Fibrina: An Active-Infused Protein Carrier Delivery System, and its Automated Production for Cosmetics Use

A Major Qualifying Project Report

Submitted to the Faculty of

Worcester Polytechnic Institute

in partial fulfillment of the requirements for the

Degree of Bachelor of Science.

By

____

Peyton Delgorio

____

Drew DeRubeis

____

Conrad Ruiz

April 2017

Approved By:

____

Prof. Glenn Gaudette, Advisor

____

Prof. Kristen Billiar, Advisor

This report represents the work of Worcester Polytechnic Institute undergraduate students submitted to the faculty as evidence of completion of a degree requirement.

Abstract

Human skin, the largest organ in the body, is consistently exposed to outside elements, making it one of the body's most vulnerable organs. Factors that contribute to deterioration of the cosmetic appearance of the skin include disease, injury, the environment, age, and genetic conditions. In some cases, physical damage to the skin may result in scarring of the skin. The cosmetic industry consistently seeks new products to improve skin appearance and conditions. A Worcester Polytechnic Institute (WPI) team has discovered that fibrin, an insoluble protein and first responder in wound healing, can encapsulate retinol, a popular active ingredient commonly found in cosmetics skincare. Fibrin can also be degraded using bromelain, a natural enzyme also found in cosmetics, to control the release of retinol. This demonstrates the opportunity for fibrin's use as a novel delivery carrier for cosmetic product ingredients. Utilizing an automated manufacturing process, fibrin microthreads may be infused with popular active ingredients in the cosmetic industry, and ground into smaller particles to be incorporated into a topical cream. The team developed a prototype of a fibrin-based skin cream and a mechanism to automate the process of producing infused fibrin microthreads.

Table of Contents

Abstract ... i
Table of Contents ... ii
Acknowledgements ... vi
Authorship ... vii
List of Figures ... ix
List of Tables ... xiii
Chapter 1: Introduction ... 1
Chapter 2: Literature Review ... 6
2.1 What is Fibrin? ... 6
2.2 Current Applications of Fibrin ... 6
2.3 What are Fibrin Microthreads? ... 8
2.3.1 How are Fibrin Microthreads made? ... 8
2.3.2 What are the Applications of Fibrin Microthreads? ... 8
2.4 VitaThreads ... 9
2.5 Current IP status of Fibrin Microthreads ... 9
2.6 Food and Drug Administration ... 10
2.7 Cosmetic Industry ... 13
2.8 Current Cosmetic Products on the Market ... 14
2.9 IP Coverage on Powder and Cosmetics ... 16
2.10 Cosmeceuticals and the Consumer ... 17
2.11 Cosmetic Dermatology ... 18
2.11.1 Skin Physiology Pertinent to Cosmetic Dermatology ... 18
2.11.2 Delivery of Cosmetic Skin Actives ... 23
2.11.3 Creams ... 27
2.11.4 Cosmeceuticals ... 28
2.11.5 Cosmetic Dermatology and Fibrin-Based Skin Cream ... 33
2.12 Previous Designed Mechanisms to Manufacture Fibrin Microthreads ... 34
2.13 Good Manufacturing Standards ... 36
Chapter 3: Project Strategy ... 38
3.1 Initial Client Statement ... 38
3.2 Design Requirements of Validating Fibrin's Value for a Cosmetic Cream ... 39

3.2.1 Objectives 40
3.2.2 Constraints 42
3.2.3 Functions of Additive-Infused Fibrin Threads 42
3.2.4 Standards and Regulations 43
3.3 Design Requirements of the Automated Manufacturing Machine 43
3.3.1 Objectives 43
3.3.2 Constraints 45
3.3.3 Functions and Specifications 46
3.3.4 Standards and Regulations 47
3.4 Conversion of Fibrin Microthreads into a Usable Form 48
3.4.1 Objectives 48
3.4.2 Constraints 49
3.4.3 Functions and Specifications 49
3.4.4 Standards and Regulations 50
3.5 Revised Client Statement 50
Chapter 4: Design Process and Approach 51
4.1 Fibrina 51
4.1.1 Interviews 52
4.1.2 The Accelerate Program 57
4.2 Fibrin Microthread Extrusion Device 58
4.2.1 Machine Components and Needs Analysis 59
4.2.2 Concept Maps/Conceptual Designs and Alternative Designs 62
4.2.3 Final Preliminary Design Selection 80
4.3 Conversion of Fibrin Microthreads 82
4.3.1 Conversion Method Approach 82
4.3.2 Conversion Mechanism Design Approach 84
Chapter 5: Preliminary Design Verification 86
5.1 Fibrina Testing 86
5.1.1 Cream Composition Testing 86
5.1.2 Additive-Infused Fibrin Testing 88
5.1.3 Fibrin Degradation Properties Testing 88
5.1.4 Fibrin Surface Topography Testing 90
5.1.5 Fibrin UV Absorbance Properties Testing 91

5.1.6 Fibrin Diffusion Properties Testing 92
5.2 Preliminary Design Machine Testing 94
5.2.1 Phase 1: Pumping 94
5.2.2: Phase 2: Extrusion 95
5.2.3: Phase 3: Stretching 96
5.2.4: Phase 4: Drying 97
5.2.5: Phase 5: Removal 99
5.3 Conversion of Fibrin Microthreads 100
5.3.1 Conversion Method Application Preliminary Imaging Results 100
5.3.2 Conversion Mechanism Preliminary Analysis 101
Chapter 6: Final Design Validation 106
6.1 Fibrina Testing 106
6.1.1 Composition Results 106
6.1.2 Additive-Infused Fibrin Experiment 108
6.1.3 Fibrin Degradation Experiment Results 110
6.1.4 Surface Roughness Results 114
6.2 Final Machine Design 117
6.2.1 The Extruder Head 117
6.2.2 The Tub 118
6.2.3 The Conveyor 120
6.2.4 The Magnetic Clamps 121
6.3 Conversion of the Fibrin Microthreads 122
6.4 Project Impact 123
6.4.1 Economics 123
6.4.2 Environmental Impact 124
6.4.3 Societal Influence 124
6.4.4 Political Ramifications 124
6.4.5 Ethical Concerns 124
6.4.6 Health and Safety Issues 125
6.4.7 Manufacturability 125
6.4.8 Sustainability 125
Chapter 7: Discussion 127
7.1 Fibrina 127

7.1.1 The Fibrina Business Model 127
7.1.2 Fibrina Experimentation and Learning 129
7.1.3 Composition 129
7.1.4 Additive-Infused Threads 130
7.1.5 Degradation 131
7.1.6 Surface Topography 132
7.1.10 Future Considerations 133
7.2 The Machine Design 133
7.2.1 Design and Assembly of the Machine 134
7.3 Conversion of Fibrin Microthreads 136
Chapter 8: Conclusions and Recommendations 137
8.1 Conclusion 137
8.2 Recommendations 139
8.1.1 Future of Fibrina 139
8.2.2 Machine Recommendations 146
8.2.3 Recommendations for the Conversion of Fibrin Microthreads 150
References 151
Appendices 163
Appendix I: Additive-Infused/Regular Fibrin Microthread Protocol 163
Appendix II: Fibrina Interview Insights and Takeaways 166
Appendix III: Protocols for Fibrina Experiments 177
Appendix IIIa Protocol for Composition Experiment 177
Appendix IIIb Protocol for Exploratory Degradation Experiment: 178
Appendix IIIc Protocol for UV Spectroscopy Degradation Analysis Experiment 179
Appendix IV: Data and Results from Fibrina Experiments 182
Appendix IVa: Fibrin Exploratory Experiment Images 182
Appendix IVb: UV Spectroscopy Data 194
Appendix IVc: AFM Surface Roughness Images 195
Appendix IVd: Channels UV Sub-Experiment Results 202
Appendix V: CAD drawings for Automated Fibrin Microthread Production Mechanism 206
Appendix VI: Conversion Preliminary Testing Results and Design Analysis 216

Acknowledgements

Our team would like to thank Professor Glenn Gaudette and Kristen Billiar for their help, guidance, and support throughout the duration of this project. We would also like to thank the WPI Biomedical Engineering and Mechanical Engineering Departments for their assistance and guidance in providing our team with the necessary tools and materials to complete our project. The team would also like to thank the Technology Advisory Network (TAN) for allowing us to take part in the pilot WPI Accelerate Program.

Special thanks are given to Marek Travnikar, sophomore robotics engineering student, who was invited to work with us as part of an independent study project. Marek's assistance in the development of the team's mechanical design objectives were essential to creating a successful final prototype.

Section 2.11 was heavily influenced in both organization and content by *Cosmetic Dermatology: Products and Procedures*, which was edited by Zoe Draelos. Information from this book accelerated the team's comprehension of cosmetology to a height capable of successfully completing this project's goals. The team recognizes and thanks Dr. Draelos for her intellectual knowledge on the subject.

Authorship

Chapter and Section	Author	Editor
Chapter 1: Introduction	Conrad Ruiz, Drew DeRubeis, Peyton Delgorio	Drew DeRubeis
Chapter 2: Literature Review 2.1: What is Fibrin?	Conrad Ruiz	Peyton Delgorio
2.3: What are Fibrin Microthreads?	Conrad Ruiz	Peyton Delgorio
2.4: VitaThreads	Conrad Ruiz	Drew DeRubeis
2.5: Current IP Status of Fibrin Microthreads	Drew DeRubeis	Peyton Delgorio
2.6: Food and Drug Administration	Drew DeRubeis	Peyton Delgorio
2.7: Cosmetic Industry	Peyton Delgorio	Conrad Ruiz
2.8: Current Cosmetics Products on the Market	Peyton Delgorio	Conrad Ruiz
2.9: IP Coverage on powder and cosmetics	Drew DeRubeis	Peyton Delgorio
2.10: Cosmeceuticals and the Consumer	Conrad Ruiz	Drew DeRubeis
2.11: Cosmetic Dermatology	Conrad Ruiz	Drew DeRubeis
2.12: Previous designed mechanisms to manufacture Fibrin Microthreads	Peyton Delgorio	Drew DeRubeis
2.13: Good Manufacturing Standards	Drew DeRubeis	Peyton Delgorio
Chapter 3: Project Strategy 3.1 Initial Client Statement	Peyton Delgorio, Drew DeRubeis, Conrad Ruiz	Peyton Delgorio
3.2 Design Requirements of Validating Fibrin's Value for the Cosmetic Cream	Conrad Ruiz	Drew DeRubeis
3.3 Design Requirements of the Automated Manufacturing Machine	Peyton Delgorio, Drew DeRubeis	Conrad Ruiz
3.4 Design Requirements of the Conversion of Microthreads into a Usable Form	Peyton Delgorio	Drew DeRubeis
3.5 Revised Client Statement	Peyton Delgorio, Drew DeRubeis, Conrad Ruiz	Drew DeRubeis
Chapter 4: Design Process and Approach 4.1 Fibrina	Drew DeRubeis, Peyton Delgorio	Peyton Delgorio

