot formation.

Figure 1. Tissue engineering scaffold
(Huanga et al., 2011).



Surface Modifications of Ti and Ti-Based Alloys against Thrombosis Formation

Surface modifications can be conducted either by passive or active coating layer on Ti surface. Passive layer such as TiN or Dimond-like carbon or 2-methacryloyloxyethylphosphorylcholine (MPC) polymer deposited on Ti surface constitute barrier and ~~itthus~~ prevents the interaction between the blood and Ti alloy. Active layer such as Heparin or endothelial cells (ECs) directly interacts with the blood and prohibits the formation of intimal hyperplasia (Wieneke et al., 2002). Tissue engineering provides a reasonable strategy of fabricating porous polymer scaffold on Ti which could attract endothelial cells and construct confluent layer of cells. Subsequently, EC cell layer works as anticoagulant agent for inhibiting thrombus formation. Figure 1 illustrates polymeric porous scaffold interacting with ECs (Huanga et al., 2011). Various polymers whether natural or synthetic or hybrid has been tried for fabricating scaffolds as well as several techniques have also been attempted for fabricating scaffold such as melted-polymer based, particle-leaching, microsphere-template, freeze-drying and electrospinning, gas-foaming and rapid prototyping (Chen et al., 2008).

Surface Treatment of Ti Substrate to Increase Interaction with Deposited Layer

Mechanical surface treatment is essential for preparing a metallic implant surface for further surface modification methods, like physical or chemical processes, and takes the form of machining, grinding and polishing. Mechanical treatment of the implant surface can also improve implant biocompatibility by altering surface roughness and topography. Blasting (Wennerberg et al., 1996) is a method of altering the surface roughness, refining and removing the contaminations of the surface. Abrasive particles such as alumina, titania and HA particles are used to blast the surface of the biometallic material to improve stability and biocompatibility. Surface mechanical attrition treatment (SMAT) has also been utilized in the biomedical field to enhance implant surface characteristics, creating nanostructures (Tao et al., 2003). Treatment of the surface of metallic biomaterial can be chemically achieved using Acid, Alkali and H_2O_2 treatment. In fact, a mixture of HNO_3 and HF of ratio 10 to 1% has been utilized for cleaning the surface of titanium. Along the same lines, a mixture of the three acids $Na_2S_2O_8$, H_2SO_4 and HCl has been ~~employed~~ for the surface treatment of titanium implants and it has been pointed out that HCl reacts only with the oxide surface layer, removing it without affecting Ti itself. On top of that, Wen et al. demonstrated that consecutive processes of acid ($HCl+H_2SO_4$) and alkaline treatment of titanium could potentially improve its bioactivity (Nanci et al., 1998). Titania gel thin film was fabricated on titanium when treated in a solution of H_2O_2 /0.1M HCl or $H_2O_2/TaCl_5$ (Tengvall et al., 1989). After that, the bioactivity of the treated surface increased .However, heat treatment is essential for the transformation

of the amorphous phase to the crystalline phase. Anodic oxidation has also been employed for treating the surface of Ti by creating porous layer on the surface (Han et al., 2014).

VAD Pumps

It has already been discussed that the VADs are very helpful when the patient require medical treatment. VAD pumps are basically the micropumps, because of their micro-size and they are completely inserted into the VADs. These micropumps help operating the entire VAD system.Original study relating to the micropumps began in 1970s and the advancement regarding the micro-fabrication technology started in 1980s. In 1990s, MEMS (Micro Electronic Mechanical System) based micropumps were built. Regarding to the drug delivery method, micropumps are the foremost part of that technique, which allows the actuation principles to supply exact amount of drugs from the tank. Basically a micropump contains diaphragm, membrane, chamber, actuator, microchannels, microvalves, inlet, outlet etc (Ashraf, Tayyaba, & Afzulpurkar, 2011). Conversely, the definition of micropumps by MEMS reveals that thetiny pumping components are made-up using micromachining techniques are known as micropumps. Generally, two types of micropumps are available (Amirouche, Zhou, & Johnson, 2009; Ashraf, et al., 2011; Nisar, Afzulpurkar, Mahaisavariya, & Tuantranont, 2008):

- Mechanical Micropumps;
- Non-mechanical Micropumps.

Mechanical Micropumps

Mechanical micropumps consist of movable components. As a result, these pumps need a physical actuator in order to complete the pumping procedure. The most frequent mechanical micropumps are the displacement type, which contain a pumping chamber and it is attached with a movable diaphragm(Ashraf et al., 2011). The categorizations of these micropumps (depending on the actuation mechanisms) are described in short (Amirouche et al., 2009; Ashraf et al., 2011; Nisar et al., 2008). Likewise, these micropumps can be additionally separated into two more types namely, displacement and dynamic pumps; whether the additional mechanical energy is given regularly in order to raise the pressure to start the movement of the fluid or constantly to raise the speed of the fluid (Amirouche et al., 2009). Table 2 demonstrates the characteristics, pros and cons of the mechanical micropumps.

