Modulation of Fibrin Gel Extracellular Matrix Properties by Fibrinogen and Thrombin Concentrations for Angiogenesis Assay

by Irza Sukmana

Submission date: 04-Dec-2019 03:04PM (UTC+0800)
Submission ID: 1226703986
File name: 3774.pdf (157.34K)
Word count: 2109
Character count: 11452

Advanced Materials Research Vol. 911 (2014) pp 342-346
© (2014) Trans Tech Publications, Switzerland
doi:10.4028/www.scientific.net/AMR.911.342

Modulation of Fibrin Gel Extracellular Matrix Properties by Fibrinogen and Thrombin Concentrations for Angiogenesis Assay

Siti Amirah Ishak[1, a] and Irza Sukmana[1, 2, b]

[1]IJN-UTM Cardiovascular Engineering Centre (CEC) and Medical Devices Technology Research Group (MEDITEG), Faculty of Biosciences & Medical Engineering, UTM Skudai, 81310 Malaysia

[2]Department of Mechanical Engineering, Faculty of Engineering, University of Lampung, Jalan Prof. Dr. Soemantri Brojonegoro No. 1, Bandar Lampung 35143, Indonesia

[a]myra_huhu1990@yahoo.com.my, [b]irza.sukmana@gmail.com

Corresponding author

Keywords: Angiogenesis assay, Fibrin gel, Extracellular matrix, Biophysical properties.

Abstract. Angiogenesis is the formation of new microvascular network from the pre-existing blood vessel. In tissue engineering approaches, angiogenesis is essential for the promotion of micro-vascular network inside engineered scaffold construct, mimicking a functional blood vessel in vivo. In the in vivo system, the formation of new blood vessels depends on the properties fibrin gel extracellular matrix. In this study, we have investigated the effect of different fibrinogen and thrombin composition on the biophysical properties of fibrin gel. Higher concentration of thrombin (4.0 Units/milliliter) yields a shorter clotting time of the fibrin gel and result in better water uptake property while at lower concentration of thrombin (0.5 Units/milliliter), the clotting time takes much longer. Also, at lowest concentration ratio of fibrinogen to thrombin (0.5 milligram/milliliter to 4.0 Units/milliliter), the turbidity study shows the lowest absorbance compared to other samples. Different concentration of fibrinogen and thrombin also affect the microstructure of the fibrin gel. The variation of these properties will be then manipulated to be used for in vitro angiogenesis. This study opens broader application of fibrin extracellular matrix in regenerative medicine and tissue engineering researches.

Introduction

There have been growing interests in the development and investigation of new biomaterials for biomedical and tissue engineering fields. Tissue engineering is a new multidisciplinary research field. It involves the use of living cells and developing a biological substitute for implantation into the human body [1]. The development of biomaterial science and tissue engineering fields open a great demand for the replacement of damaged tissue and organ in this growing population [2]. These medical concepts represent a new era in our efforts to treat problems associated with failing tissues and organs.

Angiogenesis is a dynamic process, start with endothelial cells (ECs) migration to develop new micro-capillaries which will not only depend on the cytokines present but also the cells organization and extracellular matrix (ECM) properties [2,3]. During micro-capillaries development, the interaction between extracellular matrix and cell are very crucial, and as well as for further required steps during vessels' maturation [3]. Fibrin is one of the native ECM components that involved during EC morphogenesis and angiogenesis development. In this context, fibrin matrix has been widely used for the investigation of angiogenic factors involving in ECs differentiation with and without growth factors [4,5].

The objective of this present study is to investigate the effect of different fibrinogen/thrombin compositions on their biomechanical and biophysical properties. The variation of those properties will be proposed to be used as a standard for further in vitro angiogenesis assay.

Materials and Methods

Chemicals and fibrin gel preparation. Phosphate Buffered Saline (PBS, P3813), Fibrinogen (F4753), and Thrombin (T4648) were purchased from PromoCell (Germany), Sigma-Aldrich). Fibrin gel was prepared by mixing fibrinogen and thrombin solutions in four different concentrations of Fibrinogen (mg/mL) to Thrombin (Units/mL or U/mL), they are including: 2.0/1.0, 2.0/4.0, 0.5/4.0, 4.0/0.5.