4.2 Fibrin Microthread Extrusion Device	Drew DeRubeis, Peyton Delgorio	Peyton Delgorio
4.3 Conversion of Fibrin Microthreads	Peyton Delgorio	Conrad Ruiz
Chapter 5: Preliminary Design Verification 5.1 Fibrina Testing	Conrad Ruiz	Drew DeRubeis
5.2 Preliminary Design Machine Testing	Drew DeRubeis	Peyton Delgorio
5.3 Conversion of Fibrin Microthreads	Peyton Delgorio	Drew DeRubeis
Chapter 6: Final Design Validation 6.1 Fibrina Testing	Conrad Ruiz	Drew DeRubeis
6.2 Final Machine Design	Drew DeRubeis, Peyton Delgorio	Peyton Delgorio
6.3 Conversion of Fibrin Microthreads	Peyton Delgorio	Conrad Ruiz
6.4 Project Impact	Peyton Delgorio, Drew DeRubeis	Conrad Ruiz
Chapter 7: Discussion 7.1 Fibrina	Conrad Ruiz	Peyton Delgorio
7.2 The Machine Design	Peyton Delgorio, Drew DeRubeis	Drew DeRubeis
7.3 Conversion of Fibrin Microthreads	Peyton Delgorio	Drew DeRubeis
Chapter 8: Conclusions and Recommendations 8.1 Conclusion	Drew DeRubeis	Peyton Delgorio
8.2 Recommendations 8.2.1 Future of Fibrina	Conrad Ruiz	Drew DeRubeis
8.2.2 Machine Recommendations	Drew DeRubeis, Peyton Delgorio	Peyton Delgorio
8.2.3 Recommendations for the Conversion of Fibrin Microthreads	Peyton Delgorio	Conrad Ruiz
References	Peyton Delgorio, Drew DeRubeis, Conrad Ruiz	Peyton Delgorio
Appendices	Peyton Delgorio, Drew DeRubeis, Conrad Ruiz	Drew DeRubeis

List of Figures

Figure 1: Objective Tree for Fibrina ... 39
Figure 2: Objective Tree for the Automated Manufacturing Extrusion Device ... 44
Figure 3: Objective Tree for the Conversion Method of Fibrin Microthreads ... 48
Figure 4: Design Concept 1 ... 63
Figure 5: Piston Rod Syringe Pump ... 64
Figure 6: SIMCO Aerostat XC Air Blower ... 65
Figure 8: Design 2 channel system for organizing and stretching threads ... 67
Figure 7: Design 2 ... 67
Figure 9: Design Concept 3 ... 72
Figure 10: Conveyor submerged within HEPES solution concept ... 73
Figure 11: Top-view of design concept 3 ... 74
Figure 12: Blocks coupled to chain for clamping threads ... 75
Figure 13: Drawing of closed clamping mechanism ... 76
Figure 14: Drawing of opened clamp ... 76
Figure 15: CAD assembly of Design 3 concept ... 77
Figure 16: CAD part of stretching platform ... 78
Figure 17: Objective Tree for the Specific Methods of Converting the Fibrin Microthreads ... 83
Figure 18: Over-the-Counter Bromelain Capsules (500mg per Capsule) ... 89
Figure 19: A simple design of a Franz Diffusion Cell Apparatus. Produced by means of cutting two media bottles of different sizes and appropriately creating an inner chamber separately surrounded by a water jacket ... 93
Figure 20: Drying of the Microthreads with warm air at a medium fan speed ... 98
Figure 21: Machine Prototype ... 99
Figure 22: Grinding or Kneading Results at 10x Magnification, Scale bar = 0.1 mm ... 100
Figure 23: Rotational Disks Image Results at 10x Magnification, Scale bar = 0.1 mm ... 100
Figure 24: Manual Trimming Image Results at 10x Magnification, Scale bar = 0.1 mm ... 101
Figure 25: Automated Dual-Blade Disks Image Results at 10x Magnification, Scale bar = 0.1 mm ... 101
Figure 26: Design 1 Conceptual Diagram ... 102
Figure 27: Design 2 Conceptual Diagram ... 102
Figure 28: Design 3 Conceptual Diagram ... 103
Figure 29: 70% Water-Based Cream, water separating from oil base at bottom of jar ... 107
Figure 30: 100ul Retinol in Ethanol (25ug/ml) pre-mixed into 900ul Thrombin/CaCl2 Solution. ... 109
Figure 31: Left: 1mg fibrin particle degradation via 10mg bromelain exposure after 1 hour. Right: 1mg fibrin particle degradation via 10mg bromelain exposure after 3 hours. ... 111
Figure 32 Results of UV Spec Analysis for Bromelain Enzyme. Significant Outlier for the 9mg dish at hour 5. ... 112
Figure 33: Results from running the MATLAB code for the Two-way ANOVA. Circled is the calculation indicating no statistical significance ... 113
Figure 34 Standard Curve of retinol in ethanol mixed with PBS. The retinol in ethanol amounts represent the amount of retinol in ethanol within a 500ml PBS solution ... 113
Figure 35: 3D printed multi-channel tub. 5ml HEPES within each channel. ... 114
Figure 36: Surface imaging of Fibrin Particle under AFM ... 115

Figure 37 Thread Surface image with Area Roughness Calculations 115
Figure 38 Thread Surface image with Area Roughness Calculations 2 116
Figure 39: Final Machine Assembly 117
Figure 40: Top View of the Assembled Tub 119
Figure 41: Isometric View of the Tub 119
Figure 42: Conveyor Base 121
Figure 43: Assembled Clamps and Conveyor Incorporated in Final Design 121
Figure 45: Set-up of the Mechanism without the Funnel 122
Figure 44: Final Mechanism with the Funnel to Bundle the Fibrin Microthreads 122
Figure 46: Image Results Using the Manual Trimming Method with the Mechanism, Scale bar = 0.1 mm 123
Figure 47: Image Results Using the Automated Dual-Action Trimmer Blades with the Mechanism, Scale bar = 0.1 mm 123
Figure 48: Clamp made through rapid prototyping in the open position 147
Figure 49: Clamp made through rapid prototyping in the closed position 148
Figure 50: Stretching platform prototype made of birch plywood 149
Figure 51: Cutting of Threads into particles using small scissors. Threads cut onto weigh plate for measuring 180
Figure 52: Tissue Culture Dishes all prepped with 5mg fibrin particles and 5ml PBS. On the Right: Eppendorf Tubes prepped with varying amounts of protease 180
Figure 53: Plate Reader Setup. 6,7,8,9 and mg protease degradation measured over 1,3,5, and 7 hours. Actual Standards used: 1,3,5,10,20 and 30 ul retinol in ethanol. Control group was PBS only 181
Figure 54: Fibrin Control Hour 0, Pre-PBS 182
Figure 55: Fibrin 1mg Hour 0, Pre-PBS 183
Figure 56: Fibrin 2.5mg Hour 0, Pre-PBS 183
Figure 57: Fibrin 5mg Hour 0, No PBS 183
Figure 58: Fibrin 10mg Hour 0, No PBS 184
Figure 59: Fibrin Control Hour 0 184
Figure 60: Fibrin 1mg Hour 0 184
Figure 61: Fibrin 2.5mg Hour 0 185
Figure 62: Fibrin 5mg Hour 0, PBS Added 185
Figure 63: Fibrin 10mg Hour 0, PBS Added 185
Figure 64: Fibrin Control Hour 1 186
Figure 65: Fibrin 1mg Hour 1 186
Figure 66: Fibrin 2.5mg Hour 1 186
Figure 67: Fibrin 5 mg Hour 1 187
Figure 68: Fibrin 10mg Hour 1 187
Figure 69: Fibrin Control Hour 3 188
Figure 70: Fibrin 1mg Hour 3 188
Figure 71: Fibrin 2.5mg Hour 3 188
Figure 72: Fibrin 5mg Hour 3 189
Figure 73: Fibrin 10mg Hour 3 189
Figure 74: Fibrin Control Hour 5 190
Figure 75: Fibrin 1mg Hour 5 190

Figure 76: Fibrin 2.5mg Hour 5 190
Figure 77: Fibrin 5mg Hour 5 191
Figure 78: Fibrin 10mg Hour 5 191
Figure 79: Fibrin Control Overnight 192
Figure 80: Fibrin 1mg Overnight 192
Figure 81: Fibrin 2.5mg Overnight (Image 1) 192
Figure 82: Fibrin 2.5mg Overnight (Image 2) 193
Figure 83: Fibrin 5mg Overnight 193
Figure 84: Fibrin 10mg Overnight 193
Figure 85: AFM Top-down image of Fibrin Particle Sample Plate 195
Figure 86: Side Image of Fibrin Particle Sample Plate under AFM 196
Figure 87: AFM image of Fibrin Particle with Data Info 196
Figure 88: Derived Data of the AFM image in Figure 79, with Area Roughness Calculation for near-center cross section. 197
Figure 89: Derived Data of the AFM image in Figure 79, with Area Roughness Calculation for lower-left cross section. 197
Figure 90: Near-center area roughness calculations from backward scan of Figure 79 198
Figure 91: Near-Edge area roughness calculations from forward scan of Figure 79 198
Figure 92: Center cross section area roughness analysis on forward scan of Figure 79 199
Figure 93: Near Center cross-section area roughness calculations from backward scan 199
Figure 94: Top-Right Cross section area roughness calculations from backward scan 200
Figure 95: Whole image area roughness calculations for forward scan 200
Figure 96: Whole Image area roughness calculations from backwards scan 201
Figure 97: Unreadable imaging of cut face of Fibrin Particle 201
Figure 98: Attempted area roughness calculation for cut face particle 202
Figure 99: A custom 3D Printed multi-channel design (has 19 channels) 203
Figure 100: Channels 1-12 transferred over to a 12-well plate 204
Figure 101: Top-row:Channels 13-16; 205
Figure 102: SpectraMax 250 template for UV Spec testing; CH - Channel; C# - Control/Standard, amount of ROL in EtOH (ul); T - Threads+Bromelain; M - Droplets from threads; B - 370mg Bromelain 205
Figure 103: Results from UV Spectroscopy reading 206
Figure 104: CAD image for Automated Fibrin Microthread Production Mechanism Final Design 206
Figure 105: CAD of 80/20 Frame 207
Figure 106: CAD of Chain Guiding Platform 207
Figure 107: CAD of Stepper Motor 1 Holster 207
Figure 108: CAD Assembly of Drive Shaft Bracket and Ball Roller Bearing 208
Figure 109: CAD Assembly of Drive Shaft Bearing Assembly, Sprockets, and Hex Bar 208
Figure 110: CAD Assembly of Bearing, Bracket, and Sprocket 208
Figure 111: CAD Assembly of Chain and Magnetic Clamps 209
Figure 112: CAD of 80/20 Gantry Frame 209
Figure 113: CAD of Slider for Extruder 209
Figure 114: CAD of Holster for Stepper Motor 2 210
Figure 115: CAD Assembly of Tub and Conveyor 210

Figure 116: CAD Assembly of Conveyor 210
Figure 117: CAD of L-frame 1 211
Figure 118: CAD of L-frame 2 211
Figure 119: CAD of Bearing for Conveyor Roller 212
Figure 120: CAD of Urethane Conveyor Belt 212
Figure 121: CAD of PVC for Conveyor Roller Assembly 212
Figure 122: CAD of Threaded Rod for Conveyor Drive Roller Assembly 213
Figure 123: CAD of 3/8 in Hex Bolt 213
Figure 124: CAD of 3/8 in Hex Nut 213
Figure 125: CAD of 3/8 in Washer 214
Figure 126: CAD for Rubber Bung to Provide Friction Between Threaded Rod and PVC of Conveyor Drive Roller 214
Figure 127: CAD of Acrylic Tub 214
Figure 128: CAD of Pulley for Conveyor Drive Roller for Coupling with Hex Bar 215
Figure 129: CAD of Pulley for Hex Bar Drive Shaft 215