Non-Mechanical Micropumps

Non mechanical micropumps do not contain any movable components. As a result, these micropumps require a method, which can alter the non-mechanical force into the kinetic energy. Basically no physical actuation parts are required for these micropumps and that is why the shape, size, pattern and production of these pumps are quite easy and straightforward. However, these micropumps have some shortcomings too, as the utilization of lone low conductivity fluids and the actuation principles hinder with the pumping fluids (Ashraf, et al., 2011). Usually, the rate of flow for these pumps is below 10 l/min and it varies from 10 l/min to some millilitres/min for the mechanical micropumps (Amirouche, et al., 2009). The categorization for the non-mechanical micropumps can be found from (Amirouche et al., 2009;

Ashraf et al., 2011; Nisar, et al., 2008). Table 3 demonstrates the characteristics, pros and cons of the non-mechanical micropumps.

Although the VAD systems provide significant lifeline-support to the patients, but these devices are also prone to some complications (Arefin, 2015; Friedman et al., 2011; Goldstein et al., 1998; Pamboukian, 2011):

- Size of the device,
- Durability,
- Driveline infections,
- Open-chest operation,
- Right-sided heart failure,
- Thromboembolism,
- Bleeding issues.

Recent Development of the VAD System and Need for a "Next Generation" VAD System

To prevent the limitations, to date, the overall VAD system has been upgraded and still moving forward. Moreover, substantial work and progress for the mechanical VADs have been attained and particularly for the people having acute cardiac failure. To be precise, higher stability, simple pumping mechanisms devoid of bearings orfew bearings and incessant advancement in the valves are the prime outcomes in the modern day VAD system. Different VAD systems are currently available to the cardiac failure patients (Arefin, 2015; Molina & Boyce, 2013):

- HeartMate II,
- HeartWare HVAD,
- Jarvik 2000,
- MicromedCardioVascularHeartAssist 5,
- Synergy LVAD.

Subsequently, different generations for the left ventricular assist devices have been found, as (Arefin, 2015; Garbade et al., 2011; Rodriguez, Suarez, Loebe, & Bruckner, 2013):

- **First Generation:** Utilizes pulsatile pumps for the blood flow. VAD systems include numerous moving parts, for instance: HeartMate I, HeartMate XVE and so on.
- **Second Generation:** Utilizes continuous blood pumps/axial pumps such as: HeartMate II, Jarvik 2000 and many more.
- **Third Generation:** Incorporates non-contacting bearings and continuous flow pumps/centrifugal pumps such as: HeartWare LVAD, HeartMate III and so on.

Additionally, further improvements of the VAD system have been reported by Ostrovsky, 2006 that the Myotech Cardiovascular was experimenting devices using an enclosure/cup on the outer surface of the cardiac system which will aid the heart in contraction and expansion similar to the natural functionalities of the heart. Moreover, this device includes a small outer control unit and stretchable/flexible polymer

Table 2. Mechanical micropumps established on different actuation mechanism with advantages and disadvantages

Pump	Author	Principle	Advantage	Disadvantage
Electrostatic	Nisar et al. (2008), and Ashraf et al. (2011)	• Coulomb luring force between two opposite charged platers was the main cause of actuation for this pump.	• Uses small amount of power, around 1mW and also quick response of time. • By giving a voltage, the movement of the diaphragm can be guided quite nicely.	• Composes of a miniature actuator stroke and that is restricted to 5μm including the given voltage of 200V.
Piezoelectric	Pol et al. (1990), (Pol van de, Lintel van, Elwenspoek, & Fluitman, 1990), and Ashraf et al. (2011)	• The exchange process between mechanical energy to voltage (electrical signal) and vice-versa is recognized as the piezoelectric effect.	• Bigger rate of actuation and quick time response. • Displays miniature stroke volume when the applied voltage is elevated.	• Needs greater input voltages (around 100V).
Thermopneumatic	Pol et al. (1990), (Pol van de, et al., 1990), and Ashraf et al. (2011)	• Thermal expansion technique is the main cause of act for these micropumps.	• Produces comparatively robust pressure and movement of the membrane.	• The desired power needs to uphold always over a specific level.
Shape Memory Alloy (SMA)	Benard et al. (1998), (Benard, Kahn, Heuer, & Huff, 1998), Nguyen et al. (2002), and Ashraf et al. (2011)	• SMA shows two exclusive matters as, pseudo elasticity and the shape memory (SM) stimulation. Moreover, when an outer effect is applied, then they can alter their form.	• However, theresistivity of these films is appropriate for the Joule heating. This high temperature permits nonstop electrical manipulation of the actuator and at the same time, able to accomplish elevated force and strains.	• Bigger dimension.
Bimetallic	Ashraf et al. (2011)	• Bimetal indicates to an item, which consists of two distinguished metals, is linked as one. However, the thermal expansion coefficients are dissimilar.	• Needs small amount of voltage than other types of micropumps.	• Not efficient when the frequency increases.
Ionic Conductive Polymer Film (ICPF)	Nisar et al. (2008), and Ashraf et al. (2011)	• Polymer MEMS actuators can work on aqueous surroundings including big deflection/movement and it need small amount power than the usual MEMS actuators.	• Generally, this actuator is known as artificial muscle due to its very flexible movement, small amount of actuation voltage and the biocompatibility. • Demonstrates greater speed stimulation. • Ideal for different purposes as, soft gel actuators, servo actuators, integrated sensor actuators etc..	• The position/ movement- control is very complex.
Electromagnetic	Nisar et al. (2008), and Ashraf et al. (2011)	• An usual electromagnetic micropump has a chamber including inlet and outlet valves, a movable membrane, a stable magnet along with the coils. • The power of an electromagnet can be diverted by altering the flow of electric current using the coils.	• This actuation is better and can encompass extended spaces than the electrostatic actuation. • Small amount of voltage is required.	• Actuation mechanism depends on outer source, for example, permanent/ stable magnet. • Excessive usage of power and heat loss.
Phase Change Type	Nisar et al. (2008), and Ashraf et al. (2011)	• Consists one heater, a diaphragm along with an efficient fluid compartment. • The working theory for these actuators is the vaporization and condensation phenomenon.	• Comprises small amount of flow rate, which is necessary for the lab-on-a-chip technology needing lower flow rate (not more than few μl/min) along with the back pressure below 68.9kPa.	
Optical actuation	Amirouche et al. (2009)	• Light works as the source and it can be changed into mechanical alterations.	• Biocompatible and can be handles without any difficulties using outer fields. • Promising for blood transport.	