Measurements. To measure the clotting time, after mixing the solutions of fibrinogen and thrombin, the gel was left at room temperature. The clotting time were observed in naked eye and when the tip of pipette cannot penetrate the gel that will be the clotting time of that particular fibrin gel. The clotting time of the gel was recorded using a stop timer. Water uptake property was measured at room temperature as follow: 1mL of PBS is transferred into the gel using pipette and soaked in the fibrin gel. After 2 hour, PBS which does not diffuse to the gel will be collected using pipette and volume is measured. The same procedure is repeated for intervals of 4 hour, 6 hour, and 24 hour. Turbidity was measured using fibrin sample inside the cuvette then turbidity was recorded using UV-VIS Spectrophotometer (Shidmadzu-UV 1201). The wavelength was 550nm, as suggested elsewhere [6].

Results and Discussions

Clotting time. The fibrin gel can be easily formed by mixing solutions of Fibrinogen and Thrombin. After clotting for a several minutes, the solution will lost its fluidity and transformed into gel. From the graph, the concentration of 4.0/0.5 has the highest clotting time which is 329 seconds while the lowest clotting time is for the concentration of 0.5/4.0, which is 59 seconds. The concentration of 2.0/1.0 and 2.0/4.0 has the clotting time of 199 second and 92 seconds respectively. The graph shows that along with an increase of the thrombin concentration, the clotting time at room temperature decreases rapidly from about 300s for 0.5U/mL thrombin concentration to about 100-50s for 4.0U/mL.

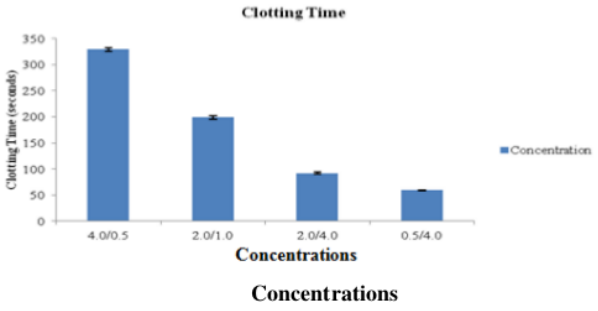

Fig. 1. Clotting time properties in different fibrin/thrombin concentration

Physiologically, fibrin gel is formed by the reaction of fibrinogen and thrombin. Fibrinogen is a rod-shaped protein in which it will be converted into fibrin monomers in the presence of enzyme called thrombin. During the reaction, thrombin will cleave at specific peptide bond in the fibrinogen to produce fibrin clots [7,8]. With the increase of thrombin concentration, more fibrinopeptides will be produced due to the cleavage of fibrinogen by thrombin which leads to a shorter clotting time. Meanwhile, no significance difference can be found when the concentration of fibrinogen increased. Figure 1 shows that as the thrombin concentration increases, the clotting time will decreases significantly. Our results are in good agreement with other previous study [8].

Water Uptake Property. Higher thrombin concentration will results in better water uptake property of the fibrin gel. Figure 2 shows that water uptake of all samples increased with time until 24 h. From

the graph, the concentration 0.5/4.0 has the highest water uptake property followed by the concentration of 2.0/4.0, concentration 2.0/1.0 and finally the concentration of 4.0/0.5 which has the lowest water uptake property.