List of Tables

Table 1: Common Ingredients Used in Skin Care Products [26] 15
Table 2: Initial Value Proposition and Customer Segment 57
Table 3: Current Value Proposition and Customer Segment 57
Table 4: Definition of the Different Phases of the Machine 59
Table 5: Summary of the Needs and Wants of the Machine Design 59
Table 6: Design Matrix for the System Needs 61
Table 7: Design Matrix for the System Wants 62
Table 8: Decision Matrix for the Individual Machine Components 81
Table 9: Decision Matrix for the Overall Machine Designs 81
Table 10: Advantages and Disadvantages of the Grinding or Kneading Method 83
Table 11: Advantages and Disadvantages of the Dual-Action Flat Blade Disks Method 84
Table 12: Advantages and Disadvantages of the Manual Trimming Method 84
Table 13: Advantages and Disadvantages of the Dual-Action Automated Trimmer Blades Method 84
Table 14: Needs and Wants of the Conversion Mechanism 84
Table 15: Objective Descriptions 85
Table 16: Basic Composition of Oil-in-Water Cream [93] 87
Table 17: Results from Testing the Removal of Threads at Different Time Points 95
Table 18: Variables for the Drying Test 98
Table 19: Fibrin Particle Average Lengths for Each Thread 101
Table 20: Example Pairwise Comparison Chart 104
Table 21: Total Pairwise Comparison Results 104
Table 22: Example Design Matrix Used in Calculating the Total Design Matrix Score for each Design 105
Table 23: Total Design Matrix Score 105
Table 24: Composition Experiment, 50% Water-Based Cream 106
Table 25: Composition Experiment, 60% Water-Based Cream 107
Table 26: Weight % calculations for a baseline moisturizer with additive-infused fibrin particles 108
Table 27: Surface Roughness Values for the cross-sections in Figures 37 and 38 116
Table 28: First Test Tub Leakage Results 119
Table 29: Second Test Tub Leakage Results 120
Table 30: First Dermatologist Interview Insights and Takeaways 166
Table 31: Second Dermatologist Interview Insights and Takeaways 167
Table 32: Cosmetic/Plastic Surgeon Interview Insights and Takeaways 169
Table 33: Cosmetic Marketing and Branding Interview Insights and Takeaways 170
Table 34: Low-End Store Interview Insights and Takeaways 172
Table 35: Wholesale Manufacturer and Distributor Interview Insights and Takeaways 174
Table 36: SpectraMax 250 Data Values for UV Spec. Analysis Experiment 181
Table 37: Standard Curve Values for Retinol in Ethanol Solutions within PBS Solution 182
Table 38: Raw UV Spectroscopy data from UV spectroscopy experiment 194
Table 39: Corresponding data to Figure 32 from UV Spectroscopy Results 194
Table 40: Corresponding data to Figure 34 from UV Spectroscopy Standard Curve 194

Table 41: Initial Testing Conversion Method Image Analysis - Fibrin Particle Length Measurements 216
Table 42: Design Matrices for the other teammates 217
Table 43: Pairwise Comparison Charts for the other Teammates 217

Chapter 1: Introduction

Fibrin is a protein developed at Worcester Polytechnic Institute (WPI) that have the potential to affect the field of biomedical research and tissue engineering through a variety of applications. Fibrin, which is composed of fibrinogen and thrombin, is produced within the body when a person suffers an injury that results in bleeding. The protein compound plays an integral role in blood clotting by initiating the regenerative process of wound healing [1]. Fibrin has already been reproduced in a laboratory setting where it has been manufactured into microthread-like structures. A number of experiments, ranging from human mesenchymal stem cell (HMSC) engraftment to closing heart defects, have explored the potential use of these microthreads in cell therapy applications and in wound closing [2]. The expansion of the microthreads' studies in research has presented new opportunities for future medical device products and other industries. The current process to develop the microthreads in research laboratories, however, only enables manual production of fibrin microthreads in small quantities per batch. In order to ease the production of fibrin microthreads for research, and to further enhance market opportunities for the invention, a manufacturing machine that can mass produce fibrin microthreads in large, consistent quantities is required.

The team, consisting of three WPI students recognized that due to Food and Drug Administration (FDA) regulations, certain applications of fibrin microthreads, such as human mesenchymal stem cell (HMSC) engraftment to enclose heart defects, are not currently commercially plausible. Although such applications are desirable by stakeholders in the future of fibrin microthreads, the team determined that current commercial promise exists in the cosmetics field.

Although background and cosmetic market research provided substantial information on fibrin and its potential value in the cosmetics realm, it was decided that more could be done with regards to customer discovery. The team opted for a consumer-driven design approach to producing Fibrina. Over 30 interviews were conducted with professionals in the cosmetics industry. These professionals included dermatologists, manufacturers, distributors, retailers, and cosmetics marketers. Every-day consumers were also interviewed within low, middle, and high-end stores. The resulting takeaways provided enough information to identify a customer segment and value proposition which drove much of the experimentation work for this project.

Using background literature on cosmetic dermatology and the insights taken from customer discovery, a series of experiments were created to validate Fibrina's as a valuable cosmetic ingredient. The first experiment involved identifying a cream composition which could work as a baseline for current and future testing with fibrin-based particles. The second experiment was broken down into two parts: part one identified a way to degrade fibrin and subsequently release retinol introduced to it; part two quantified the release of retinol from degraded fibrin particles using UV spectroscopy. An additional sub-experiment was performed to try and identify any potential losses in retinol during the extrusion process. Finally, an experiment utilizing atomic force microscopy was performed in an attempt to discover other properties of fibrin which could enhance active delivery to the skin. Through the customer discovery process, two more experiments were identified, but ultimately not undertaken by the team. These two experiments were designed to identify fibrin's skin diffusion and UV absorbance properties.

Results from the Fibrina experiments highlighted fibrin's capabilities to exist and be tested within a baseline cream composition. Using a natural fibrinolytic such as bromelain, fibrin

can be degraded. This degradation allows for the release of actives encapsulated within fibrin, and with different concentrations of bromelain, fibrin's degradation rate can be controlled. However, UV spectroscopy analysis performed in conjunction with the degradation experiments was unable to match degradation with the release of retinol. Atomic force microscopy imaging was able to show nanoscale surface roughness properties of fibrin particles.

In order to implement fibrin particles within our Fibrina product, the team realized that fibrin microthreads, due to their extrusion process, high surface area to volume ratio, and advantageous mechanical structure, could be utilized in this production process. The team set out to design and prototype an automatic fibrin microthread extrusion device that fulfilled the need for mass production of fibrin microthreads to be implemented into our novel skin cream. The benefits of a fibrin microthread extrusion device include minimizing production time for threads, eliminating human error in the formation of consistent, customizable threads, and creating a method of mass production for future commercial applications of the invention. Using a precise engineering design process and computer-aided-design, the team aimed to create a device that replicates the current, manual fibrin microthread extrusion process into an automated system with greater accuracy and consistency. The ability to manipulate the ingredients of the microthreads, their properties, and to form a desired end product opens greater opportunity to have an efficient supply of additive infused fibrin for our product.

The final design of the team's fibrin microthread manufacturing device incorporated a stepper-motor driven chain mechanism that completely automated the extrusion process for the production of fibrin microthreads. The mechanism can be attached to a syringe pump, which is commonly found in laboratories, via tubing, to deliver a fibrinogen, thrombin, and additive solution to the extrusion phase of the production process. The design incorporated magnetic

clamps that guide a drawn fibrin microthread through its production process. The fibrin microthreads were drawn into a tub of HEPES solution, a buffering solution used to polymerize the fibrin microthreads, across the surface of two open magnetic clamps and a submerged conveyor belt. The magnetic clamps were attached to chain links that moved in tandem with the submerged conveyor belt. Once the threads reached the end of the conveyor belt, the clamps were guided shut and lifted out of the HEPES tub via sprockets. From there, two chain guiding platforms guided the clamps away from one another, stretching the fibrin microthreads to double their original length. A dryer was placed within the frame of the mechanism to dry the threads during this process. At the end, the threads could be removed manually by a user. Utilizing this mechanism, additive infused fibrin microthreads could be mass produced. From here, the fibrin microthreads needed to be converted into fibrin-based particles.

Next, the team designed a process of converting the produced fibrin microthreads into particle form within a range of 0.1 – 1 mm. In order to do this, the team explored four methods of conversion. The four methods were grinding and kneading, rotational flat disks, manual trimming, and dual-action automated blades. After conducting initial testing and doing image analysis, the team identified three methods: grinding, manual and automated trimming, which successfully converted the fibrin microthreads to a particle form. The second part of this objective included creating a mechanism that utilized successful methods in order to create a space for a more equal and cleaner conversion. The team performed a design analysis and came up with a final design that included two clamps with PVC coated prongs that were attached to a ring stand. The first clamp has bundled fibrin microthreads mounted to them with tape to stabilize them, while the second clamp is holding a 50 mm circular piece with mesh to filter out debris and particles larger than 1 mm. A 60 x 15 mm petri dish collects the desired particles. The

first clamp can also hold a small funnel that can bundle and group the fibrin microthreads as well. Utilizing this method, fibrin-based particles were able to be introduced as an ingredient in Fibrina.

In regard to the entrepreneurial pursuit, customer discovery was essential to defining the scientific metrics needed to validate fibrin as a valuable cosmetic ingredient. Given the lack of any research into fibrin's use as a functional cosmetic ingredient, much of the approach to this project existed as trial and error. Background literature, insights from industry professionals, and continuous learning helped to guide the team throughout its scientific endeavors. Modeling fibrin particles as carriers for actives proved to be difficult, and more testing is required to better validate its use within cosmetics.

Recommendations towards furthering the customer discovery process of Fibrina, repeating and expanding upon Fibrina experiments, and upscaling and refinement of the automated machine can be made. The team is hopeful with more research that Fibrina can see itself in the cosmetics market relatively soon. Milestones for entrepreneurial success need to be established to help guide future work in adding value to fibrin as a skincare ingredient.

In the report herein, the team conveyed the research, methodology, findings, and results for the validity of fibrin in the cosmetics market, the design of the fibrin microthread production process, and the conversion of fibrin microthreads into particles used for implementation into Fibrina.

Chapter 2: Literature Review

2.1 What is Fibrin?

When the body suffers an injury such as a cut or wound, the first major protein to act as the blood clotting agent is fibrin. Fibrin is composed of a chain of fibrinogen monomers. These monomers exist solubilized within the blood until they are activated by a protein called thrombin. Fibrinogen is produced in by both the liver and blood plasma. When the fibrinogen monomers bind together, they form a mesh-like structure which traps surrounding blood cells at a wound site to form a clot and prevent further bleeding [1].