Table 3. Non-mechanical micropumps established on different actuation mechanism with advantages and disadvantages

Pump	Author	Principle	Advantage	Disadvantage
Magnetohydrodynamic (MHD)	Nisar et al. (2008)	• Electrically operating fluid in both magnetic and electric fields. • Lorentz force is the prime force for this micropump.	• Highly suitable in order to driving fluid with superior conductivity • Highly useful for medical and biological purposes.	• Ionication effect creates bubble and it is considered as an obstacle for this micropump.
Electrohydrodynamic (EHD)	Fuhr et al. (1992), Fuhr et al. (1992), and Nisar et al. (2008)	• Conversion of electrical to mechanical energy is done by using electric field operating on stimulated charges in the liquid. • The conductivity of the liquid for this micropump has to be minimal and dielectric at the same time.	• Able to drive the conductive fluids for example, water and feeble electrolyte mixtures. • Parts are fixed.	
Electroosmotic (EO)	Laser et al. (2003), Laser et al., Nisar et al. (2008), Amirouche et al. (2009), and Ashraf et al. (2011)	• The movement of the fluid in EO is stimulated by an applied voltage crosswise the capillary tube or other microchannel. • Also known as electrokinetic incident and it is very useful to drive the electrolyte mixtures.	• Functional voltage is under 200mW and mechanical parts are fixed, for example: check valves.	• A specific pH mixture is needed for this micropump.
Electrowetting	Nisar et al. (2008)	• Wettability alters when electric potential is employed.	• Minimal usage of power and voltage, no fluid-heating.	
Bubble Type	Yin and Prosperetti, (2005), (Yin & Prosperetti, 2005), and Nisar et al. (2008)	• Driving outcome depends on the cyclic growth and breakdown of the bubble, produced in the microchannels.	• Easy to manufacture and mechanical parts are fixed.	• Wide usage becomes restricted if the procedure of hearting is not permitted or ideal.
Flexural Planar Wave (FPW)	Ashraf et al. (2011)	• These micropumps functions ultrasonically. • The liquid- movement stimulated by the FPW can be manipulated for the transportation of fluids.	• Minimal/low functioning potential is essential for the audio streaming.	
Electrochemical	Nisar et al. (2008), and Ashraf et al. (2011)	• Bubble force is produced after the electrochemical reaction in electrolysis.	• Minimal functioning voltage and power usage.	• The bubble can get smashed and as a result it will produce water. This water can begin an erratic discharge of drugs
Evaporation Type	Guan et al. (2006), (Guan, Xu, Dai, & Fang, 2006), Nisar et al. (2008), and Ashraf et al. (2011)	• Similar working method as xylem transportation in the trees. • Regulated vaporization of fluid is employed.	• Especially built for the microfluidics system using the chemiluminance (CL) exposure. • Easy to manufacture, cheap, consist of reduced shapes and above all adaptable flow rates.	

enclosure which are to be placed on the outer surface of the heart just in three minutes time (Arefin, 2015; Ostrovsky, 2006). Recently, wireless technology has been utilized by Leviticus Cardio and they developed the wireless coplanar energy transfer (CET) system which is able to meet the usual requirements of energy of a VADs system. The group also mentioned that their innovative CET technology would play a significant role in the advancement of the VAD system as it is less sensitive to the movement of the body, minimum risk for skin-heating complications and very easy to implant (Arefin, 2015; Cardio, 2014). Consequently, Free- Range Resonant Electrical Energy Delivery (FREE-D) System have been utilized by Waters and his group and they tested this system with the axial VAD and VentrAssist VAD. Their results show that these VADs could be controlled wirelessly by employing the FREE-D system (B. H. Waters, Sample, Bonde, & Smith, 2012). They also mentioned that, for higher usability with the VADs, this FREE-D system requires the inclusion of single frequency operation and superior resonators (Arefin, 2015; B. Waters et al., 2013). Moreover, wireless power transmission in between the source and an implantable device has been studied by Kim et al., 2012 (Kim, Ho, Chen, & Poon, 2012). Also, Ho et al, 2013 investigated the idea of midfield wireless power system and demonstrated that for a cardiac-implant (Arefin, 2015; Ho, Kim, & Poon, 2013).

