FMRI RET 2016-Fibrin Gel Strength Modulated by Dip Coating Technique

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Abstract

Fibrin gel is made up of the same proteins our blood uses in the process of blood coagulation. The protein fibrinogen when mixed with thrombin, the enzyme, will then proceed to become a fibrin gel. While the fibrinogen optimizes the strength of the gel, thrombin can increase or decrease the reaction rate when combined together. This fibrin gel has the potential to be very useful in wound healing and furthering cell research within the area of medications in relation to blood thinners. In order to see cell reactions under the microscope we focused on designing a very thin layer of the fibrin gel in order to optimize the visibility of cell growth and response by dip coating a coverslip into our two materials.

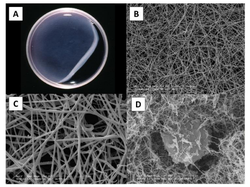


Figure 1. Fibrin Gel. The fibrin gel is show in section A. Section B, C and D show the networking of the fibrin gel under a microscope.

Background

Thrombin is primarily used as an enzyme for promoting blood clotting. Thrombin can also be used as therapeutic release in different topical creams. [1] In addition, parenteral direct thrombin inhibitors (DTIs) may be used in pediatric patients with contraindications to heparin therapy, such as heparin-induced thrombocytopenia. [4] Lastly, fibrinogen- and thrombin-impregnated collagen can be used to prevent air leakage. There is still further investigation of its impact on lung healing.

Figure 2. Thrombin. Crystal structure of thrombin with inhibitor.

Thrombin is an enzyme that converts the substance fibrinogen to fibrin in order to promote blood clotting. This process is extremely important in mammalian wound healing. Thrombin is not specific to one organism. Thrombin from one mammal will clot the fibrinogen of any other mammal. The only site of thrombin cleavage of fibrinogen occurs on the Arg-Gly bonds of fibrinogen. Thrombin should be stored at -20˚C. The active enzyme thrombin has a molecular weight of 36,000 Da. [5]

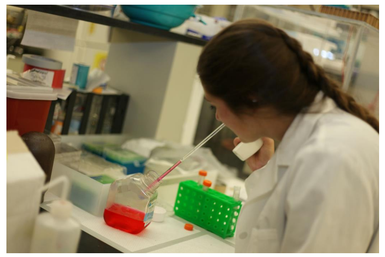
Objectives

- Alter a solution to have the optimal strength and desired reaction rate in order to create a fibrin gel with an ideal thickness.
- Test the fibrin gel for optimal cell growth and response by using the plasma etching method and a dip coating method.

Approach

Formula

Our fibrin gel solution is made up of fibrinogen stock, thrombin stock, CaCl and DMEM. Within our fibrin gel solution, we found the more fibrinogen stock we used the stronger our gel would be. We found that putting between 5 mg/mL and 20 mg/mL of fibrinogen stock was our ideal stiffness.
The thrombin stock is our enzyme. The amount of thrombin stock that we add directly correlates with the speed of the reaction. Because we are making the thinnest layer of fibrin gel we needed to have time to transfer the solution to a dish or coverslip before the solution started to gel. Our ideal amount of thrombin was 2 nm/mL.

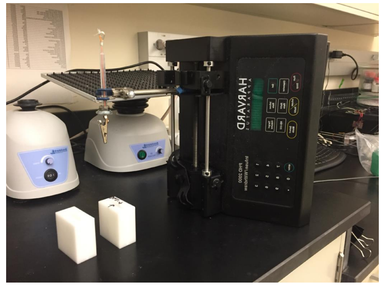


Plasma Etching Method

The first approach we took to making a thin fibrin gel layer was using the plasma etching method. This is a cleaning treatment used on the coverslip where we were putting the fibrin gel solution. Instead of the fibrin gel solution creating a raindrop like bubble due to surface tension on the coverslip, it allows the solution to completely cover the coverslip creating a thin layer of gel. We used as little as 20 nm/ml.

Dip Coating Method

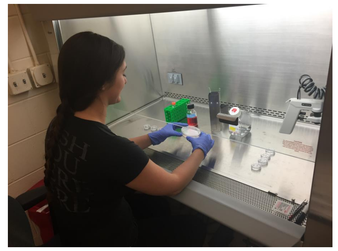
The second approach we followed was the dip coating method. Using a syringe pump on its side, we connected the coverslip to a glass slide being grabbed a clip that when moved it will vertically move up and down. We would carefully dip the coverslip into the fibrinogen stock, let it dry for about a minute then dip the coverslip into the thrombin stock creating a thin layer of fibrin gel.
When dipping the coverslip into the solution, there is no drastic change or difference as to how many time you dip the coverslip.



Conclusions

Cell Cultures

In order to see which method worked best we tested our thin fibrin gels by using cell cultures.
We found that the cells growth and response was greater on coverslips that were dip coated verses using the plasma etching method.



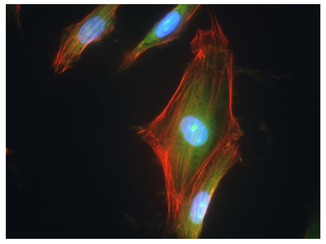


Figure 3: Cell on fibrin gel. Blue represents the nucleus, red represents the cytoplasm and green represents proteins on the out membrane of the cell.

Future Work

- Finding out the actual thickness of the fibrin gel using the dip coating method.
- Not all of our methods were done using all sterile materials. We would like to have a completely sterile run through from the start to the beginning.

Referenced Resources

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