THE EFFECT OF FIBRIN EXTRACELLULAR MATRIX PROPERTIES FOR THE IN VITRO ANGIOGENESIS ASSAY

SITI AMIRAH BINTI ISHAK

UNIVERSITI TEKNOLOGI MALAYSIA

THE EFFECT OF FIBRIN EXTRACELLULAR MATRIX PROPERTIES FOR THE IN VITRO ANGIOGENESIS ASSAY

SITI AMIRAH BINTI ISHAK

This thesis is submitted in fulfillment of the
requirements for the award of
degree of Master of Engineering (Biomedical)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JANUARY 2015

Special dedication and thanks to:

My ever present and inspiring family:

My father, Ishak Bin ABD Wahab
My mother, Che Aishah Bte Che Wang
My brother, Ahmad Syafiq Akmal Bin Ishak
My sister, Siti Khalida Binti Ishak
My sister, Siti Maisarah Binti Ishak
My youngest brother, Ahmad Luqman Hakim Bin Ishak
My youngest sister, Siti Nurfathihah Binti Ishak

My loving and supporting friend:

Nur Najiha Binti Saarani
Nik NurZuliyana Binti Rajdi
Nur Syazana Binti Rashidi
Noor Jasmawati Binti Mat Noor

ACKNOWLEDGEMENT

First and foremost, I would like to give praise and grateful to ALLAH, the Almighty, for giving me patience and hardiness in completing my research. With Allah blessings and guidance especially on the most difficult time, I was able to complete my Master project.

Next, I would like to express my sincere gratitude to my supervisor, Prof. Dr. Ir.Mohammed Rafiq Bin Dato' Abdul Kadir and my co-supervisor Dr. Irza Sukmana for being an outstanding advisor which made them a backbone of this research as well as to this thesis. Their constant support, advice, supervision and guidance from the very early stage of this research have made this work successful. Without their help and guidance, the success of this project would not have been possible. They have been extremely helpful and inspiring as well.

My thanks also go out to all my friends for being there for me, helping me as well as guiding me in conducting the research. Their presence gave me a lot of encouragement to overcome the problems I faced during the research with sheer determination. I would also like to take this opportunity to acknowledge Universiti Teknologi Malaysia (UTM) Skudai, faculty of Biosciences & Medical Engineering and Ministry of Higher Education (MOHE) for my master scholarship. Last but not least, to my parents and siblings I dedicate this dissertation to you. Your heartfelt support and unconditional love give me strength and comfort. Words cannot express how grateful I am for all the years of character building and encouragement I receive.

ABSTRACT

Angiogenesis, the formation of new vascular network from the pre-existing vasculature, has been studied in the connection to the normal developmental process as well as numerous diseases. In tissue engineering research, angiogenesis is also essential to promote micro-vascular network inside engineered tissue constructs, mimicking a functional blood vessel in vivo. Besides, the formation of new blood vessels depends strongly on the extracellular matrix such as fibrin gel. In this study, we investigate the effect of four different ratio of fibrinogen (mg/mL) to thrombin (Units/mL) composition (i.e., 2:1, 1:2, 1:8, 8:1), on the physical properties of fibrin gel and the role of fibrin gel for angiogenesis assay with HSF. Some properties of fibrin gel such as clotting time, water uptake property, turbidity and microstructure observation was studied. Higher concentration of thrombin (i.e., 8:1 mg/U) will yields a shorter clotting time of the fibrin gel when compared to the lower concentration of thrombin (i.e 1:8 mg/U). Fibrin gel with higher thrombin and lower fibrinogen concentration (i.e., 1:8 mg/U) are not stable enough to withstand the amount of PBS subjected to it hence it cannot absorb water well compared to other fibrin gel concentration. Meanwhile, at higher concentration of thrombin, the turbidity study shows the lowest absorbance compared to other samples. Different ratio of fibrinogen to thrombin may affect the microstructure of the fibrin gel produced. Microstructure observation proved that fibrin gel produced in this study shows the fibrous structure. The last part of this experiment also proved that fibrin gel with ratio of 2:1 mg/U of fibrinogen to thrombin reveal the best tube-like structure which has the highest number of nucleus as well as highest number of tube-like structure formed after 48 hours.