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

Technology certainly facilitates our daily life and it has also helped in the development of the micropumps and sizes, including the flexibility to experiment and produce new types of VAD micropumps and simultaneously minimizing the defects. It is yet to finalize to produce a pump which has low defects overall, but the momentum is rapidly going towards it. Moreover, the cost is also a bigger issue, as many patientsface difficulty due to much cost. Therefore, special attention needs to paid on minimize the cost and make it available for long term use to people (Givertz, 2011). Moreover, these micropumps can be converted into nanopumps for VAD systems, ~~because,~~ nanotechnology offers ~~huge~~ flexibility in terms of fabrication, low cost, miniaturization etc. ~~As~~ this technology is still in research, however, it surely offers a great promise and thus it will eventually help to make suitable VAD systems. Furthermore, one of the new trends would be the power consumption; voltage has to be low and to use new actuation methods or new materials(Amirouche et al., 2009). However, biocompatibility is another issueand lot of works are required to be performed in this area. In case of microchannel cooling and pumping system that could dissipate heat, design of an efficient micropump is always tough and researchers can work into this area ~~as~~. As a whole, significant amount of research is required to find an appropriate VAD system which can help the patients, especially the elders.Also, for the betterment of the VAD, some special factors should be considered:

- Small, thus it can operate both in adult and children;
- Cost effective;
- Can be easily constructed;
- Low weight;
- Portability;
- Hemodynamics including no blood-clotting factors.

On the other hand, people are specially focusing on the inclusion of wireless system which will certainly minimize the driveline infections of the VAD (Arefin, 2015). Although, researchers have started working on the wireless VAD system but it is still yet to be completed. With the inclusion of 'next generation' wireless power transfer method, the advancement of the VAD and artificial-heart-development system holds a promising future (Arefin, 2015; Miller, 2012). With the inclusion of wireless electricity acting as the source (Glass & Ponsford, 2014), wireless technology oriented control unit for different medical devices and implants (HospiMedica, 2012) and also the wireless charging will be very imperative in the development of different medical devices and various implants including the VADs in the near future (Arefin, 2015). More recently, Prof. Morsi's group in Swinburne University of Technology have been working in the development of the *next generation wireless VAD* system. An *enclosure* is currently under development, which will be placed around the cardiac system and it will be operated using wireless technology (Arefin, 2015). VADs are particularly useful for those people who have cardiac complications and/or weakened cardiac muscle – mainly the elders. In a nutshell, the VADs are constantly getting upgraded but still there are few complications which need to be avoided. Once these problems can be evaded, especially the next generation wireless VADs, patients including the elder people will enjoy more comfortable life. Also, with the advancement in the VAD micropumps and low cost, it ~~would be very to handle and~~ can be widespread to poor people. Furthermore, Ti and Ti-based alloys have received ever-increasing attention as biomaterials for VAD system due to their suitable biomechanical properties and biocompatibility. Nonetheless, surface modifications of these alloys by suitable porous polymer is still challenging task. Eventually, the manufacture and the technological control of the device together could lead to suitable and efficient VAD for widespread community of heart disease especially elderly people.

REFERENCES

Agarwal, S., & High, K. M. (2012). Newer-generation ventricular assist devices. *Best Practice & Research. Clinical Anaesthesiology*, *26*(2), 117–130. doi:10.1016/j.bpa.2012.01.003 PMID:22910085

Ahn, C. B., Son, K. H., Lee, J. J., Choi, J., Song, S. J., & Jung, J. S. et al. (2011). The effect of fluid viscosity on the hemodynamic rnergy changes during operation of the pulsatile ventricular assist device. *Artificial Organs*, *35*(11), 1123–1126. doi:10.1111/j.1525-1594.2011.01350.x PMID:21954946

Amirouche, F., Zhou, Y., & Johnson, T. (2009). Current micropump technologies and their biomedical applications. *Microsystem Technologies*, *15*(5), 647–666. doi:10.1007/s00542-009-0804-7

Anita, K., Jorma, R., Anatoli, D., & Juha, T. (2001). Effect of nickel–titanium shape memory metal alloy on bone formation. *Biomaterials*, *22*(18), 2475–2480. doi:10.1016/S0142-9612(00)00435-X PMID:11516078

Arefin, M. S. (2015). *Fluid Structure Interaction (FSI) of the Left Ventricle (LV) in developing the Next Generation Ventricular Assist Device (VAD) System.* Swinburne University of Technology.