Fibrin formation begins at a wound site in which the interaction between the two proteins (fibrinogen and thrombin) commences. When the body recognizes an injury, prothrombin, which is produced in the liver, becomes proteolytically cleaved into its active version known as thrombin. Thrombin then catalyzes the hydrolytic removal of two fibrinopeptides (Type A and B) on fibrinogen molecules [2]. The removal of fibrinopeptides A and B reveal binding sites, which interact with complementary sites at the ends of other fibrinogen molecules. Soluble fibrinogen molecules can then bind to each other freely to form fibrin. Fibrin monomers will continue to assemble as long as thrombin activates more fibrinogen molecules. A clot then forms when a large amount of fibrinogen monomers are bound together into a fibrous branch structure [3,4].

2.2 Current Applications of Fibrin

Fibrin has been utilized in a variety of ways within the biomedical field. It has been used to create scaffolds for tissue engineering applications and as a sealant material in a plethora of surgeries. Together, along with other existing applications, these uses reveal the broad range of benefits of which Fibrin is being used for today.

Fibrin's use as a scaffold for tissue engineering has taken multiple forms including: gels, glues, and microbeads, which are used for engineered bone and skin tissues [5]. These scaffold choices can be modified to incorporate several biologically active peptides to increase the local concentrations of growth factors and mimic the natural milieu surrounding the cells [5]. Fibrin is used extensively to support three-dimensional scaffolds in tissue engineering. Such examples include its use for adipose, bone, cardiac, cartilage, liver, nervous, ocular, skin, tendon, and ligament tissues [5].

Fibrin sealant or "glue", is a unique hemostatic/adhesive material used in a number of surgical applications [6]. Fibrin sealant devices work much like a two-for-one syringe, where fibrinogen and thrombin are at separate in the device, but once injected, are injected simultaneously. The amount of fibrinogen and thrombin within these devices is in high concentrations; thus promoting immediate clotting [6]. The first fibrin sealant that was approved for use by the Food & Drug Administration (FDA) was in 1998. However, in countries such as Europe, fibrin sealants have been around for much longer. Fibrin sealants have predominately made a beneficial impact in surgical procedures that require site-specific blood clotting [6].

Fibrin gels have also taken stride in the biomedical industry, providing the same positive benefits of blood clotting and natural wound healing. Their nonlinear elastic properties, in conjunction with their soft compliance at small strains and stiffening to resist large deformations, make them useful for their function as hemostatic plugs and matrices for cell migration and wound healing [7]. Their current clinical uses stem from general surgical wound repair to also drug/cell delivery [7].

These applications demonstrate the extent to which fibrin can be altered to improve the medical field, and further advance the biomedical sciences. A number of commercial fibrin

sealants and glues have already been FDA approved, while others are awaiting FDA approval [6] [8]. While these applications have shown the extent at which fibrin has taken certain forms to enter the commercial market, one application that has not been mentioned is the application of fibrin in the form of microthreads.

2.3 What are Fibrin Microthreads?

Fibrin microthreads are a recent invention in the biomedical field of research. These laboratory-developed threads are used in a variety of experiments relating to wound healing and cell therapy engraftment. Currently, biomedical researchers are investigating the use of these microthreads on the medical market. However, the time and resources required to push fibrin microthreads into the market make this invention less profitable. It is still vital to learn and understand the process by which fibrin microthreads are made in order to understand the promising applications for fibrin microthreads.

2.3.1 How are Fibrin Microthreads made?

The general process of making these threads involves the extrusion of fibrinogen and thrombin into a specially crafted extruder device (see Appendix I). Once the fibrin microthreads are extruded from the device, the threads sit in a 10mM HEPES solution. This particular HEPES solution also contains 10mM $CaCl_2$, which helps activate thrombin, allowing it to hydrolyze the removal of the fibrinogen peptides A and B. The threads are then left in the HEPES for 10 minutes before removal from the bath for drying. Once the threads are dried completely, they are stored in an aluminum foil wrapping, or used immediately for other experiments.

2.3.2 What are the Applications of Fibrin Microthreads?

Fibrin microthreads have been used in a number of laboratory experiments, mainly focusing on experiments surrounded cellular therapy and delivery. In one experiment, the threads were engrafted with human mesenchymal stem cells (HMSCs) to determine whether they were

an effective cell delivery method [9]. The purpose of this experiment was to determine if this method effectively localized cell delivery [9, 10]. The threads were woven into grafts and implanted into diseased animal hearts as a means of treating myocardial infarctions [2]. Other scaffolding design strategies were explored. Threads were woven into a braid, or aligned together in a sheet. The purpose of these various weave structures was to mimic the alignment of certain tissue structures within the body [9, 10]. The results of these experiments identify potential for use of fibrin microthreads as suitable cell delivery carriers as well as scaffolds for aiding in tissue recovery.

2.4 VitaThreads

VitaThreads is a limited liability company that was founded in 2012. Vitathreads focuses on the potential for fibrin microthreads to be used as medical-grade sutures. Thus far, VitaThreads has aligned its marketing of fibrin threads toward facial reconstructive surgery [11]. Due to fibrin's naturally decomposing properties, fibrin-based sutures would be ideal in closing facial skin. With ownership of the intellectual property on fibrin microthreads, VitaThreads has pushed to get the threads out into the medical market [12].

2.5 Current IP status of Fibrin Microthreads

The research and development of fibrin microthreads has broken ground in the field of tissue regeneration. The realm of this discovery includes its use in wound and cardiovascular tissue development. Due to the extent of these discoveries, the team's institution, Worcester Polytechnic Institute (WPI), and professors who have developed the threads, including Professor George Pins, and the team's advisor, Professors Glenn Gaudette, have claimed the rights to their discoveries through filed patents with the World Intellectual Property Organization (WIPO) and the United States Patent and Trademark Office (USPTO).

These filed patents, which are all assigned to WPI, claim several developmental methods and applications for fibrin microthreads that is optimal for future commercialization efforts. For example, a WIPO published patent, WO 2007109137A1, claims a method for producing fibrin microthreads along with its composition. Specifically, it claims using fibrinogen and another molecule, which for research purposes refers to thrombin, to create a mixture that is extruded into a medium to produce a thread [13]. Since WPI is assigned the patent, this enables the team to use this method for consideration of a large scale manufacturing method for these threads.

Several patents that have been filed on fibrin microthreads enables the team to explore different opportunities for application of the threads. United States patent application 20130096610A1, “Biopolymer microthreads with microscale surface topographies,” discloses a method for creating textured threads [14]. The development and change of texture on these threads allows its application to different kinds of damaged tissues. Additionally, a filed patent application, 20150005739A1, discloses the use of a fibrin microthread coupled with a needle in order to suture a region of damaged tissue. By removing the needle from the thread, the fibrin thread is retained in the body, which allows it to treat damaged tissue. This includes, but is not limited to, cardiovascular tissue [15]. Finally, a WO patent publication, 2015148993A1, discloses the use of fibrin microthreads to suture a damaged tissue, which can be absorbed by the tissue rapidly for treatment. This patent has been assigned to Vita Threads, LLC [16]. Although these published patents and filed applications provide exclusive rights to the development of fibrin microthreads, certain limitations to the use of these threads still exist.

2.6 Food and Drug Administration

Although the intellectual property assigned to VitaThreads contains novel claims in the field of tissue engineering, the commercialization of fibrin microthreads presents several

obstacles. Since fibrin microthreads present ample opportunity in the field of sutures, wound healing, and potentially, in the future, cardiovascular procedures. Since the Food and Drug Administration (FDA) regulates the commercialization process of inventions of this nature, VitaThreads is required, under the Food, Drug, and Cosmetic Act (FDCA), to disclose the device to the FDA for approval. The financial implications and timeliness of this process proves unfavorable for VitaThreads.

The FDA defines products that are regulated either as a device, drug, or biologic. For purposes fully understanding the scope of potential regulation of fibrin microthreads, it is important to be knowledgeable of the regulatory processes of both biologics and devices. In one definition, the FDCA defines a medical "device" as an implant "intended to affect the structure or any function of the body [17]." However, depending on the risk the medical device presents, the degree of FDA regulation may differ. Three different classes exist, (Class I, Class II, and Class III), for identifying the degree of necessary regulation in order to receive FDA approval for commercialization. Specifically, a Class III medical device, the classification that requires the most taxing approval process, claims that "insufficient information exists [on the disclosure] to assure that general controls and special controls will provide reasonable assurance of safety and effectiveness" [17]. Further, a Class III medical device claims that the disclosure may be "represented to be life threatening or life supporting, those implanted in the body, or those presenting potential unreasonable risk of illness or injury" [17]. Considering these parameters, the potential commercial applications of fibrin microthreads, the risk these procedures could have on a human subject, and fibrin's origin in the blood classifies the invention as a biologic under the FDCA.

In order to obtain FDA approval on a Class III medical device, an approved Premarket Approval Application (PMA) is required. The fee of a PMA application alone, reported on the FDA website under 21 CFR part 814 for fiscal year 2003 was $154,000 [18]. The PMA, however, is not merely an application preceded by a user fee; rather, an in depth process that involves experimentation and overwhelming proof of intended effectiveness of the device. Expanding upon this process, an Investigational Device Exemption (IDE) is required to receive PMA approval. The IDE involves sponsored human subject research that is approved by the FDA and the Institutional Review Board (IRB). In the case of fibrin microthreads, where studies comprise obvious risk to the subjects, the difficulty of FDA and IRB approval increases. PMA approval exists on the terms that the sponsored research assured safety as defined in the FDCA, and that experts in the field of research can "reasonably conclude" that the disclosure presents effectiveness in achieving its purpose [17]. Finally, prior to commercialization of a Class III medical device, a Quality System Regulation (QS)/Good Manufacturing Practices (GMP) examination, as defined in 21 CFR part 820, must occur in regard to the process and standards of mass production of the medical device [18].

Fibrin is a blood product. If the fibrin microthreads were applied without the use of another implantable device, it would be classified as a biologic. A product classified as a biologic engages in premarket approval through regulations outlined in the Public Health Services (PHS) Act. Specifically, regulations for biologics based upon guidelines stated in section 351(a) of the PHS Act are enforced by the FDA's Center for Biologics and Research (CBER). Under section 351(a), a Biologics License Application (BLA), which cost itself exceeds $250,000, may be issued by the FDA if the product, "meets standards designed to ensure the continued safety, purity, and potency of such products" [19,20]. In order for this determination to

be reached, the licensure must disclose convincing data show through preclinical and clinical research.

Finally, a product may be defined as a combination product, that is, a product composed of a combination of a drug, device, or biological product [21]. In the case of fibrin microthreads, the FDA may define an application for the invention as a biologic/device under a combination product categorization. In the case of a combination product, the product is assigned to an agency center after it is determined what the primary jurisdiction is based on which constituent, (device, biologic, or drug), has a primary role in delivery the performance of the product [18]. Therefore, a biological/device may have the biologic constituent determined to attribute primarily to the end-result of the product, and therefore would be assigned to a biologic agency for regulation.

2.7 Cosmetic Industry

When dealing with a new potential product, it is important to define the scope and use of the product's final form. In order to determine the best way to utilize the product, the team researched its current regulatory guidelines and understands the products on the market in the industry of interest.

Cosmetic products and cosmeceuticals are not regulated by the FDA. The Food, Drug, and Cosmetic Act was passed in 1938 and became the official regulation document for cosmetic products [21]. An independent panel known as the Cosmetic Ingredient Review (CIR) also assesses the safety of cosmetic products through medical and scientific expertise. Their feedback is considered by the FDA when determining the safety of cosmetic products. Even though the majority of cosmetic products (aside from color additives) do not require FDA approval in order

to be sold and marketed to buyers, the FDA still monitors the safety and effects of the products [22].

