Recombinant Production of Fibrinogen for Wound Healing Studies

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Introduction

Wounding healing is broken down into four phases: Hemostasis, Inflammation, Proliferation, and Remodeling. A detailed explanation can be seen in Figure 1. Once wounded, fibrinogen is then recruited to the site during the hemostasis phase, where it is then cleaved by thrombin to form a fibrin matrix that it is replaced with collagen.

The 4 phases of wound healing

Phase of healing	Time post injury	Cells involved in phase	Function or activity	Analogy to house repair
Hemostasis	Immediate	Platelets	Clotting	Utility workers cap-off broken utilities
Inflammation	Day 1–4	Neutrophils Macrophages	Phagocytosis	Unskilled labourers clean up the site Contractors direct activity
Proliferation (granulation and contraction)	Day 4–21	Macrophages Lymphocytes Angiocytes Neurocytes Fibroblasts Keratinocytes	Fill defect Re-establish skin function Closure	Subcontractors start work: • framers; • plumbers; • electricians; and • roofers and siders
Remodelling (maturation)	Day 21–2 years	Fibrocytes	Develop tensile strength	Interior finishing

Figure 1: The Phases of Wound Healing [1]

The structure of fibrinogen is composed of two sets of three chains: alpha, beta, and gamma chains. See Figure 2. The alpha chain is primarily involved in forming the matrix, the beta chain in addition to helping form the matrix it possesses antimicrobial properties, and the gamma chain helps with stabilizing the matrix and adhering to the fibroblast cells.

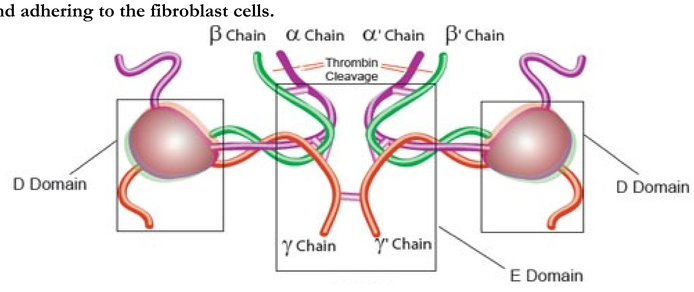

Figure 2: Structure of Fibrinogen [3]

Disorders associated with fibrinogen are presented in Figure 3 and primarily come in two forms: Fibrinogenemia (low or absent levels of fibrinogen) and Dysfibrinogenemia (normal levels of fibrinogen but lacks proper function). Both of these are often treated with fibrinogen concentrates or factor replacements to help ensure healthy levels of functional fibrinogen.

	Quantitative fibrinogen disorders		Qualitative fibrinogen disorders	
	Afibrinogenemia	Hypofibrinogenemia	Dysfibrinogenemia	Hypodysfibrinogenemia
Bleeding	Classically starting in neonatal period, spontaneous and in all tissues	Depending on the fibrinogen activity, mainly following trauma	Usually mild, frequent in mucosa, mainly related to surgery, injury, or delivery	Frequent, may be spontaneous and in all tissues
Thrombosis	In both arterial and venous territories, often in large vessels	Less frequent than in afibrinogenemia	In both arterial and venous territories, markedly increased with some fibrinogen mutations	Frequent, in both arterial and venous territories
Pregnancy	Early fetal loss[a] Placental abruption Postpartum hemorrhage	Depending on the fibrinogen activity level	Miscarriage Postpartum hemorrhage	Miscarriage Placenta abruption Postpartum hemorrhage
Other	Bone cysts Spleen rupture Poor wound healing	Fibrinogen storage disease[b]	CTEPH[b] Hereditary fibrinogen A α-chain amyloidosis[b]	None

Figure 3: Types of Fibrinogen Disorders [2]

Introduction (cont.)

The primary purpose of this study is to develop the materials and protocols necessary to enable production of "clean" and functional samples of fibrinogen in the lab for wound healing studies. A main challenge is to transfer the genes to express the fibrinogen chains in either bacterial cells or mammalian cells. Bacterial cells are less expensive to maintain in the lab and can potentially grow faster, but they typically do not modify proteins after translation the same way that animal cells do, a set of steps that is crucial for fibrinogen's function. Mammalian cells can form the correct structure with the appropriate post-translational modifications necessary but are far more expensive to culture and maintain in the lab.