ABSTRAK

Angiogenesis, pembentukan rangkaian vaskular baru dari vaskulatur yang sedia ada, ianya telah dikaji berkaitan dengan proses perkembangan yang normal dan juga banyak penyakit. Dalam penyelidikan kejuruteraan tisu , angiogenesis juga penting untuk menggalakkan rangkaian mikro vaskular dalam membina kejuruteraan tisu. Selain itu, ia juga mampu menyerupai fungsi saluran darah *in vivo*. Tambahan itu pula, pembentukan saluran darah yang baru bergantung sepenuhnya pada matriks luar sel seperti gel fibrin. Dalam kajian ini, kami turut mengkaji kesan empat nisbah fibrinogen (mg/mL) dan thrombin (Unit/mL) yang berbeza komposisi terhadap sifat-sifat fizikal fibrin gel dan juga peranannya kepada assay *angiogenesis* dengan menggunakan HSF. Masa pembekuan, penyerapan air, kekeruhan dan pemerhatian mikrostruktur telah dikaji. Gel fibrin pada kepekatan thrombin yang lebih tinggi (i.e., 8:1 mg/U) akan menghasilkan masa pembekuan yang lebih pendek jika dibandingkan dengan kepekatan thrombin yang rendah (i.e 1:8 mg/U). Selain itu, gel fibrin dengan kepekatan thrombin yang tinggi dan fibrinogen yang rendah (i.e., 1:8 mg/U) tidak cukup stabil untuk menahan PBS yang menyebabkan penyerapan yang tidak baik berbanding dengan gel fibrin lain. Sementara itu, thrombin pada kepekatan yang lebih tinggi menunjukkan tahap kekeruhan yang paling rendah berbanding dengan sample yang lain. Perbezaan nisbah fibrinogen dan thrombin juga memberi kesan kepada struktur mikro gel fibrin . Pemerhatian mikrostruktur juga membuktikan bahawa gel fibrin menunjukkan struktur yang berserabut. Bahagian terakhir eksperimen ini juga membuktikan bahawa gel fibrin pada nisbah 2:1 mg / U menunjukkan struktur tiub yang paling sempurna kerana menunjukkan jumlah nukleus dan struktur tiub yang tertinggi selepas 48 jam.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CVD	-	Cardiovascular Diseases
HSF	-	Human Skin Fibroblast
VEGF	-	Vascular Endothelial Growth Factor
BFGF	-	basic fibroblast growth factor
ECM	-	extracellular matrix
FGF	-	fibroblast growth factor
PLGF	-	placental growth factor
TGF-β	-	transforming growth factor beta
CT	-	computated tomography
MRI	-	magnetic resonance imaging
PET	-	positron emission tomography
ELISA	-	enzyme linked immunosorbent assay
PBS	-	Phosphate buffered saline
BSA	-	Bovine serum albumin
FESEM	-	Field Emission Scanning Electron Microscopy

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Tissue engineering research plays an important role to support clinicians and medical experts in increasing human life quality through the development of engineered tissues and biological substitutes for a desired human organ. It also gives promises in regenerative medicine through the treatment of variety diseases by providing some biomaterials for organ transplant. To this context, tissue engineering aims to repair, replace or regenerate damaged tissues or organ in the body that was incapable to regenerate. Although there has been tremendous success in tissue engineering to support treatment of broad variety of diseases, some challenges still remain. One of the challenges is to develop a vascularized engineering tissue construct that facilitate blood microvessel network within the thick scaffold. Vascularized tissue is an essential factor for cell survival as well as tissue growth and integration (Anderson *et al*., 2011). Another challenge is also to generate an in vitro angiogenesis assays to study the step-

by-step development of microvasculature network inside engineering tissue construct (Sukmana, 2011).

Although massive progress has been made, some major problems on mass production of human tissue are still remaining. The lack of a sufficient supply of nutrients and oxygen to the implanted tissue as well as waste removal are the limiting factors for the successful implantation of tissue engineered constructs and leads in many cases to the failure of the implant (Atala, 2004). Pre-vascularization of the tissue-scaffolds construct is the most favorable trend to deal with that problem. The idea of pre-vascularization is mainly incorporation of endothelial cells or vascular-like structures in the tissue-engineered constructs and then to implant the material at the desired site. This approach could favor a link between the existing vasculature from the host and the endothelialized tissue construct and furthermore accelerate the formation of functional micro-vessels in the inner parts of the implant (Godbey and Atala, 2002).