One way the FDA monitors cosmetics products on the current market is through their level of transparency with buyers: the product labeling. According to Section 602 of the Food, Drug, and Cosmetic Act, a cosmetic is considered a misbranded item if it contains one of the following: the label contains false information and/or is misleading, the label does not include information such as the origin of manufacturer, packer, and distributor and the amount of contents of the product that was produced, the label does not clearly state the information in a way consumers can understand, and the container or bottle is filled with a product different from what is labeled [23]. Product labelling is important for the consumers' safety as well as the product's legitimacy.

Some ways the FDA encourages cosmetic manufacturers to confirm the validity of their products is through the Voluntary Cosmetic Registration Program [20]. This program gives manufacturers the opportunity to release information regarding their products to a public database. Based on the feedback from the CIR and industry data, the FDA often performs manufacturer inspections (factory and products), while specifically examining products that have been reported to cause consumer problems. Even though the FDA cannot prevent cosmetic products from being marketed globally, they still inquire levels of transparency and honesty to ensure the products are safe for usage.

2.8 Current Cosmetic Products on the Market

Current cosmetic products offered on the market include facial cleansers, skin creams and moisturizers, and scar treatments. According to the Cosmetic, Toiletry, and Perfumery Association (CPTA), the purpose of these products are to replenish and protect the skin, alter the

external appearance (i.e. face) to give the user a rejuvenated and cleaner look, and remove the excess oils from the external surfaces of the body [23]. Skin care products in particular have been the source of interest for many buyers because they are cheaper than medical alternatives and can be easily accessed. In 2015, it was shown that the anti-aging creams in the cosmetic industry held the biggest market share of global skin care products due to their varied uses [24]. According to an article written in September 2016 [25], it is predicted that the sales of cosmetic products will rise globally due to their diversified uses. The appealing effects of these skin care products such as the minimization of wrinkles, minimization of acne scars, and dark spot correction motivate major brand name companies to constantly reevaluate the contents and ingredients of their skin care products.

The major ingredients included in skin care products vary from product to product and from company to company. The ingredients that are frequently used in skin care products are Alpha-hydroxy acids (AHAs), retinol, hyaluronic acid, copper peptide, alpha-lipoic acid, and dimethylaminoethanol (DMAE) [26]. These ingredients are utilized in skin care products that produce anti-aging effects. In Table 1, a summary of these ingredients can be found:

Table 1: Common Ingredients Used in Skin Care Products [26]

Product	**Description**
Alpha-hydroxyacids (AHA)	AHAs are used in lotions and creams to help correct the discolored pigmentation on the skin as well as remove the appearance of age spots. It is found in many over-the-counter products. Glycolic Acid is a type of AHA
Retinol	Retinol is an ingredient that is a derivative of vitamin A and is used to produce anti-aging effects. Skin responds better to retinol than other ingredients due to the molecular structure of Vitamin A. It is small enough to penetrate deep within the skin where it combines with the collagen and elastin to initiate the rejuvenation process by correcting skin pigmentation, texture, and tone.
Hyaluronic Acid	Hyaluronic acid is often an ingredient mixed with vitamin C to increase the amount of skin permeation. This ingredient is found in the body naturally in young skin. The natural aging process lowers the amounts of hyaluronic acid in the body. Reapplying hyaluronic acid into the skin helps to maintain a youthful appearance.

Copper Peptide	Copper peptide is a relatively newer ingredient used in skin care products as it has been on the market since 1997. This ingredient promotes the growth of collagen and elastin in the body while encouraging the effectiveness of the body's tissue regeneration process. It also has the ability to smoothen, soften, and toughen up the skin in a shorter amount of time than other anti-aging skin care ingredients.
Alpha-lipoic Acid	Alpha-lipoic acid has been called the universal antioxidant because it can dissolve in oil and water. It has the ability to repair past damage to the skin and prevent future damage. It has the ability to protect the skin from harmful agents and reduce the lines in the skin.
Dimethylaminoethanol (DMAE)	DMAE is in foods such as salmon, sardines, and anchovies. It is produced in the brain and important for mental health. In skin care, it is found to be effective in reducing lines and wrinkles on the skin when applied as a topical agent.

Many of these ingredients found in skin cream have alternative purposes aside from the anti-aging and rejuvenating effects when used in skin care products. Fibrin, as mentioned earlier in this chapter, can help to repair damaged tissues as a blood clotting factor and is a naturally occurring protein in the body. Due to its small molecular size, it can potentially be utilized as a skin care agent to help promote rejuvenation and replenishment.

2.9 IP Coverage on Powder and Cosmetics

The application for fibrin microthreads in which the team pursued commercialization is in the field of cosmetics. Specifically, the team's method involves a composition containing fibrin microthreads infused with another agent, such as retinol, to improve the performance capabilities of the infused agent. The infused fibrin microthreads are converted into particle form for use as an active ingredient in skin cream. Therefore, it was important to explore the prior art within these embodiments to determine what limitations the art presents. This helped the team determine what factors to improve upon.

Methods and compositions used for cosmetic purposes are well known in the art. For example, US patent application 11761893, "Skin care compositions and treatments," teaches of a composition containing growth agents to be used in pharmaceutical or cosmetic applications. In this disclosure, the method of caring for skin cells is utilizing cultured skin agents synthesized from cultured skin. A cell medium is utilized to capture these skin agents, and are incorporated

into a product to be applied to the skin of a patient [27, 28]. Skin care compositions of this nature may be commercialized and marketed within the cosmetics industry.

Further, fibrin powder is well known in the art. For example, US patent 9119897B2, "Dry powder fibrin sealant" teaches of a powder sealant composed of fibrinogen, thrombin, and an additional biocompatible material. Although relevant to the team's desire to create fibrin powder, strict limitation exists in the claims in the powder's application to open wounds to serve as a sealant. Recognizing this limitation to the patent proved important in ensuring our future concepts would not infringe on a previously claimed fibrin powder [29].

2.10 Cosmeceuticals and the Consumer

A crucial part of the cosmetics industry is the sub-group named "cosmeceuticals." Cosmeceuticals, coined by Dr. Albert Kligman, claims that cosmetics products under this label contain pharmaceutical like properties. In particular, Dr. Kligman recognized that "some cosmetic products" had impact on the structure of skin. Since cosmeceutical products must have sufficient scientific evidence to back these claims, consumers contain high expectations. Due to an increase in consumer expectations of scientific claims in cosmeceutical products, the FDA has contained authority over these claims. The FDA acts against cosmeceutical companies that claim false principles, as defined by the scientific reasoning behind claims, and false labeling. Additionally, the Federal Trade Commission (FTC) presides over marketing and advertisements that cosmeceutical companies in the industry claim in order to protect consumers from deceptive advertising regarding products in the field [30]. The team recognizes that sufficient scientific research exists as a necessity in order to efficiently pursue the commercialization of a product cosmeceutical market. However, it is important to first understand cosmetic dermatology.

2.11 Cosmetic Dermatology

Existing knowledge on the field of cosmetic dermatology can greatly benefit the team's approach of scientific experiments, which will ultimately lead to the final product of a novel skin cream. This section focuses on the subfields of cosmetic dermatology that pertain to this project. These subfields include some of the basic concepts of skin physiology, the delivery of cosmetic skin actives, the science of moisturizers, and finally, developments related to anti-aging and the world of cosmeceuticals.

2.11.1 Skin Physiology Pertinent to Cosmetic Dermatology

Four main concepts play a primary role cosmetic dermatology as it relates to skin creams. The first concept discusses the epidermal barrier and the corresponding layers of the skin which contribute to its general function and permeability. The next topic covers information related to the skin's somatosensory system. Next, the team addresses topical agents and their effect on skin. Finally, different techniques for evaluating cosmetic products is introduced and discussed.

2.11.1a The Epidermal Barrier

The skin acts as the physical barrier between the body and the external environment. There are three main components of the skin: the epidermis, the dermis, and the hypodermis [31, 32]. The epidermis is the outermost layer of the skin, and is comprised of several layers of epithelial tissue. The stratum corneum (SC) is the outermost layer of the epidermis [33]. The SC acts as a barrier which retains water and electrolytes [34].

The epidermal barrier is responsible for protection against other factors such as UV rays and disease immunity [35]. The epidermal barrier changes with age. The external environment also plays a role in this change. Along with other factors related to health and lifestyle, the barrier will either sustain or lose elastic, retentive, and otherwise youthful properties [35]. The

team's objective in creating a novel skin cream primarily interacts with the biological properties of the epidermal barrier. These properties include water evaporation, skin hydration and moisturization, desquamation, and potentially other barrier properties and protein functions.

Properties such as water evaporation, skin hydration and moisturization, and desquamation are vital to the epidermis. The SC takes part in providing hydration and moisturization to skin via natural moisturizing factors (NMFs). NMFs are a collection of water-soluble compounds which are vital to maintaining hydration, barrier homeostasis, desquamation, and plasticity [36]. The SC acts as the lipid barrier to the NMF, thus preventing its loss. The NMF provides hydration to the skin, even in harsh external environments [35, 37]. Desquamation is the shedding of the outermost layer of the skin. This process is vital for skin homeostasis [37]. Another sub process of the SC involves the regulation of proteases, which cause desquamation [38].

There exists a number of cosmetics ingredients that help restore skin barrier properties. Products labelled as moisturizers have shown to contribute positively towards restoring the SC [38]. The main ingredients used within these moisturizers are labelled as humectants, emollients, barrier compatible lipids, and antioxidant/anti-inflammatory agents. Humectants consist of a range of amino acids and their metabolites. Humectants aid to relieve dry skin conditions. Emollients prevent excessive moisture loss from the skin surface. Barrier compatible lipids replace the loss of lipids naturally synthesized in the skin that are responsible for barrier recovery. Finally, antioxidants and anti-inflammatories protect skin from UV and decrease skin redness [39].

While modifying the properties of the epidermal barrier can be beneficial to the health of one's skin, one must caution from applying substances which penetrate deeply into the skin. Said

substances can disrupt cellular processes existing in the lower layers [40]. Interrupting maturation of these cells or production of barrier lipids can result in undesired changes to the skin's desquamation process and ultimately the thickness of the SC [41].

2.11.1b The Somatosensory System and Skin Sensitivity

The somatosensory system of the skin covers the nerve impulses, which result in the brain responding to feelings of touch, temperature, pleasure, pain, and itch. Applications of topical products produce nerve responses that tell a person how an application is affecting their skin. Understanding skin sensitivity is essential to creating a good product that induces a positive feeling for the consumer.

The skin recognizes its surrounding environment by means of multiple receptors that respond to different stimuli. The perception of pressure, vibration, and texture, all rely on a group of four different receptors, known as low threshold mechanoreceptors (LTMs). Together, these receptors are effectively responsible for the perception of touch [41].

Pain is a response to irritation, stinging, burning, soreness, or painful sensations arising from the skin. The perception of pain is not solely dependent upon receptor input; it is also a result from other processes and pathways that provide information about emotional or contextual components [42]. Itch was thought to be a response of weakly activated pain nerves. Recent findings show the feeling of itch has a set of primary afferent neurons [42]. Pain and itch are both useful skin responses that indicate a product is having a negative response to the skin. These receptors can be signaled off by agents within a skin cream, or are a response to how the product was put on.