Ashraf, M. W., Tayyaba, S., & Afzulpurkar, N. (2011). Micro Electromechanical Systems (MEMS) based microfluidic devices for biomedical applications. *International Journal of Molecular Sciences*, *12*(6), 3648–3704. doi:10.3390/ijms12063648 PMID:21747700

Awad, H., Dayem, M. A. E., Heard, J., Dimitrova, G., Yu, L., & Sun, B. C. (2010). Initial experience with off-pump left ventricular assist device implantation in single center: Retrospective analysis. *Journal of Cardiothoracic Surgery*, *5*(123). PMID:21134285

Badii, C. (2012). *Hypertensive Heart Disease*. Retrieved from http://www.healthline.com/health/hypertensive-heart-disease#Overview

Baryalai, W., Wang, Y., AlMalki, A. A., & Masoud, M. B. (2011). *The electro-mechanical stimulation of cardiac processes*. Swinburne University of Technology. Australia.

Basçiftçi, F., & Incekara, H. (2011). Web based medical decision support system application of coronary heart disease diagnosis with Boolean functions minimization method. *Expert Systems with Applications*, *38*(11), 14037–14043. doi: doi:10.1016/j.eswa.2011.04.211

Benard, W. L., Kahn, H., Heuer, A. H., & Huff, M. A. (1998). Thin-film shape-memory alloy actuated micropumps. *Microelectromechanical Systems*. *Journalism*, *7*(2), 245–251.

Bender, J. R. (1992). *Heart Valve Disease.* Retrieved from http://www.scribd.com/doc/39785094/HEART-VALVE-DISEASE

Boucek, M. M., Mashburn, C., Dunn, S. M., Frizell, R., Edwards, L., Pietra, B., & Campbell, D. (2008). Pediatric heart transplantation after declaration of cardiocirculatory death. *The New England Journal of Medicine*, *359*(7), 709–714. doi:10.1056/NEJMoa0800660 PMID:18703473

Bronzino, J. D. (2006). *Biomedical Engineering Fundamentals* (3rd ed.). CRC Press.

Buehler, W. J., Gilfrich, J. V., & Wiley, R. C. (1963). Effect of low temperature phase changes on the mechanical properties. *Journal of Applied Physics*, *34*(5), 1475. doi:10.1063/1.1729603

Cardio, L. (2014). *The Need.* Retrieved from http://trendlines.com/portfolio/leviticus-cardio/

Center, U. o. C. S. F. M. (2014). *Heart Failure Treatment.* Retrieved from http://www.ucsfhealth.org/conditions/heart_failure/treatment.html

Channel, B. H. (2014). *Heart disease - risk factors explained.* Retrieved May 28, 2014, from http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/Heart_disease_your_risk-factors_explained

Chen, Q., Harding, S. E., Ali, N. N., Lyon, A. R., & Boccaccini, A. R. (2008). Biomaterials in cardiac tissue engineering:Ten years of research survey. *Materials Science and Engineering R Reports*, *59*(1), 1–37. doi:10.1016/j.mser.2007.08.001

Council, N. H. a. M. R. (2014). *Cardiovascular Disease (NHPA).* Retrieved from http://www.nhmrc.gov.au/grants/research-funding-statistics-and-data/cardiovascular-disease

Emeto, T. I., Moxon, J. V., Rush, C., Woodward, L., & Golledge, J. (2011). Relevance of urocortins to cardiovascular disease. *Journal of Molecular and Cellular Cardiology*, *51*(3), 299–307. doi:10.1016/j.yjmcc.2011.06.002 PMID:21689660

Fogoros, R. N. (2014a). *Heart Failure - The Basics.* Retrieved from http://heartdisease.about.com/od/livingwithheartfailure/a/heart_failure.htm

Fogoros, R. N. (2014b). *What Is Diastolic Dysfunction and Diastolic Heart Failure?* Retrieved from http://heartdisease.about.com/od/livingwithheartfailure/a/diastolic_HF.htm

Foundation, H. (n. d.). *Data and statistics*. Retrieved Friedman, P. A., Kushwaha, S. S., Bruce, C. J., Park, S. J., Ladewig, D. J., Mikell, S. B., et al. (2011). Use of the aortoatrial continuity as means of providing Left Ventricular Assist Support without entering the ventricle: A feasibility study. Journal of Cardiac Failure, 17(6), 511–518. doi: PubMed10.1016/j.cardfail.2011.01.014

Fuhr, G., Hagedorn, R., Muller, T., Benecke, W., & Wagner, B. (1992). Microfabricated electrohydrodynamic (EHD) pumps for liquids of higher conductivity. *Microelectromechanical Systems*. *Journalism*, *1*(3), 141–146.

Garbade, J., Bittner, H. B., Barten, M. J., & Mohr, F.-W. (2011). Current trends in implantable Left Ventricular Assist Devices. *Cardiology Research and Practice*, *2011*, 1–9. doi:10.4061/2011/290561 PMID:21822483

Geiger, J., Arnold, R., Csatari, Z., Langer, M., & Markl, M. (2011). Whole heart 4D hemodynamics in patients with transposition of the great arteries after switch procedure. In Proeedings of the International Society for Magnetic Resonance in Medicine.19.