Angiogenesis is a key mechanism on the development of new blood vessels. It was firstly pioneered by Folkman and his colleague over the last two decades when demonstrated a new capillary blood vessels in tumor progression (Folkman, 1980). Disorder angiogenesis also founded in more than 70 pathogenic problems as reported by Carmeliet (Carmeliet, 2000). The development of new blood vessels is also very important in the process of organ growth and during wound healing (Carmeliet, 2000). Some researchers have been developed a system to fabricate capillary-like network and angiogenesis in a 3-dimensional environment of bio-gel assays (Sanders 2002). Although there are some aspects of angiogenesis modulations can be described, but the formation of micro-vessels and lumen are often intracellular rather than surrounded and multi-cellular (Nakatsu *et* al., 2003).

This study aims to explore the potential use of fibrin gel as extracellular matrix for the angiogenesis assay. Angiogenesis is a complex process by which blood micro vessels developed from the pre-existing vascularate (Linnes *et al*., 2007). In this

research, the effect of different ratio of fibrinogen to thrombin for fibrin gel has been evaluated with respect to the tube-like structure development of human skin fibroblast (HSF). Fibrinogen is one of the most important natural proteins in the body that support the vascular growth during development.

1.2 Problem Statement

Tissue engineering research involves on the development of specific tissues in order to replace the damage tissue inside the body when the body is incapable of regenerating it back (Lanza *et al*., 2007). However, a vascular supply is typically required in order to support the growth and survival of growing cell within the implant (Anderson *et al*., 2011). Therefore, it is highly important to promote angiogenesis in tissue engineering so that the new blood vessel growth can support the survival of growing tissue.

The use of natural polymer scaffold is one of the possible ways to create a vascularized tissue construct within the implant. Natural polymers have been widely used in many applications in biomedical as well as tissue engineering compared to synthetic polymer. Collagen is one of the popular natural polymers that have been used in research that related to the development of angiogenesis. However, there are still drawbacks of using collagen as the scaffold because collagen can be very difficult to purify while maintaining their architecture (Dubiel *et al.*, 2011). During the process of purification, small amounts of impurities may cause unexpected cell response that will influence the result in culture system. Besides collagen, fibrin gel is another natural polymer that seems to have potential in supporting the development of angiogenesis (Sukmana, 2012). In this research, instead of using collagen, the biophysical properties of fibrin gel were investigated in order to observe their effect toward the development of angiogenesis study.

Angiogenesis is one of the important process and fundamental to many physiological and pathological diseases especially in cardiovascular diseases, chronic wound healing and also to overcome problem related to tissue engineering. All of these diseases are characterized by dependence on a new blood vessel formation for adequate supply of oxygen, nutrients as well as removal of waste (Griffioen and Molema, 2000). Although there have been some aspects on the development of angiogenesis research, however, the step-by-step of tube-like structure formation is still lacking such as the effect of fibrinogen to thrombin concentration on the tube-like structure development.

1.3 Research Objective

The objectives of this study were:

a) To modulate the effect biophysical properties of fibrin gel for three dimensional angiogenesis assays.

b) To study the role of fibrin gel on angiogenesis development of Human Skin Fibroblast (HSF)

1.4 Scope of Research

The study is conducted to produce fibrin gel for the *in vitro* angiogenesis assays of HSF by modulation of fibrinogen and thrombin concentrations. Different ratio of fibrinogen as well as thrombin will be studied in order to investigate their effect toward the biophysical properties of fibrin gel produced. The variation of these properties will

be then manipulated to be used for *in vitro* angiogenesis with HSF (human skin fibroblast).

1.5 Significance of Research

Fibrin gel has broad applications in various biomedical engineering fields, including haemostate glue and wound repair, drug delivery, cell delivery, cell differentiation and also tissue engineering. In addition, fibrin gel continue revealing its fascinating features of self-assembly and soft elasticity by which it can resist stretching to more than five times its resting length without breakage. Although fibrin gel provide many potential in clinical application especially wound healing, but the healing of any skin wound other than the most superficial cannot occur without angiogenesis. This is because, not only the damage vasculature need to be repaired but in order to increased local cell activity, increased supply of nutrients from the bloodstream is typically required in the process of wound healing. This is why, the biophysical properties of fibrin gel is very important as its function is normally used to support the angiogenesis process. Therefore, this study will help to expand the research and development in regenerative medicine and tissue engineering.