Pleasure is felt in response to low force, slowly moving mechanical stimuli affecting particular afferent neurons in the skin [43]. These afferents, alongside key myelinated and

unmyelinated fibers found in the skin, respond to parts of the nervous system which signal for pleasant touch [44, 45]. This is a highly sought after response for a skin cream product.

The somatosensory system plays a key role in the development of a quality cosmetic product. Interactions between different stimuli create the sensation for positive, neutral, and negative responses to the skin. This can result in a favorable or unfavorable experience to the user of a skin product.

2.11.1c Contact Dermatitis and Topical Agents

Within the application of topical agents onto the skin, there exists the possibility of having adverse reactions. Contact dermatitis is among the most predominant type of adverse reactions that have occurred as a result of cosmetics products [46]. Various forms of contact dermatitis exist. Irritants, allergens, phototoxic exposure, and foreign body reactions all have potential in causing contact dermatitis. Common irritants include everything from environmental elements to various chemical compounds. Notable allergen groups include fragrances, preservatives, and botanicals, all of which can be found in nearly all cosmetic products, especially creams [47].

Contact dermatitis is a negative response from the skin due to contact with undesired substances. The reaction with the undesired substances typically produces an inflammatory response to the skin. This reaction couples with disruption of the stratum corneum, and epidermal cellular changes [48]. Allergic contact dermatitis works much like regular allergenic responses in that a person needs to be pre-sensitized to the causative agent in order for there to be an allergic reaction [47]. Phototoxic dermatitis is a particular type of dermatitis resulting from using plant-derived photosensitizing compounds followed by exposure to UV light.

Fragrances are a common cause of contact dermatitis [48]. Preservatives are a common skin care product allergy inducer [49]. With respect to moisturizers, the most common irritants and allergens include fragrances, preservatives, vitamin E, essential oils, benzyl alcohol, propylene glycol, and lanolin [50]. In short, a certain population of consumers will run into difficulty in trying to use products with fragrances and preservatives. While there are products that don't use fragrances, nearly every skin cream product uses some form of preservative, as it is necessary for good shelf life.

Treatment of contact dermatitis primarily relies on discovering the agent which is affecting an individual. From there, the objective is future avoidance of the allergenic agent. While not every consumer might be able to apply a skin cream product due to the adverse effects resulting from its ingredients, it may be important to have variations of product which allow for a larger consumer pool.

2.11.1d Evaluation of Cosmetic Products

Any kind of skin care product needs to be tested for its safety and efficacy. Non-invasive, cheap, and accurate testing protocols are the most ideal. One of the most effective ways of measuring the change in someone's appearance through the use of cosmetics products is digital photography, although other means of evaluating cosmetics products exist.

There are two challenges for photography in a clinical setting. The first challenge is to choose the best photographic technique relative to the aims of the study. The second challenge is to maximize the consistency of the imaging at each clinic visit throughout the trial. In choosing the right photography technique, there exist a number of options. These options are visible light photography, raking light photography, polarized photography, UV reflectance, and finally UV

fluorescence. Each technique helps to focus on a particular area or aspect of the skin that is being aimed at for study purposes [51].

There has been demand for new technology in the field of photography as a result of the need for more objective, quantitative, and noninvasive tools for skin assessments. Some of these methods include new light spectrophotometric techniques in which light is penetrating the skin at up to 2 mm. Images taken of this light penetration are able to provide a better view into the changes happening under the skin as a result of product use. Improved 3D imaging systems are being used to better capture skin sagging conditions and wrinkle depth.

Photography and other non-invasive techniques are useful when assessing the safety and efficacy of cosmetic products. As the subject of evaluating products drives forward the type of ingredients used in cosmetics skin products, there begins the discussion of the active ingredients being used in cosmetics skin products.

2.11.2 Delivery of Cosmetic Skin Actives

With recent developments in new technology, new advances in delivering substances through the skin have arisen. The delivering of cosmetic skin actives has been primarily done through delivery by penetration of the stratum corneum into the skin. With thorough understanding of skin physiology and structure, it is possible to create vehicles for passage by physical and/or chemical means.

Thorough the understanding of skin physiology must accompany with knowledge of the active being delivered into the skin. Measurable characteristics that help in determining an active ingredient’s potential in entering the skin include molecular weight, dissociation constant, solubility, octanol/water partition coefficient, and net ionic charge [52].

Permeation of an active across the stratum corneum (SC) can be approximated using an equation known as Fick's Law, which defines steady state flux. Fick's law can be seen here, in Equation 1:

$$J = \frac{DK}{L}(C)$$ *Equation 1: Fick's Law*

D is noted as the diffusion coefficient of the SC. L is the diffusional path length or membrane thickness. K is the partition coefficient between the SC and the vehicle. C is the applied drug concentration, which acts as a constant in this equation. Through manipulations of K and C, scientists have been able to increase drug diffusion, increase drug solubility, and also increase the degree of saturation of drugs in formulations [53].

2.11.2a Vehicles

One of the most commonly used vehicle types for topically delivered actives is either oil-in-water, or water-in-oil based emulsion. There is a difference between oil-in-water and water-in-oil based emulsions. These differences are more effective in certain formulation strategies. Theoretically, high solubility and affinity for the hydrophilic and lipophilic components of the SC allow for faster partitioning of the active into the skin. This can be done using chemical adjuncts, supersaturation, eutectic blends, skin occlusion, and penetration enhancers which come in chemical or physical forms [54].

Vectors for aiding in penetration enhancement include liposomes, niosomes, solid lipid nanoparticles, and nanocapsules. Liposomes are useful for encapsulating active ingredients for passage through the SC [55]. Liposome effectiveness varies by size, lipid composition, surface charge, mode of application, and total lipid concentration [55]. Niosomes have an infrastructure consisting of hydrophilic, amphiphilic, and lipophilic moieties. Together these properties allow for a wide range of actives to be encapsulated within them at various solubilities [56].

Solid lipid nanoparticles require no solvents for production processing. They were originally developed as an alternative carrier system to emulsions, liposomes, and polymeric nanoparticles. Currently, they are being investigated as carriers for skin delivery of sunscreens, vitamins A and E, and other actives [57]. Nanocapsules are a type of submicron delivery system (SDS). Their small size allows for easy penetration into the skin. They have shown potential to be safe for the delivery of actives, and can also control the level of delivery to the skin [58].

2.11.2b Devices for penetration enhancement

In terms of devices used for skin penetration enhancement, moderately invasive to slightly invasive systems exist. Among these systems are ultrasound waves, patches, microneedles, and iontophoresis. Ultrasound waves have shown to change the skin barrier properties through mechanical and thermal energy [59]. Patches have been in existence and are useful for localized delivery [60]. Microneedles are a more invasive route. Various micron sized needles composed of different materials penetrate the skin for direct active delivery. Iontophoresis is the process of using current to manipulate charge properties on the skin, allowing for better passage of actives [61].

2.11.2c Assessment of Cosmetic Active Delivery

Assessment of cosmetic active delivery in vivo and in vitro can be done through different forms of testing. These tests include Franz cell, tape stripping, microdialysis, and confocal Raman microspectroscopy. A key evaluation metric with particular respect to skin creams and moisturizers is the rate of diffusion at which active ingredients are passing through the stratum corneum and into the deeper layers of the skin. This kind of evaluation is important for understanding whether an active added to a formulation is able to enter the skin at all, and if so, at what rate, due to the carriers which exist as part of the formulation. One particular method of

measuring the rate of diffusion through skin that will be discussed more in detail is known as Franz Diffusion Cell [62].

A Franz Diffusion Cell permeation assay functions by taking a membrane (in many cases a skin sample), and placing it between two chambers. The top chamber is known as the donor chamber. In this chamber, the donor compound (typically a formulation) is introduced above the skin sample. The bottom chamber is known as the receptor chamber. Here, fluid (typically PBS) is added and sampled through a sampling port. A stir bar sits at the bottom of the chamber to create circulation. A heater and circulation section surrounding receptor chamber is filled with fluid that is maintained at 37 degrees Celsius. The donor compound, or test product, is applied to the membrane via the donor chamber. Fluid within the bottom chamber collects ingredients which permeate through the membrane. Using the sampling port, samples can be taken at different time intervals.

Some of the drawbacks of this kind of assay include not being able to determine how much of an active ingredient is needed to provide a beneficial effect. Advantages of the test include its low cost and replicability. For most test products, this assay is used to evaluate the ideal formulations to be tested in vivo.

While the manner in which actives are delivered into the layers beyond the stratum corneum, it’s important to show precaution towards penetrating actives deep enough into the skin that they begin to interrupt corneocyte functions which affect the properties of all the above layers in the epidermis. Testing can demonstrate the effect of active delivery. Delivery of actives is a balancing act of localization, penetration, and the cost of laboratory techniques.

2.11.3 Creams

Creams are emulsions. An emulsion is a colloidal dispersion comprising of two immiscible liquids (for example, oil and water), one of which is dispersed as droplets within the other [63]. An emulsion also contains emulsifiers or dispersing agents that are responsible for keeping the two different phases together for a long time. There are two subcategories of emulsions which separate the oil-in-water and the water-in-oil type of emulsions. Only the oil-in-water based emulsions will be discussed, as they are the preferred emulsion type for making creams.

2.11.3a Composition of a Cream

Oil-in-water (O/W) creams are the most popular type of emulsions used in skincare products. O/W emulsions are composed of a mixture of ingredients which make up the water phase, and a mixture of ingredients which make up the oil phase. Along with the ingredients which comprise of these phases, there are additional fragrances or essential oils which can be added, additional preservatives, color, and pH adjustments. Together these ingredients create a complete O/W cream.

There are five general components that have an individual effect on the aesthetic of an O/W cream. These components are the emulsifiers, emollients, active ingredients, humectants, and thickeners. Emulsifiers play a role in determining the pH of the emulsion. They affect the application and stability of the emulsion, as well as the delivery of materials into the skin. There exists a popular group of emulsifiers to use which are "cationic": said emulsifiers have a net positive charge, which attaches more readily to the net negatively charged skin. [64]

Emollients play a major role in the feel, application, and delivery of the active ingredients to the skin. There has been an increased use in silicone and natural emollients as of recent. These have given rise to much more possible emollients to choose from. The most difficult part of

choosing an emollient is in picking the one which delivers the right initial, middle and end feel for a user.

Humectants can be a part of the solvent system for the active ingredients in an emulsion. They also play a role on “skin cushion”, and may function to help with freeze/thaw stability. Thickeners control the viscosity and rheology of an emulsion. They also help in maintaining the stability of the emulsion. The choice of thickener is very dependent on the compatibility of the thickener with the rest of the ingredients, as well as the pH of the formulation. For O/W emulsions, the most common types of thickeners are acrylic based polymers.

Together, these ingredients form an emulsion, alongside any fragrances, essential oils, additional color, preservative, and pH adjustment (used as required for emulsion formation). It is essential to know the roles existing ingredients within skin creams play. In addition to the main ingredients that formulate a baseline skin cream, there is one final type of added ingredient: cosmeceuticals.