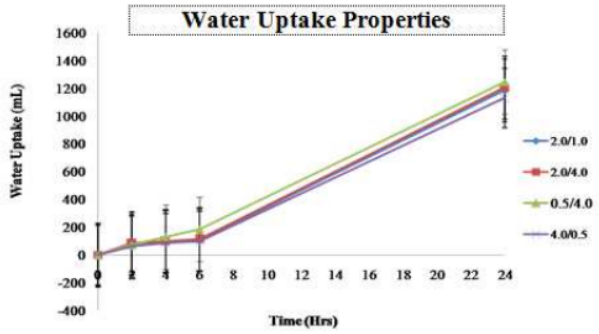

Fig. 2. Water uptake properties over time

In Figure 2, concentration 0.5/4.0 shows the highest water uptake property due to the slower shrinkage process of the gel thus it can adsorbed more water. While, at the concentration of 4.0/0.5, it shows the lowest water uptake property compared to other concentration because of the progressive shrinkage of fibrin gel. Therefore, it cannot absorb water well. Higher thrombin concentration will results in better water uptake property of the fibrin gel. This is due to the fact that, at higher concentration of thrombin, the cross-link fibrin gel should have a more complete cross linking structure which has stronger ability to resist the shrinkage [9]. Therefore, more water can be absorbed into the fibrin gel compared to the lowest concentration. After 24 hour, the remaining volume of PBS is increased exceeding the initial volume. This is because of the shrinkage process of fibrin gel, where the gel will finally shrink and released the water content.

Turbidity. Turbidity known as the decreased in the transparency of solution caused by the suspended solution that generally invisible to the naked eye, which causes the light to be scattered as transmitted in a straight lines. The higher the light scattered, the higher the value of turbidity. In this study, turbidity measurement was monitored by UV-VIS spectroscopy. Figure 3 shows that absorbance at 550nm increased with time for all samples with different concentration of fibrinogen and thrombin. Based on the graph, concentration of 4.0/0.5 shows the highest absorbance followed by the concentration of 2.0/1.0, 2.0/4.0 and finally concentration of 0.5/4.0 which has the lowest value of absorbance.

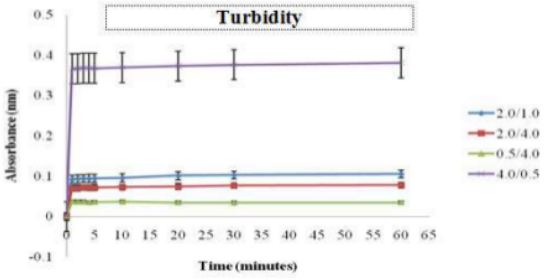

Fig. 3. Turbidity property presented as absorbance in different Time

There are 2 types of fibrin clot which are known as rigid clot and malleable clot. Rigid clots have long and thicker fibrin fibres while malleable clots have thinner and shorter fibrin fibers [9-10]. The rigidity and thickness of fibrin gels can be determine through the turbidity measurement which can be measured using UV-VIS Spectroscopy. At higher concentration of fibrinogen, more fibrinopeptides

will be produced by the cleavage of fibrinogen which prevents the light from moving in a straight line. Therefore, more light is scattered and absorbed by the fibrin gel which increase the value of absorbance thus increased the value of turbidity. Besides, it can be clearly seen that fibrin gel at concentration of 4.0/0.5 is the cloudiest as compared to other concentration. At concentration of 4.0/0.5, the gel is more turbid while at concentration of 0.5/4.0, the gel is less turbid because the absorbance is the lowest. According to the figure 3, all values of absorbance increased and becomes almost constant as the polymerization process of fibrin gel is completes. Besides, fibrin gels that are malleable and low-absorbance can stimulate the formation of tube like structure but at the same time inhibit the cell migration whereas fibrin gels that are rigid and high absorbance can promote cell migration but inhibit capillary formation as concluded by other researchers [10,11].

Conclusions

This study is to investigate the effect of different fibrinogen and thrombin compositon on the physical properties of fibrin gel. The clotting time of the fibrin gels is significantly influenced by different concentration of thrombin, while less influenced by the concentration of fibrinogen. The freeze dried also shows the fibrous structure of fibrin gel in which the porosities and stiffness can be seen well. Higher thrombin concentration result in better water uptake property of fibrin gels due to the fact that it can resist the process of shrinkage and maintain its structure. Fibrin gel properties are greatly influenced by the concentrations of thrombin and less influenced by the concentration of fibrinogen. Besides, *in vitro* cell culture found that HUVEC could normally proliferate well in the fibrin gel while forming tube like structure.