REFERENCES

Anderson, S.M., Siegman, S.N., Segura, T. (2011). The effect of vascular endothelial growth factor (VEGF) presentation within fibrin matrices on endothelial cell branching. *Biomaterials*. 32, 7432-7443.

Atala, A. (2004). Tissue engineering and regenerative medicine: concepts for clinical application. *Rejuvenation Research*. **7**, 15-31

Auerbach, R., Lewis, R., Shinners, B., Kubai, L., Akhtar, N. (2003). Angiogenesis Assays : A Critical Overview. *Clinical Chemistry*. 49, 32-40

Alessi, P., Ebbinghaus, C., Neri, D. (2004). Molecular targeting of angiogenesis. *Biochimica et Biophysica Acta*. 1654, 39-49.

Bauer, S.M., Bauer, R.J., Velazquez, O.C. (2005). Angiogenesis, Vasculogenesis, and Induction of Healing in Chronic Wounds. *Vascular and Endovascular Surgery*.39, 293-306

Bayless, K.J., Davis, G.E. (2003). Sphingosine-1-phosphate markedly induces matrix metalloproteinase and integrin –dependant human endothelial cell invasion and lumen formation in three-dimensional collagen and fibrin matrices. *Biochemical and Biophysical Research Communications*. 312, 903-913

Benjamin, CJ., Rebecca, SM., Daniel, LC. (2006). GCSF and AMD3100 mobilize monocytes into the blood that stimulate angiogenesis in vivo through a paracrine mechanism and AMD3100 mobilize monocytes into the blood that stimulate angiogenesis in vivo through a paracrine mechanism. *Blood*. 108, 2438- 2445.

Berger, A.C., Wang, X., Zalatoris, A., Cenna, J., Watson, J.C. (2004). A murine model of ex vivo angiogenesis using aortic disks grown in fibrin clot. *Microvascular Research.* 68, 179-187

Blomback, B., Bark, N. (2004). Fibrinopeptides and fibrin gel structure. *Biophysical Chemistry*. 112, 147-151

Blomback. B., Carlsson. K., Hessel, B., Liljeborg, A., Procyk, R., and Aslund, N. (1989). Native fibrin gel networks observed by 3D microscopy, permeation and turbidity. *Biochim. Biophys. Acta*. 997, 96-110Chaidez, C., Lopez, J., Campo, N.C. (2007). Quaternary ammonium compound : an alternative disinfection method for fresh produce wash water. *Journal of Water and Health*. 52, 1-8.

Briganti, E., Spiller, D., Mirtelli, C., Kull, S., Counoupus, C., Losi, P., Senesi, S., Stefano, R.D., Soldani, G. (2010). A composite fibrin-based scaffold for controlled delivery of bioactive pro-angiogenetic growth factors. *Journal of Controlled Release*. 142, 14-21

Caiado, F., Carvalho, T., Silva, F., Castro, C., Clode, N., Dye, J.F., Dias, S. (2011). The role of fibrin E on the modulation of endothelial progenitors adhesion, differentiation and angiogenic growth factor production and the promotion of wound healing. *Biomaterials*. 32, 7096-7105

Carmeliet, P. (2000). Mechanisms of angiogenesis and arteriogenesis. *Nature Medicine.* 6, 389-395

Carmeliet, P and Tessier-Lavi gne, M.(2005). Common mechanisms of nerve and blood vessel wiring. *Nature*. 436, 195-200.

Christ, PC., Jacquesmorel, CM., Asifamin, M., Matthew, CA., Lisa, HA., & Alisa, KE. (2001). Evidence of IL-18 as a novel angiogenesis mediator. *The Journal of Immunology.* 167, 1644 1653.

Chung, A.S., and Ferrara, N. (2010). The Extracellular Matrix & Angiogenesis. *Pathway*. 11,1-5.

Cheong, W., Prahl, S.A., Welch, A.J. (1990). A Review of the Optical Properties of Biological Tissues. *Journal of Quantum Electronics*. 26, 2166-2185.