2.11.4 Cosmeceuticals

The term cosmeceutical describes any cosmetics product which contains biologically active ingredients claiming to have medical or drug-like properties. There exists a large variety of cosmeceutical ingredients. Each will be discussed for their value brought to the cosmetics realm, as it possible for some of these ingredients to eventually become incorporated into the product of which this project is focused. Listing the types of cosmeceuticals currently in existence, there are botanicals, antioxidants and anti-inflammatories, peptides and proteins (of which fibrin can fall under as a new ingredient), cellular growth factors, retinoids, topical vitamins, and hydroxyacids.

2.11.4a Botanicals

Botanicals refer to any of the herbal-based products which exist in today's market. Botanicals have been shown to have beneficial effects on photoaging. They have been shown to target aging pathogenesis occurring from a disrupted/impaired stratum corneum barrier, and chronic inflammation [65]. There are also botanicals which have active ingredients to brighten or lighten skin, tighten skin, modulate nucleic acids, and protect from light exposure [66]. Botanicals research is still developing, with more herbal ingredients being tested through clinical trials. The botanical realm is extremely vast, and advantageous to understand for potential opportunities in emulsion formulations.

2.11.4b Antioxidants and Anti-Inflammatories

The role of antioxidants for use in both oral and topical applications is in part to reduce the number of free radicals and reactive oxygen species which exist in the body and cause damage to proteins, DNA, and lipids [67]. Anti-inflammatories bind to DNA regulatory elements which in part inhibit specific pro-inflammatory genes that set off the inflammation cascade [68]. The important aspect of both antioxidants and anti-inflammatories is their potential role in minimizing the damage caused by aging. Unfortunately, many anti-inflammatory products currently out on the market have shown negative side effects when applied to the skin. These side effects range from the lowering of collagen production which reduces fibroblast proliferation, to causing immunosuppressive effects which can lead to cancer. Overall, new, safer anti-inflammatory technologies need to be discovered in order for them to become viable for use [69].

2.11.4c Peptides and Proteins

Peptides play a major part in many biological signal functions. Peptide hormones already control important biological activities such as the regulation of blood sugar concentration, blood

pressure regulation, and much more [70]. With the right carriers, it is possible to introduce peptides topically into the epidermis, where biological signals can be activated (or inactivated) within keratinocyte cells [71]. With the right peptides, certain biological signals can alert cells to produce desirable actions, such as faster repair, reduction of skin sensitivity to a variety of stresses, or an increase in elastin synthesis [72].

Proteins offer much less capability in the cosmeceutical realm. They are much larger and more difficult to carry through to the deeper layers of the skin. Proteins are the vital 3-dimensional structures which form muscles as well as the skin. Enzyme proteins in particular speed up biochemical reactions that would otherwise occur too slowly for the body to function. Within the realm of cosmeceuticals, there are particular protein enzymes which have been used for chemical peelings, photoprotection, and in some newer cases infrared protection. As a protein compound, fibrin faces the same difficulty of diffusion through the skin. Despite this, it is advantageous to show fibrin's inability to diffuse through the skin. Fibrin can act as a reservoir for other drugs or actives to pass through the skin as fibrin degrades over time, without penetrating the SC.

2.11.4d Cellular Growth Factors

Cellular growth factors have some of the greatest potential in anti-aging. Studies have shown their major advantages of accelerating wound healing. Studies have also shown disadvantages in potentially promoting cancerous cell formations [73]. Other disadvantages and obstacles include difficulty in maintaining activity of growth factors through product shelf-life. Cellular growth factors also have an issue with successfully passing any large proteins through the skin [73]. While there are some alternative methods of getting large molecules into the skin (mainly in the form of physical skin penetration techniques), it would be more ideal if simple

topical application was the only requirement. From there, control over the acceleration of biological activity would aid in preventing issues associated with carcinogenic activity.

2.11.4e Retinoids

Topical cosmeceutical retinoids are a class of substances composed of vitamin A and its natural and synthetic derivatives. Retinoids are lipophilic molecules that can pass through the stratum corneum, where they then bind to nuclear receptors. This binding results in the modulation of gene expression involved in cellular differentiation and proliferation [74, 75]. The different classes of retinoids provide certain advantages and disadvantages. Retinoids have been used in cancer treatment and prevention due to their role in increasing tumor-preventive transcription factors while also decreasing factors linked to proliferation and inflammation [74].

Hyaluronan has been shown to be a valid partner for cosmeceutical retinoids [76]. Hyaluronan (HA) is the major component of the extracellular matrix (ECM) and is found in high quantities in the skin. HA can accommodate many water molecules due its negatively charged areas. This helps to maintain hydration and viscoelasticity of the skin [77]. Studies have shown that topical retinoids, particularly retinaldehyde, can restore epidermal functions by stimulating HA synthesis and biological functions [78].

One of the major issues resulting from the use of retinol and retinyl esters (such as retinyl palmitate) in topical use is the induced irritant dermatitis that occurs at the same concentrations required to induce any measurable biological action similar to that of retinoic acid [79]. To counteract this, combination with other topical agents can be used to mitigate irritant response from skin. Overall, cosmeceutical retinoids can be seen as an excellent potential additive to be mixed in with fibrin as a cosmeceutical. Retinol's ability to pass through the membrane while

subsequently delivering positive effects to the skin is highly promising, given a low threshold to avoid irritation.

2.11.4f Topical Vitamins

Based on form and concentration, many vitamins can be applied topically to the skin to improve skin appearance. Aside from Vitamin A (retinoic acid), there is vitamin B3, B5, C, E, as well as several forms of vitamin D, K, and P. Each type of vitamin poses its own set of formulation challenges. For vitamin B3, the main issue is hydrolysis of niacinamide into nicotinic acid, which causes intense skin reddening [88]. Panthenol (B5) can produce sticky/greasy feeling formulations which may be undesirable to users. While the antioxidant benefits among most of these vitamins are attractive, there remains the problem of getting a measurable amount through the skin in a form that is both aesthetically pleasing and cosmeceutically beneficial.

2.11.4g Hydroxyacids

Hydroxyacids (HAs) are low pH substances that exfoliate the SC and modulate the process of keratinization [81]. Hydroxyacids can be categorized into five groups. The most widely studied and commercialized group is the alpha-hydroxyacids (AHAs). The simplest of AHAs, glycolic acid, can be derived from sugar cane. AHAs are great for targeting oily and acne-prone skin. Certain HAs, such as malic and tartaric acid, double as antioxidants.

Other benefits from HAs include antiglycation, zero change in sun sensitivity. Certain HA groups such as PHAs and bionic acids are non-stinging and non-burning [82]. For clinical uses, HAs aid dry skin and hyperkeratinization, keratoses and dyspigmentation, wrinkles, and photoaging. As mentioned before with retinoids, HAs have already been shown to possess synergistic qualities with topical drugs.

2.11.5 Cosmetic Dermatology and Fibrin-Based Skin Cream

The purpose of detailing some of the many possible formulation ingredients, as well explaining the science of cream formulation down to the individual ingredients, was to better understand the complexity and science behind producing a skin cream. The role which fibrin can play as an additional ingredient of choice within the skin cream realm is a top priority for this project. Formulations using the various ingredients mentioned above alongside fibrin can be designed so as to have a complete cream.

As a protein, fibrin faces the challenge of passing through the stratum corneum and into the epidermis of the skin. This is because of its relatively large size. However, this may not be the desired intent for fibrin within a skin cream. Due to the nature of the extrusion process in which fibrin micro threads are created, it is more likely that fibrin can be beneficial as a natural vehicle for other cosmetic actives. The results of this could mean a longer-lasting release of actives into the skin.

The process of using an anti-aging regimen follows this order: Cleanse, exfoliate, protect, and treat. Cleansing involves the removal of dirt and other debris on the skin. Exfoliation by definition is the removal of dead skin cells on the outermost surface of the SC. Protection is the use of photoprotective ingredients to help avoid damage caused by sun exposure. Treatment involves the use of ingredients which aid in the skin's look and health.

It is possible for fibrin to aid in more than one of these anti-aging regimen steps. With regards to exfoliation, it is possible for fibrin particles to have a positive mechanical abrasion effect on dead skin. As a potential photoprotective, fibrin absorbs a set amount of UV radiation before crosslinking. There are endless possibilities for fibrin to be used an effective skin cream.

2.12 Previous Designed Mechanisms to Manufacture Fibrin Microthreads

In order to develop a feasible design that will successfully extrude fibrin microthreads, an analysis of previous Major Qualifying Projects (MQP) related to automated manufacturing machines is important to comprehend because it will help the team eliminate non-viable design options and specify the important objectives and components needed. This will also help the team decide upon what specific design mechanisms to include in the final design. The team evaluated two MQPs from 2010 and 2012 that worked to design automated manufacturing machines of collagen microthreads and fibrin microthreads respectively. The team focused on the groups' approaches to their design process as well as their final design outcome and results.

In general, both teams had similar obstacles when deciding on important design objectives for their machine. The manual extrusion process of collagen and fibrin microthreads is extremely time consuming and does not always produce threads that are uniform and of similar diameters [83]. It also requires additional labor to manually extrude the threads and due to inconsistencies in the microthreads' properties, not all the threads are viable following the extrusion process [84]. The 2010 MQP team's objectives was to design an automatic manufacturing machine that would yield precise and reproducible collagen microthreads, produce threads that can withstand uniaxial loads and can be applied towards therapeutic effects, and allow for thread modifications following the extrusion process [84]. The 2010 team approached their design by focusing on automating the entire thread extrusion and drying process. The final design was comprised of a motorized bi-directional extrusion system, an extrusion bath, the removable anchor system, and drain system [83]. They modified the machine to allow for a variation in the output of the collagen threads. One aspect of the final design that the 2010 MQP team focused on was the anchoring system. This system allowed them to dry and stretch the threads, while creating a

smooth transition platform from the extrusion phase to the post-production phase of the machine [83]. The removal and drying process, however, was not automated and did require manual labor. Despite this drawback, the team did achieve success in automating the main extrusion process, which saved collagen thread production time and money.

While many of the design challenges for both teams were similar, the project team from the 2010 MQP required additional design parameters because they dealt with collagen threads. For example, the team had to add an additional heating element set at 37 degrees Celsius (body temperature) because that temperature is required for the collagen to remain viable following the extrusion process [84]. On the other hand, the 2012 MQP team dealt with different constraints because they were working with fibrin, which is a smaller thread and required different extrusion parameters (i.e. the extrusion process did not require a heated water bath since Fibrin can thrive at room temperature) [84]. The 2012 team also referenced the 2010 MQP and aimed to try to automate both the extrusion and removal/drying process. The 2012 team had many design ideas including ways to secure the threads and remove them without breaking them. One of the ideas involved the use of a Velcro material to hold the threads in place [84]. They performed experiments to test the reliability and durability of the Velcro's ability to stabilize the threads without breaking them. Even though many of the 2012 team's ideas were worth trying, constraints such as budget and accessibility to design materials led to a final design of only a semi-automated machine for fibrin microthreads [84].

Understanding the design processes of previous MQPs can help future projects increase their chances of success in the final design. Understanding manufacturing processes can also help the team design products that adhere by FDA and safety regulations and to ensure success in the manufacturing gold standards.