Acknowledgements

The authors would like to acknowledge the financial support from UTM Tier-1 (vote# 03H12), MOHE-FRGS (vote# 4F128) and UTM RU (vote# 00G670).

References

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

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

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

[4] H. Duong, B. Wu, B. Tawil, Modulation of 3D Fibrin Matrix Stiffness by Intrinsic Fibrinogen-Thrombin Compositions and by Extrinsic Cellular Activity, Tissue Engineering. 15 (2009) 1865-1876.

[5] I. Sukmana, Microvascular guidance: A challenge to support the development of vascularised tissue engineering construct, The Scie World J. 1 (2012) 1-10.

[6] I.N. Chernysh, C. Nagawami, J.W. Weisel, Visualization and identification of the structures formed during early stages of fibrin polymerization, Blood. 117 (2011) 4609-4614.

[7] L. Ma, C Y. Gao, Z W. Mao, J, Zhou, J C. Shen, X Q. Hu and C M. Han, Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering, Biomaterials. 24 (2003) 4833–4841.

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

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

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

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

Modulation of Fibrin Gel Extracellular Matrix Properties by Fibrinogen and Thrombin Concentrations for Angiogenesis Assay

ORIGINALITY REPORT

19%	11%	17%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Posters", Polymers for Advanced Technologies, 2013. Publication	2%
2	fcee.utm.my Internet Source	2%
3	Jasmawati, Noor, Joy R.P. Djuansjah, Mohammed Rafiq Abdul Kadir, and Irza Sukmana. "Porous Magnesium Scaffolds for Bone Implant Applications: A Review", Advanced Materials Research, 2015. Publication	2%
4	Haiguang Zhao. "A polylactide/fibrin gel composite scaffold for cartilage tissue engineering: fabrication and an in vitro evaluation", Journal of Materials Science Materials in Medicine, 01/2009 Publication	2%
5	Haiguang Zhao, Lie Ma, Changyou Gao, Jinfu Wang, Jiacong Shen. "Fabrication and	1%

properties of injectable β-tricalcium phosphate particles/fibrin gel composite scaffolds for bone tissue engineering", Materials Science and Engineering: C, 2009
Publication

6	www.icrmm.org Internet Source	1%
7	Safuan, Nadia, Irza Sukmana, Mohammed Rafiq Abdul Kadir, and Deni Noviana. "The Evaluation of Hydroxyapatite (HA) Coated and Uncoated Porous Tantalum for Biomedical Material Applications", Journal of Physics Conference Series, 2014. Publication	1%
8	Robert M. Nerem. "Tissue Engineering: The Hope, the Hype, and the Future", Tissue Engineering, 05/2006 Publication	1%
9	eprints.uthm.edu.my Internet Source	1%
10	Submitted to University College London Student Paper	1%
11	iopscience.iop.org Internet Source	1%
12	Sukmana, I, and J R P Djuansjah. "Sandwiched polymer fibre in fibrin matrices for the dictation	1%

of endothelial cells undergoing angiogenesis", Journal of Physics Conference Series, 2013.
Publication

13	Submitted to University of Florida Student Paper	1%
14	hdl.handle.net Internet Source	1%
15	Yu-Tong Hong, Daniel T. Bregante, Johnny Ching-Wei Lee, Yongbeom Seo et al. "Catalytic Microgelators for Decoupled Control of Gelation Rate and Rigidity of the Biological Gels", Journal of Controlled Release, 2019 Publication	<1%
16	ethesis.inp-toulouse.fr Internet Source	<1%
17	Greeshma Ratheesh, Jayarama Reddy Venugopal, Amutha Chinappan, Hariharan Ezhilarasu, Asif Sadiq, Seeram Ramakrishna. "3D Fabrication of Polymeric Scaffolds for Regenerative Therapy", ACS Biomaterials Science & Engineering, 2017 Publication	<1%
18	es.scribd.com Internet Source	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		