Givertz, M. M. (2011). Ventricular Assist Devices. *Circulation*, *124*(12), e305–e311. doi:10.1161/CIRCULATIONAHA.111.018226 PMID:21931095

Glass, N., & Ponsford, M. (2014). *Wireless electricity? It's here.* Retrieved from http://edition.cnn.com/2014/03/14/tech/innovation/wireless-electricity/

Goldstein, D. J., Oz, M. C., & Rose, E. A. (1998). Implantable Left Ventricular Assist Devices. *The New England Journal of Medicine*, *339*(21), 1522–1533. doi:10.1056/NEJM199811193392107 PMID:9819452

Guan, Y.-X., Xu, Z.-R., Dai, J., & Fang, Z.-L. (2006). The use of a micropump based on capillary and evaporation effects in a microfluidic flow injection chemiluminescence system. *Talanta*, *68*(4), 1384–1389. doi:10.1016/j.talanta.2005.08.021 PMID:18970476

Han, C. M., Kim, H., & Koh, Y. (2014). Creation of hierarchical micro/nano-porous TiO2 surface layer onto Ti implants for improved biocompatibility. *Surface and Coatings Technology*, *251*(25), 226–231. doi:10.1016/j.surfcoat.2014.04.030

Ho, J. S., Kim, S., & Poon, A. S. Y. (2013). Midfield wireless powering for implantable systems. *Proceedings of the IEEE*, *101*(6), 1369–1378. doi:10.1109/JPROC.2013.2251851

HospiMedica. (2012). *Cardiac Implants Could Potentially Be Powered Wirelessly.* Retrieved from http://www.hospimedica.com/critical_care/articles/294742895/cardiac_implants_could_potentially_be_powered_wirelessly.html

Huanga, C., Chena, R., Kea, Q., Morsi, Y., Zhanga, K., & Moa, X. (2011). Electrospun collagen-chitosan-TPUnanofibrous scaffolds for tissue engineered tubular grafts, Colloids and Surfaces. *Biointerfaces*, *82*, 307–315. doi:10.1016/j.colsurfb.2010.09.002 PMID:20888196

Hunt, S. A., Abraham, W. T., Chin, M. H., Feldman, A. M., Francis, G. S., & Ganiats, T. G. et al. (2009). 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the diagnosis and management of heart failure in adults. *Circulation*, *119*(14), e391–e479. doi:10.1161/CIRCULATIONAHA.109.192065 PMID:19324966

Institute, N. H. L. a. B. (2012). *What Is a Ventricular Assist Device?* Retrieved from http://www.nhlbi.nih.gov/health/health-topics/topics/vad/

Kang, S.-M., & Choi, S.-W. (2011). Blood flow and pressure evaluation for a pulsatile conduit-shaped Ventricular Assist Device with structural characteristic of conduit shape. *Transactions of the Korean Society of Mechanical Engineers B*, *35*(1), 1191–1198. doi:10.3795/KSME-B.2011.35.11.1191

Kim, S., Ho, J. S., Chen, L. Y., & Poon, A. S. Y. (2012). Wireless power transfer to a cardiac implant. *Applied Physics Letters*, *101*(7), 073701. doi:10.1063/1.4745600

Kudrman, J., Fousek, J., Brezina, V., Mikova, R., & Vesely, J. (2007). Titanium alloys for implants in medicine. *Metallic materials, 45*(4), 199 - 208.

Laser, D. J., Myers, A. M., Yao, S., Bell, K. F., Goodson, K. E., Santiago, J. G., et al. (2003). Silicon electroosmotic micropumps for integrated circuit thermal management. Paper presented at *the The 12th International Conference on Solid Slate Sensors.* Actuators and Microsystems, Boston. doi:10.1109/SENSOR.2003.1215275

Lavu, M., Gundewar, S., & Lefer, D. J. (2011). Gene therapy for ischemic heart disease. *Journal of Molecular and Cellular Cardiology*, *50*(5), 742–750. doi:10.1016/j.yjmcc.2010.06.007 PMID:20600100

Miller, R. (2012). 'Tetherless' power-transmission technology could radically change LVAD therapy. *Heartwire.* Retrieved from http://www.medscape.com/viewarticle/758065#1

Moazami, N., Sun, B., & Feldman, D. (2011). Stable patients on left ventricular assist device support have a disproportionate advantage: Time to re-evaluate the current UNOS policy. *The Journal of Heart and Lung Transplantation*, *30*(9), 971–974. doi:10.1016/j.healun.2011.05.004 PMID:21676630

Molina, E. J., & Boyce, S. W. (2013). Current Status of Left Ventricular Assist Device Technology. *Seminars in Thoracic and Cardiovascular Surgery*, *25*(1), 56–63. doi:10.1053/j.semtcvs.2013.02.002 PMID:23800529

Morsi, Y. S. (2011). *Tissue Engineering of Aortic Heart Valve: Fundamentals and Developments*. Nova Science Publishers.