Cole, R.W., Liu, F., Herron, B.J. (2010). Imaging of angiogenesis: past, present and future. *Microscopy: Science, Technology, Applications and Education.* pp.885-895.

Donovan, D., Brown, N.J., Bishop, E.T., Lewis, C.E. (2001). Comparison of three in vitro human "angiogenesis" assays with capillaries formed in vivo. *Angiogenesis*. 4, 113-121.

Duong, H., Wu, B., Tawil, B. (2009). Modulation of 3D Fibrin Matrix Stiffness by Intrinsic Fibrinogen – Thrombin Compositions and by Extrinsic Cellular Activity. *Tissue Engineering*. 15, 1865-1875

Drinnan, C.T., Zhang, G., Alexander, M.A., Pulido, A.S., Suggs, L.J. (2010). Multimodal release of transforming growth factor-β1 and the BB isoform of platelet derived growth factor from PEGylated fibrin gels. *Journal of Controlled Release*. 147, 180-186

Elcin, A.E., and Elcin, Y.M. (2006). Localized Angiogenesis Induced by Human Vascular Endothelial Growth Factor-Activated PLGA Sponge. *Tissue Engineering*. 12(4), 959-968.

Elcin, Y.M., Dixit, V., Gitnick, G. (2001). Extensive In Vivo Angiogenesis Following Controlled Release of Human Vascular Endothelial Cell Growth Factor: Implications for Tissue Engineering and Wound Healing. *Artificial Organs.* 25(7), 558-565.

Fischer, R.S., Myers, K.A., Gardel, M.L., Waterman, C.M. (2012). Stiffness-controlled three-dimensional extracellular matrices for high-resolution imaging of cell behavior. *Nature Protocols*. 7, 2056-2066.

Francis, M.E., Uriel, S. and Brey, E.M. (2008). "Endothelial cell-matrix interactions in neovascularization". *Tissue Engineering*. 14, 19-32

Folkman, J., and Haudenschild, C. (1980). Angiogenesis in vitro. *Nature*. 288, 551-556

Geckil, H., Xu, F., Zhang, X., Moon, S., Demirci, U. (2010). Engineering hydrogels as extracellular matrix mimics. *Nanomedicine*. 3, 469-484.

Godbey W.T., Atala A. (2002). In Vitro Systems for Tissue Engineering. *Annals New York Academy of Sciences*. **961**, 10–26

Griffioen, A.W. and Molema, G. (2000). Angiogenesis: Potential for Pharmacologic Intervention in the Treatment of Cancer, Cardiovascular Diseases, and Chronic Inflammation. *Pharmacological Reviews*. 52, 237-268.

Guedez, L., Rivera, A.M., Salloum, R., Miller, M.L., Diegmueller, J.J., Bungay, P.M., Stevenson, W.G.S. (2003). Quantitative Assessment of Angiogenic Responses by the Directed in Vivo Angiogenesis Assay. *American Journal of Pathology*. 162, 1431-1439

Gu, F., Amsden, B., Neufeld, R. (2004). Sustained delivery of vascular endothelial growth factor with alginate beads. *Journal of Controlled Release*. 96(3), 463-472

Hall, H., Baechi, T., Hubbell, J.A. (2001). Molecular Properties of Fibrin-Based Matrices for Promotion of Angiogenesis *in Vitro*. *Microvascular Research.* 62, 315-326

Hoeben, A., Landuyt, B., Highley, M.S., Wildiers, H., Oosterom, A.T.V., De Bruijn, E.A. (2004). Vascular Endothelial Growth Factor and Angiogenesis. *Pharmacological Reviews*. 56, 549-580.

Ingber, D.E. (2002). Mechanical Signaling and the Cellular Response to Extracellular Matrix in Angiogenesis and Cardiovascular Physiology. *Circulation Research*. 91, 877-887.

Irza Sukmana (2010). Development of Tridimensional Cell Culture System to Orient Microvessel Formation. Doctor Philosophy. Universite De Sherbrooke, Canada.

Ishak, S.A and Sukmana, I. (2013). Modulation of Fibrin Gel Extracellular Matrix Properties by Fibrinogen and Thrombin Concentration for Angiogenesis Assay. *Advanced Materials Research.*911, 342-346

Isner, J.M. and Asahara, T. (1999). Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. *The Journal of Clinical Investigation*. 103, 1231-1236.