Rationale and Methodology

- For this initial testing phase only the fibrinogen beta gene (FBG) is being expressed
- This gene was selected due to the availability of an expression plasmid in *E. Coli* for propagation and cultivation of the matured protein
- FBG processes antimicrobial properties
- Propagating *E. Coli* bacteria transformed with FBG containing plasmid
- Extraction of FBG containing plasmid
- Preparation of liposomes for transfection (lipofection)
- Transfecting (lipofecting) plasmid into mammalian cell line
- Propagating mammalian cells, which was selected as *Mus musculus* (mouse cells)
- Expressing FBG
- Extraction and purification of mature FBG using Ni-NTA affinity resin
- Characterization of FBG via Bradford assay for protein concentration measurements and SDS-PAGE and Western blot or turbidity measures when the protein is incubated with thrombin

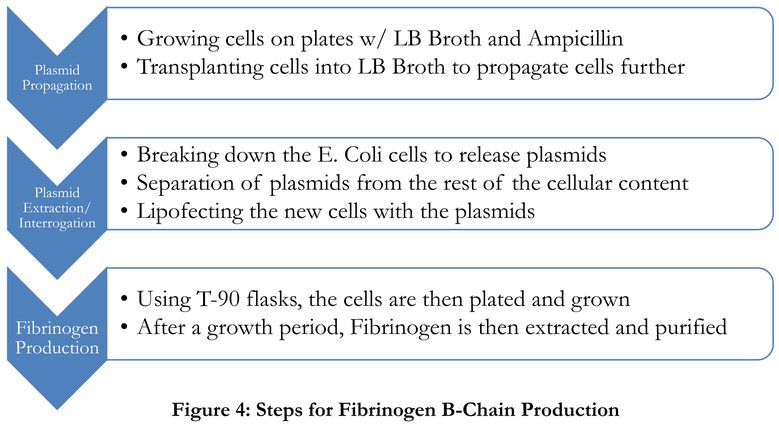

Figure 4: Steps for Fibrinogen B-Chain Production

Expected Results

- After the *E. Coli* are sufficiently grown and the plasmid lipofected into the mouse cells, large amounts should be produced as a majority of fibrinogen is typically produced in the liver cells
- We hope that after we obtain Fibrinogen B-Chain, we can form some form of fibrin gel when it is introduced to thrombin and any other clotting factors, such as Factor XIII. Figure 5 shows a fibrin gel

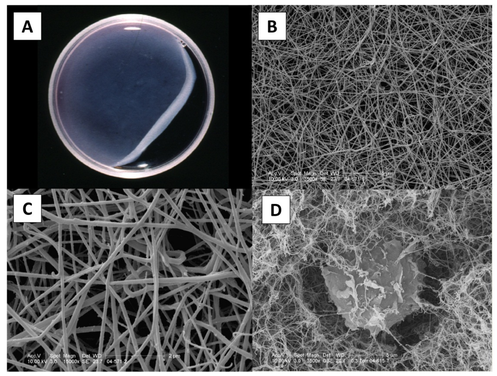

Figure 5: Fibrin Gel [4]

Discussion

- For this study, two cells types are being used: *E. Coli* (as mentioned earlier) and *Mus musculus* (mouse) liver cells
- Fibrinogen is produced mainly within the liver cells of the body. By using liver tissue, we expect to be able to provide the provide the protein with all the necessary components to fold and be modified properly
- As of now, after extensive preliminary research and sourcing of the necessary materials, we have begun by propagating the plasmid containing bacteria
- However, due to the recent global pandemic, further work has been greatly slowed

Future Work

- Propagate and extract the plasmids from the *E. Coli* cultures
- Transfect them into the mammalian cell line through the use of a liposome to transfect the cells
- Cultivate cells
- Extract mature Fibrinogen B-Chain and purify
- Evaluate for concentration and function
- Next, would be to work on incorporating all three genes into one plasmid or series of plasmids to incorporate them into one cell to produce fully formed fibrinogen protein
- The final step would be to modify the gene sequence to create different forms of fibrinogen to explore the impact on the formation of fibrin matrices to study in models of wound healing

References

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[4] Jockenhoevel, Stefan, and Thomas C. Flanagan. "Cardiovascular Tissue Engineering Based on Fibrin-Gel-Scaffolds." *IntechOpen*, IntechOpen, 17 Aug. 2011, www.intechopen.com/books/tissue-engineering-for-tissue-and-organ-regeneration/cardiovascular-tissue-engineering-based-on-fibrin-gel-scaffolds.

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