Mozes, A. (2011). *Stem cell therapy shrinks Enlarged Hearts.* Retrieved from http://consumer.healthday.com/cardiovascular-and-health-information-20/heart-attack-news-357/stem-cell-therapy-shrinks-enlarged-hearts-650937.html

Nanci, A., Wuest, J. D., Peru, L., Brunet, P., Sharma, V., Zalzal, S., & McKee, M. D. (1998). Chemical modification of titanium surfaces for covalent attachment of biological molecules. *Journal of Biomedical Materials Research*, *40*(2), 324–335. doi:10.1002/(SICI)1097-4636(199805)40:2<324::AID-JBM18>3.0.CO;2-L PMID:9549628

National Heart. (2012). Who Needs a Heart Transplant? Retrieved from http://www.nhlbi.nih.gov/health/health-topics/topics/ht/

Nguyen, N.-T., Huang, X., & Chuan, T. K. (2002). MEMS-Micropumps: A Review. *Journal of Fluids Engineering*, *124*(2), 384–392. doi:10.1115/1.1459075

Niinomi, M. (2002). Recent metallic materials for biomedical applications. *Metallurgical and Materials Transactions. A, Physical Metallurgy and Materials Science*, *33A*(3), 477–486. doi:10.1007/s11661-002-0109-2

Niinomi, M. (2003). Recent research and development in titanium alloys for biomedical applications and healthcare goods. *Science and Technology of Advanced Materials*, *4*(5), 445–454. doi:10.1016/j.stam.2003.09.002

Nisar, A., Afzulpurkar, N., Mahaisavariya, B., & Tuantranont, A. (2008). MEMS-based micropumps in drug delivery and biomedical applications. *Sensors and Actuators. B, Chemical*, *130*(2), 917–942. doi:10.1016/j.snb.2007.10.064

Okazaki, Y., Ohta, M., Ito, Y., & Tateishi, T. (1995). Corrosion resistance of implant alloys in pseudo physiological solution and role of alloying elements in passive films. *Journal of the Japan Institute of Metals and Materials*, *59*(2), 229–236.

Online, L. T. (2013). *Cardiovascular disease (CVD).* Retrieved from http://www.labtestsonline.org.au/understanding/conditions/cvd.html

Ostrovsky, G. (2006). *The MYO-VAD™. medGadget.* Retrieved from http://www.medgadget.com/2006/07/the_myovad.html

Pamboukian, S. V. (2011). Mechanical circulatory support: We are halfway there. *Journal of the American College of Cardiology*, *57*(12), 1383–1385. doi:10.1016/j.jacc.2010.11.026 PMID:21414535

Peschar, M., Vernooy, K., Cornelussen, R. N., Verbeek, X. A. A. M., Reneman, R. S., Vos, M. A., & Prinzen, F. W. (2004). Structural, electrical and mechanical remodeling of the canine heart in AV-block and LBBB. *European Heart Journal Supplements*, *6*(suppl D), D61–D65. doi:10.1016/j.ehjsup.2004.05.017

Petrovi, D., Obrenovi, R., Trbojevi-Stankovi, J., Majki-Singh, N., & Stojimirovi, B. (2011). Cardiovascular mortality in hemodialysis patients: Clinical and epidemiological analysis. *Journal of Medical Biochemistry*, *30*(4), 302–308. doi: doi:10.2478/v10011-011-0027-1

Van de Pol, F. C. M., Van Lintel, H. T. G., Elwenspoek, M., & Fluitman, J. H. J.Pol van de. (1990). A thermopneumatic micropump based on micro-engineering techniques. *Sensors and Actuators. A, Physical*, *21*(1-3), 198–202. doi:10.1016/0924-4247(90)85038-6

Rodriguez, L. E., Suarez, E. E., Loebe, M., & Bruckner, B. A. (2013). Ventricular Assist Devices (VAD) Therapy: New Technology, New Hope? *Houston Methodist DeBakey Cardiovascular Journal*, *9*(1), 32–37. doi:10.14797/mdcj-9-1-32 PMID:23519193

Rose, E. A., Gelijns, A. C., Moskowitz, A. J., Heitjan, D. F., Stevenson, L. W., & Dembitsky, W. et al. (2001). Long-Term Use of a Left Ventricular Assist Device for End-Stage Heart Failure. *The New England Journal of Medicine*, *345*(20), 1435–1443. doi:10.1056/NEJMoa012175 PMID:11794191

Schampaert, S., van't Veer, M., van de Vosse, F. N., Pijls, N. H. J., de Mol, B. A., & Rutten, M. C. M. (2011). In vitro comparison of support capabilities of intra-aortic balloon pump and impella 2.5 Left Percutaneous. *Artificial Organs*, *35*(9), 893–901. doi:10.1111/j.1525-1594.2011.01286.x PMID:21819436

Ryhanen, J., Kallioinen, M., Tuukkanen, J., Junila, J., Niemela, K. E., Sandvik, P., & Serlo, W. (1998). In vivo biocompatibility evaluation of nickel-titanium shape memory metal alloy: Muscle and perineural tissue responses and encapsule membrane thickness. *Journal of Biomedical Materials Research*, *41*(3), 481–488. doi:10.1002/(SICI)1097-4636(19980905)41:3<481::AID-JBM19>3.0.CO;2-L PMID:9659619

Suradi, H., & Breall, J. A. (2011). Successful use of the impella device in giant cell myocarditis as a bridge to permanent left ventricular mechanical support. *Texas Heart Institute Journal*, *38*(4), 437–440. PMID:21841879

Tanser, P. H. (2006). Effects of aging on the heart and blood vessels. In R. S. Porter (Ed.), The Merck Manual Home Health Handbook (Vol. 2012). Retrieved from http://www.merckmanuals.com/home/heart_and_blood_vessel_disorders/biology_of_the_heart_and_blood_vessels/effects_of_aging_on_the_heart_and_blood_vessels.html