Janmey, P.A., Winer, J.P., Weisel, J.W. (2009). Fibrin Gels and Their Clinical and Bioengineering Applications. *Journal of the Royal Society Interface*.6, 1-10

Kerbel, R.S. (2001). Clinical Trials of Antiangiogenic Drugs: Opportunities, Problems and Assessment of Initial Results. *Journal of Clinical Oncology*. 19,45-51.

Kim, K.M. and Evans, G.R.D. (2005). Tissue Engineering : The Future of Stem Cells. *VIII Stem Cells.* 2, 2-21.

Lanza, R.P., Langer, R.S., Vacanti, J. (2007). Principles of tissue engineering. 3rd ed. Boston : Elsevier Academic Press

Laurens, N., Koolwijk, P., De Maat, M.P.M. (2006). Fibrin structure and wound healing. *Journal of Thrombosis and Haemostasis*. 4, 932-939

Losordo, D.W., Dimmeler, S. (2004). Therapeutic Angiogenesis and Vasculogenesis for Ischemic Disease: Part II: Cell-Based Therapies. *Circulation*. 109, 2692-2697.

Li, J., Zhang, Y.P., Kirsner, R.S. (2003). Angiogenesis in Wound Repair : Angiogenic Growth Factors and the Extracellular Matrix. *Microscopy Research and Technique*. 60, 107-114

Linnes, M.P., Ratner, B.D., Giachelli, C.M. (2007). A fibrinogen-based precision microporous scaffold for tissue engineering. *Biomaterials*. 28, 5298-5306

Litvinov, R.I., Gorkun, O.V., Owen, S.F., Shuman, H., Weisel, J.W. (2005). Polymerization of fibrin : specificity, strength, and stability of knob-hole interactions studied at the single –molecule level. *Blood.* 106, 2944-2951.

Lijnen, H.R. (2008). Angiogenesis and obesity. *Cardiovascular Research.* 78, 286-293.

Ma L, Gao C Y, Mao Z W, Zhou J, Shen J C, Hu X Q and Han C M. (2003). Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. *Biomaterials.* 24**,** 4833–41

Makoto, N., Kimio, S., Yoshihiro, F., Yoshitaka, I., Yutaka, K., Naoto, I., Kazuo, S., & Hiroaki, S. (2007). Important role of erythropoietin receptor to promote VEGF expression and angiogenesis in peripheral ischemia in mice. *Circulation Research.* 100, 662-669.

Mckee, P.A., Mattock, P., Hill, R.L. (1970). Subunit Structure of Human Fibrinogen, Soluble Fibrin, and Cross-Linked Insoluble Fibrin. *Proceedings of the National Academy of Sciences.* 66, 738-744.

Miller, J.C., Pien, H.H., Sahani, D., Sorensen, A.G., Thrall, J.H. (2005). Imaging Angiogenesis : Applications and Potential for Drug Development. *Journal of the National Cancer Institute*. 97, 172-187

Mima, Y., Fukumoto, S., Koyama, H., Okada, M., Tanaka, S., Shoji, T.,Emoto, M., Furuzono, T., Nishizawa, Y., Inaba, M. (2012). Enhancement of Cell-Based Therapeutic Angiogenesis Using a Novel Type of Injectable Scaffolds of Hydroxyapatite-Polymer Nanocomposite Microspheres. *PLoS ONE*. 7(4)

Nakatsu, M.N., Hughes, C.C.W. (2008). An optimized three-dimensional in vitro model for the analysis of angiogenesis. *Methods Enzymol*. 443, 65-82

Nakatsu, M.N., Sainson, R.C.A., Aoto, J.N., Taylor, K.L., Aitkenhead, M., Perez-del-Pulgar, S., Carpenter, P.M., Hughes, C.C.W. (2003). Angiogenic sprouting and capillary lumen formation modeled by human umbilical vein endothelial cells (HUVEC) in fibrin gels: the role of fibroblasts and Angiopoiten-1. *Microvascular Research.* 66, 102-112.

Nehls, V. and Herrmann, R. (1996). The configuration of fibrin clots determines capillary morphogenesis and endothelial cell migration. *Microvascular Research.* 51, 347-364.

