Summer 2016

Fibrin Gel Research Paper

REHSS – UNIVERSITY OF SOUTH FLORIDA

SHARAN POONJA

Table of Contents

ABSTRACT **3**
INTRODUCTION **3**
METHODS AND MATERIALS **4**
RESULTS **5**
DISCUSSION **6**
REFERENCES **7**

Abstract

Blood coagulation is the basis of chronic wound healing and is common to all minor and major lacerations and injuries. Based on the various different proteins and particles in the blood it is common to see blood coagulation formed due to the thrombin and the fibrinogen in the blood plasma. The fibrin gel formed created a thin layer and helped attach to the cultured cells. This experiment created no direct instruction or relationship that could be obtained but it was found that the fibrin gels formed by process of dip coding had a better bond with the cultured cells than any other method.

Introduction

The depth of the research experiment can be developed and comprehended as a vast application of the biomedical and engineering fields. Blood coagulation is the basis of many organisms' survival as it protects from mass blood loss. The foundation for major blood coagulation is dependent on various conditions and proteins found directly in the blood plasma of an organism. Fibrinogen is a basic glycoprotein component used directly in blood coagulation as it develops a strong bond and determines the strength and firmness of the blood clot formed. Thrombin an enzyme catalyst used in combination with the fibrinogen helps form the blood clot as it activates the enzyme and allows for the reaction to continue and affects the rate of formation. The formation of this fibrin gel that is developed through the experimentation helps demonstrate a model for blood clotting and allows for the observation of the gel in high focus.

It was hypothesized that the thinner the fibrin gel formed the easier it is to observe the relationship between the gel and the cultured cells. This can be predicted based on the various different conditions such as the layering of the fibrin gel when viewed under the microscope is composed of many integral chains and bonds that block the observation of the cells on the top layer. Therefore, in order to observe the best reaction and relationship between the cultured cells and the fibrin gel layer, the thinnest layer is the best option to observe. In order to obtain the thinnest fibrin gel layer, two quite different procedures are used to develop and test the basis of fibrin gel formation: dip coded layer and plasma etch.

Methods and Materials

First Method derived from Sigma Aldrich: 1.8 mL of DMEM poured into a test tube along with 0.2 mL of Fibrinogen stock. 40 μL of Calcium Chloride and 2 μL of thrombin stock is poured into the test tube. The solution was mixed and 1 mL was placed into 2 mini well containers and placed in the incubator at 37 C. After 15 minutes, the gels would harden and be observed under the microscope and any observations would be recorded. This method was repeated multiple times with quantities such as 0.5 mL and 0.25 mL.

Second Method: A coverslip was obtained and placed in a plasma etch machine in order to develop the surface as hydrophilic. The same solution made of DMEM, Calcium Chloride, Thrombin, and Fibrinogen was created and only 20 μL was needed to fill a cover-slip surface. This was then again placed in mini-well container and placed in the incubator at 37 C for 15

minutes to harden. This was again observed under a microscope and the observations were recorded.

Third Method: First 2 test tubes were obtained and the first was labeled as Fibrinogen and the Second as Thrombin. 4.5 mL of DMEM was placed in both test tubes. The test tube labeled Fibrinogen had 0.5 mL of Fibrinogen poured into it and the solution in the test tube was mixed together. The second test tube labeled “Thrombin” included 100 μL of Calcium Chloride as well as 5 μL of Thrombin. The solution in the second test tube was mixed and the solutions of each test tube was placed in the dip coding containers. A linear regression based dip coder was used to dip the cover slips into the fibrinogen solution and then dipped into the thrombin solution. This would be repeated with multiple dips and all the coverslips would be placed in a mini well container and placed in the 37 C container and observed under the microscope.

Results

This research experiment did not obtain any sequential or substantial numerical data. This research just helped decipher and find an understanding for blood coagulation in terms of the speed of reaction and firmness of the gel formed. The goal as determined earlier is focused on the basis of the thinness of the fibrin gels formed and the correlational relationship between the gel and the cultured cells placed on the coverslips. It was observed that the cells that were placed on the dip coded fibrin gel layers. The fibrin gels formed on the plasma etch machines

however, did not form a strong bond because the layers of the gel were too thick and observing the bonds were not visible and made it difficult to judge the strength of the attached cells.

Discussion

The data does support our hypothesis as we had originally predicted that the thinnest gel formation would have the best relationship with the cultured cells. The dip coded cover slips did have the thinnest gel formation layer and had the best bonded structure with the cultured cells. There are no major anomalies or outliers/exceptions to our data because only 2 various different methods were tested and all the trials produced very similar results because no major numerical factor was being calculated.

It is concluded that the dip coded gel fomed reaction is most similar to the natural body reaction when the skin is lacerated as a very thin clot is formed to seal the wound and protect the body from foreign objects as well as being able to stop the blood loss. This experimentation can help develop into a great understanding of the various applications in the medical and engineering fields. This could be used in surgeries as this is a natural production of a clot in the body and can help seal internal bleeding and develop a strengthened clot. A variation of this project could focus on the different aspects of the rate of blood coagulation and this way doctors could develop a reaction that would clot based on the thrombin concentrations and help create a natural barrier when the skin is lacerated and this automatically helps develop a better medical procedure that is natural to each individual.

References

Janmey, P. A., Winer, J. P., & Weisel, J. W. (2009). Fibrin gels and their clinical and bioengineering applications. Journal of The Royal Society.

LAURENS, N., KOOLWIJK, P., & DE MAAT, M. P. M. (2006). Fibrin structure and wound healing. Journal of Thrombosis and Haemostasis, 932–939

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, http://www.pnas.org/content/66/3/738.short

Müller, M. F., & Ferry, J. D. (1984). Preparation of two-dimensionally oriented elastic films from fibrin clots and other protein gels. Biotechnology and Bioengineering, 26(2), 191–193.