Tao, N., Zhang, H., Lu, J., & Lu, K. (2003). Development of nanostructures in metallic materials with low stacking fault energies during Surface Mechanical Attrition Treatment (SMAT). *Materials Transactions*, *44*(10), 1919–1925. doi:10.2320/matertrans.44.1919

Tay, W.-B., Tseng, Y.-H., Lin, L.-Y., & Tseng, W.-Y. (2011). Towards patient-specific cardiovascular modeling system using the immersed boundary technique. *Biomedical Engineering Online*, *10*(52). doi: doi:10.1186/1475-925X-10-52 PMID:21682851

Tengvall, P., Elwing, H., Sjoqvist, L., Lundstrom, I., & Bjursten, L. M. (1989). Interaction between hydrogen peroxide and titanium: A possible role in the biocompatibility of titanium. *Biomaterials*, *10*(2), 118–120. doi:10.1016/0142-9612(89)90043-4 PMID:2706298

Timms, D. (2011). A review of clinical ventricular assist devices. *Medical Engineering & Physics*, *33*(9), 1041–1047. doi:10.1016/j.medengphy.2011.04.010 PMID:21665512

Tuzun, E., Rutten, M., Dat, M., van de Vosse, F., Kadipasaoglu, C., & de Mol, B. (2011). Continuous-Flow Cardiac Assistance: Effects on Aortic Valve Function in a Mock Loop. *The Journal of Surgical Research*, *171*(2), 443–447. doi:10.1016/j.jss.2010.05.040 PMID:20828746

Vadakkumpadan, F., Arevalo, H., Prassl, A. J., Chen, J., Kickinger, F., & Kohl, P. et al. (2010). Image-based models of cardiac structure in health and disease. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *2*(4), 489–506. doi: doi:10.1002/wsbm.76 PMID:20582162

Waters, B., Reed, J., Kagi, K., Sample, A., Bonde, P., & Smith, J. (2013). A portable transmitter for wirelessly powering a ventricular assist device using the Free-Range Resonant Electrical Energy Delivery (FREE-D) System. In J. R. Smith (Ed.), *Wirelessly Powered Sensor Networks and Computational RFID* (pp. 235–247). Springer New York. doi:10.1007/978-1-4419-6166-2_14

Waters, B. H., Sample, A. P., Bonde, P., & Smith, J. R. (2012). Powering a ventricular assist device (VAD) with the free-range resonant electrical energy delivery (FREE-D) system. *Proceedings of the IEEE*, *100*(1), 138–149. doi:10.1109/JPROC.2011.2165309

Wennerberg, A., Albrektsson, T., Johansson, C., & Andersson, B. (1996). Experimental study of turned and grit-blasted screw-shaped implants with special emphasis on effects of blasting material and surface topography. *Biomaterials*, *17*(1), 15–22. doi:10.1016/0142-9612(96)80750-2 PMID:8962942

Wieneke, H., Sawitowski, T., Wnendt, S., Fischer, A., Dirsch, O., Karoussos, I. A., & Erbel, R. (2002). Stent Coating: A New Approach in Interventional Cardiology. *Hertz*, *27*(6), 518–526. PMID:12378397

Worku, B., Naka, Y., Pak, S.-W., Cheema, F. H., Siddiqui, O. T., & Jain, J. et al. (2011). Predictors of mortality after short-term ventricular assist device placement. *The Annals of Thoracic Surgery*, *92*(5), 1608–1613. doi:10.1016/j.athoracsur.2011.06.093 PMID:22051257

Xu, Y., Liang, G., Hu, G., Yang, Y., Geng, J., & Saha, P. K. (2011). (Article in Press). Quantification of coronary arterial stenoses in CTA using fuzzy distance transform. *Computerized Medical Imaging and Graphics*. PMID:21555207

Yin, Z., & Prosperetti, A. (2005). 'Blinking bubble' micropump with microfabricated heaters. *Journal of Micromechanics and Microengineering*, *15*(9), 1683–1691. doi:10.1088/0960-1317/15/9/010

KEY TERMS AND DEFINITIONS

Biventricular Assist Device (BIVAD): It is identified when left ventricular assist device (LVAD) and right ventricular assist device (RVAD) are placed and function together inside the body.

Cardiovascular Disease (CVD): It incorporates every possible disease and condition related to the cardiac system and the blood vessel.

Coronary Heart Disease (CHD): It is referred to a condition of the coronary arteries which are obstructed by oily materials/substances, known as 'plaque' or 'atheroma'. The primary functionalities of the coronary arteries are to assist circulating the blood and oxygen to the cardiac muscle.

Hemodynamic: It is referred to the conditions of the blood motion/flow.

Total Artificial Heart (TAH): It is identified as a device which completely substitutes both ventricles (i.e. left and right) of the cardiac system.

Ventricular Assist Device (VAD): It is a mechanical device which helps pumping the ventricles.

Ventricular Assist Device Micropump: It is a micro-sized pump which can be placed inside VAD to assist it in performing total functionalities.