Palmowski, M., Huppert, J., Ladewig, G., Hauff, P., Reinhardt, M., Mueller, M.M., Woenne, E.C., Jenne, J.W., Maurer, M., Kauffmann, G.W., Semmler, W., Kiessling, F. (2008). Molecular profiling of angiogenesis with targeted ultrasound imaging : early assessment of antiangiogenic therapy effects. *American Association for Cancer Research.* 7, 101-109

Phung, M.W., and Dass, C.R. (2006). In -vitro and In –vivo assays for angiogenesis modulating drug discovery and development. *Journal of Pharmacy and Pharmacology*.58, 153-160

Risau, W., Sariola, H., Zerwes, H.G., Sasse, J., Ekblom, P., Kemler, R., Doetschman, T. (1988). Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. *Development.* 102, 471-478.

Rimann, M. and Graf-Hausner, U. (2012). Synthetic 3D multicellular systems for drug development. *Current Opinion in Biotechnology.* 23, 1-7

Rowe, S.L., Lee, S.Y., Stegemann, J.P. (2007). Influence of Thrombin Concentration on the Mechanical and Morphological Properties of Cell-seeded Fibrin Hydrogels. *Acta Biomaterial.* 3(1), 59-67.

Standeven, K.F., Ariens, R.A.S., Grant, P.J. (2005). The molecular physiology and pathology of fibrin structure/function. *Blood Reviews.*19, 275-288

Stephanou, A., Meskaoui, G., Vailhe, B., Tracqui, P. (2007). The rigidity in fibrin gels as a contributing factor to the dynamics of *in vitro* vascular cord formation. *Microvascular Research*. 73, 182-190

Shah G A, Nair C H and Dhall D P. (1985). Physiological studies on fibrin network structure. *Thromb Res.* 40 181–8.

Sukmana, I and Vermette, P. (2010). The Effect of co-culture with fibroblast and angiogenic growth factor on microvascular matura_on and multicellular lumen formation in HUVEC-oriented polymer fibre constructs. *Biomaterial*. 31, 5100-5109

Sukmana, I., and Vermette, P. (2010). Polymer fibers as contact guidance to orient microvascularization in a 3D environment. *J Biomed Mater Res* A. 92, 1587-97

Sukmana, I. (2011). Microvascular Guidance: A Challenge to Support the Development of Vascularised Tissue Engineering Construct. *The Scientific World Journal*. 10, 1-10

Sukmana, I. (2012). Bioactive polymer scaffold for fabrication of vascularized engineering tissue. *Journal of Artificial Organs*. 15, 215-224

Tahergorabi, Z. and Khazaei, M. (2012). A Review on Angiogenesis and Its Assays. *Iranian Journal of Basic Medical Sciences.* 15, 1110-1126

Vagaska B, Bacakova L, Filova E, Balik K. (2010). Osteogenic Cells on Bio-Inspired Materials for Bone Tissue Engineering. Physiological Research. 59, 309-322.

Vailhe, B., Vittet, D., Feige, J.J. (2001). In vitro Models of Vasculogenesis and Angiogenesis. *Laboratory Investigation.* 81, 439

Weigandt, K.M., White, N., Chung, D., Ellingson, E., Wang, Y., Fu, X., Pozzo, D.C. (2012). Fibrin Clot Structure and Mechanics Associated with Specific Oxidation of Methionine Residues in Fibrinogen. *Biophysical Journal*. 103, 2399-2407.

Wolberg, A.S. (2007). Thrombin generation and fibrin clots structure. *Blood Reviews*. 21, 131-142

Wolberg, A.S. and Campbell, R.A. (2008). Thrombin generation, fibrin clot formation and hemostasis. *Transfusion and Apheresis Science*. 38, 15-23.

Yancopoulus, G.D., Davis, S., Gale, N.W., Rudge, J.S., Wiegend, S.J., Holash, J. (2000). Vascular-specific growth factors and blood vessel formation. *Nature*. 407, 242-248

Zhao, H., Ma, L., Zhou, J., Mao, Z., Gao, C., Shen, J. (2008). Fabrication and physical and biological properties of fibrin gel derived from human plasma. *Biomedical Materials.* 3, 1-9.