2.13 Good Manufacturing Standards

Putting a product on the market requires specific standards, both manufacturing and safety related, in order to be viable and safe. In terms of the automated manufacturing machine and the cosmetic applications of the fibrin microthreads, the manufacturing process itself and the marketing of the cosmetic cream requires a level of strict regulation and consumer transparency. According to the United States Food and Drug Administration, the regulation system used to monitor different manufacturing facilities and practices is the current good manufacturing practice (CGMPs) regulations [85]. Based on the product, different standards and regulations apply to ensure that the product is abiding to the correct design and manufacturing procedures. Products range from biological devices to pharmaceuticals to a mix of biological specimens and biological devices.

The team desired to design a mechanism to automate and mass produce fibrin microthreads. In order to efficiently develop a design, it was important to consider devices that previously exist in the relevant art made for the purpose of the creation of other biopolymer threads. For example, US 5378469 discloses an apparatus and method of creating threads composed of collagen for certain manufacturing applications, including knitted and woven particles, to implantable prostheses. Kemp et al. teaches that the collagen solution can be extruded into a "dehydrating agent" that is under conditions to allow the extruded solution to form into a collagen thread [86]. The design includes an extrusion mechanism, a syringe, the dehydrating agent bath, and a series of pulleys that lead the thread through a drying device to be stored in a spool. In order to create a viable fibrin microthreads automated manufacturing machine and use them towards greater applications, abiding by safety and regulatory factors was important in making the product legitimate and recognizable by overarching bodies such as the

FDA. When moving forward with the design process, the team focused on adhering to good manufacturing principles while accomplishing the tasks of the fibrin microthread production mechanism.

Chapter 3: Project Strategy

This project consists of three distinct parts: validating fibrin's value in the cosmetics industry, assembling the automated manufacturing machine, and converting the fibrin microthreads into a powder form for the application of fibrin microthreads in a new cosmetic cream. Each part contains its own set of objectives and goals. The team decided to accomplish each part by separating the design objectives into three separate analyses: a breakdown of the requirements for the fibrin mixed cosmetic care skin cream, a breakdown of the requirements for the automated manufacturing machine, and a discussion of the ways the fibrin microthreads can be converted into an applicable form for the cosmetic skin cream. The team first analyzed the initial client statement with the aim to gain a sense of what direction the project was headed and what further information was needed in order to move the project forward. A qualitative and quantitative analysis was then performed to identify and rank the objectives according to importance.

3.1 Initial Client Statement

The team was tasked with an initial objective statement that established the foundation of the project. Per Professor Glenn Gaudette, the team was given the following client statement:

"The main objectives for this Major Qualifying Project is to create an automated manufacturing device that can extrude fibrin microthreads and minimize the need for continuous manual labor, while subsequently altering fibrin into a form useful for cosmetic cream applications."

In order to address these tasks, the team explored and analyzed extensive background information, discussed in detail in Chapter 2, to further develop the initial client statement and generate a revised client statement and more detailed list of objectives. The team also utilized research from past MQP projects and regulation standards in order to understand what type of

regulations each part of the project must adhere by to ensure a successful outcome for the client and the potential user(s).

3.2 Design Requirements of Validating Fibrin's Value for a Cosmetic Cream

When validating fibrin microthreads for cosmetic skin creams, the design requirements included several property-identification experiments. The main objectives of these experiments focused on demonstrating fibrin's potential as a demandable skin cream product ingredient. Constraints, such as time and supplies, were considered when planning the timeline of experiments. Validation of the experiments are explained via the protocols for the experiments (See Appendix III for more detail), as well as the decision behind running each experiment. Standards and regulations of cosmetics products will be readdressed to validate the team's pursuit towards the final product of fibrin-based skin cream. Figure 1 below lists the major objectives for our Fibrina skin cream product.

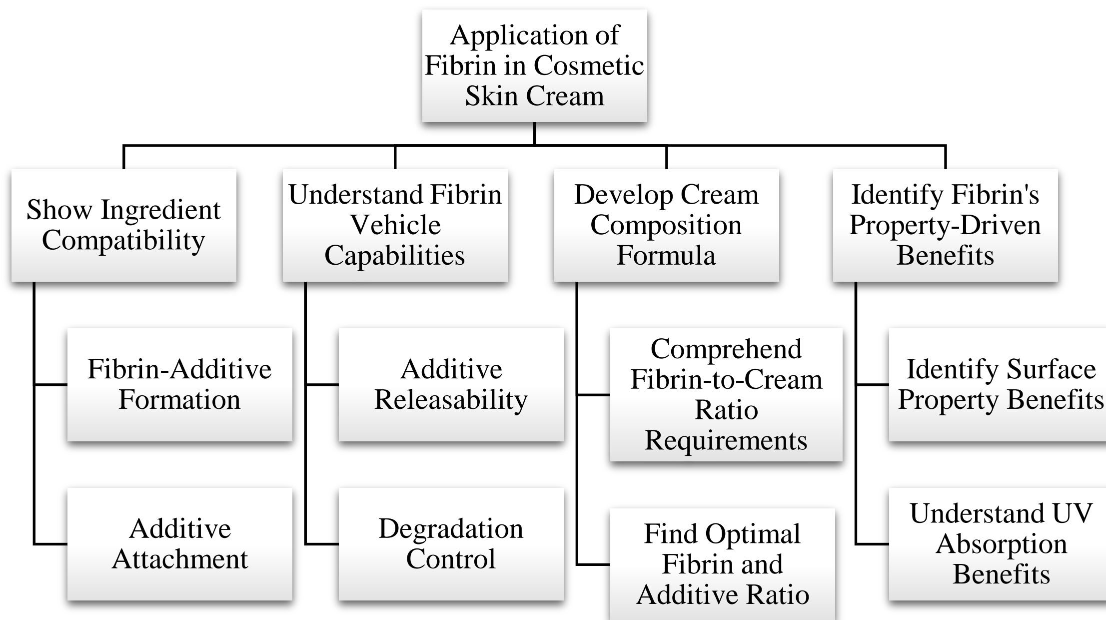


Figure 1: Objective Tree for Fibrina

3.2.1 Objectives

The overarching goal for validating fibrin's value as a cosmetic skin cream ingredient was to identify and scientifically support all benefits of fibrin for use in cosmetics. The team ran several experiments on the various properties that helped to identify the outcomes of applying fibrin along with other common skin cream additives. The following breakdown of objectives details all aspects of the overarching goal and how the team evaluated their achievements as part of the overall objective.

3.2.1a Showing Ingredient Compatibility

Since the team is introducing a new ingredient to the cosmetic industry, it is important to provide data that qualifies fibrin as a compatible ingredient. For this project, the objective of ingredient compatibility was to show that fibrin was compatible with retinol, a highly popularized active skincare ingredient. Success or failure in achieving basic compatibility with retinol creates an opportunity to create better protocols for testing fibrin's synergistic properties with other ingredients.

Basic compatibility corresponds to introducing retinol into fibrin while maintaining fibrin's compositional form as a microthread upon production. This objective breaks down further when looking at the process of attaching retinol to fibrin. Synergies among combinations of ingredients used in the cosmetics industry exist. Discovering synergies of actives alongside fibrin would further promote the use of fibrin in cosmetics.

3.2.1b Fibrin Vehicle Capabilities

Larger proteins such as fibrin are especially difficult in penetrating through to the skin, it is important to find use for fibrin in cosmetics, primarily as a carrier for other actives. The purpose of assessing fibrin's vehicle capabilities was to demonstrate that active ingredients

carried by fibrin can be delivered to the skin more optimally than it would on its own. In order to achieve this, fibrin must release any active over time, after it has been applied to the skin. With regards to the release profile of fibrin, there should also be customization. Depending on determining factors of fibrin-active release, the team wanted to control those factors in order to offer various options in time-release capabilities.

3.2.1c Cream Composition Formula

Since there are existing FDA regulations on the active percentage allowed within cosmetics products, the team wanted to understand the relative quantities of fibrin-to-active that would be required for a baseline cream. For that, there was the objective of determining how much active can be successfully carried by fibrin. Knowing the maximum carrying capacity for additives within fibrin would showcase how well the team's final product would be able to perform as a novel carrier. The resulting quantity of fibrin particles required to be used in a cream will affect overall costs, as well as the texture and appeal of the cream.

3.2.1d Fibrin's Property-Driven Benefits

The last three objectives looked to identify fibrin's capabilities as a carrier for actives. Success in achieving these objectives would be great, but perhaps not great enough in terms of differentiating fibrin from other ingredient-encapsulating products on the market. One positive product outcome identified through cosmetology research was the existence of exfoliates. Given that the team sought to ground up fibrin microthreads into particles, there was incentive to objectivize finding results on whether the surface roughness of those particles would aid in skin exfoliation. Other existing research highlights fibrin's UV absorption properties. Since UV absorbance is a sought after property for sunscreens, the team wanted to understand how fibrin's UV absorption properties could benefit a skin cream. Overall, the proposed objectives lean to

various lab experiments. With a budget shared for both laboratory work as well as mechanical work, the team had to identify constraints.

3.2.2 Constraints

Constraints included limitations on time and money. The team was tasked with focusing on three major sections of the project (validating fibrin's value through cosmetology experiments and customer discovery, mechanical engineering through automation design, and fibrin's conversion capabilities). Since the team had three major project focuses, there was little room for errors that could lead to extending certain deadlines, thus pushing the project's deadline behind schedule. Money was also a major factor. The team was given $750 for the MQP. Fortunately, through the team's participation in the WPI Accelerate Program, more funds were secured for this project ($1728.67). However, splitting the total amount of funds among experiments and machine parts would be straining for both project sections.

3.2.3 Functions of Additive-Infused Fibrin Threads

Functions for additive-infused threads were created to focus the team on what outcomes were desired for the experiments being conducted. The team focused primarily on one ingredient: Retinol (also known as vitamin A). Through a set protocol of experimenting fibrin threads with retinol, the team desired for three functional outcomes:

1. Additive-infused threads should be able to hold at least 10% of actives with respect to their overall weight.
2. Additive-infused threads should be capable of being ground into particles.
3. Additive-infused threads should be capable of releasing actives within it.

The identified outcomes cover the basic necessary functions for additive-infused fibrin threads to be commercially valuable within a skin cream.

3.2.4 Standards and Regulations

Standards and regulations for developing skin cream are covered under the background research on the FDA and its jurisdiction over cosmetic products. The FDA does not require the need for approval of a cosmetic product prior to its entry to the cosmetic market. However, the FDA monitors products for their safety and its effects on consumers. The team chose to be transparent with the ingredients it uses to develop the cream. These standards help to maintain the safety of the product by making it the job of the FDA to hold the commercialization of a product such as fibrin skin cream accountable for its effects on consumers.

3.3 Design Requirements of the Automated Manufacturing Machine

Developing an automated manufacturing machine required a detailed design plan in order to produce successful results. The team's overarching focus for the automated manufacturing machine for the extrusion of fibrin microthreads was to automate the entire extrusion process. The team generated and compiled a list of objectives, constraints, functions, and specifications of the machine, taking into consideration the feedback and suggestions of the team's client, Professor Glenn Gaudette.

3.3.1 Objectives

In order to efficiently organize the objectives and requirements of the machine, the team generated an objective tree (Figure 2) to organize and identify the tasks in each of the major objective categories (next page):

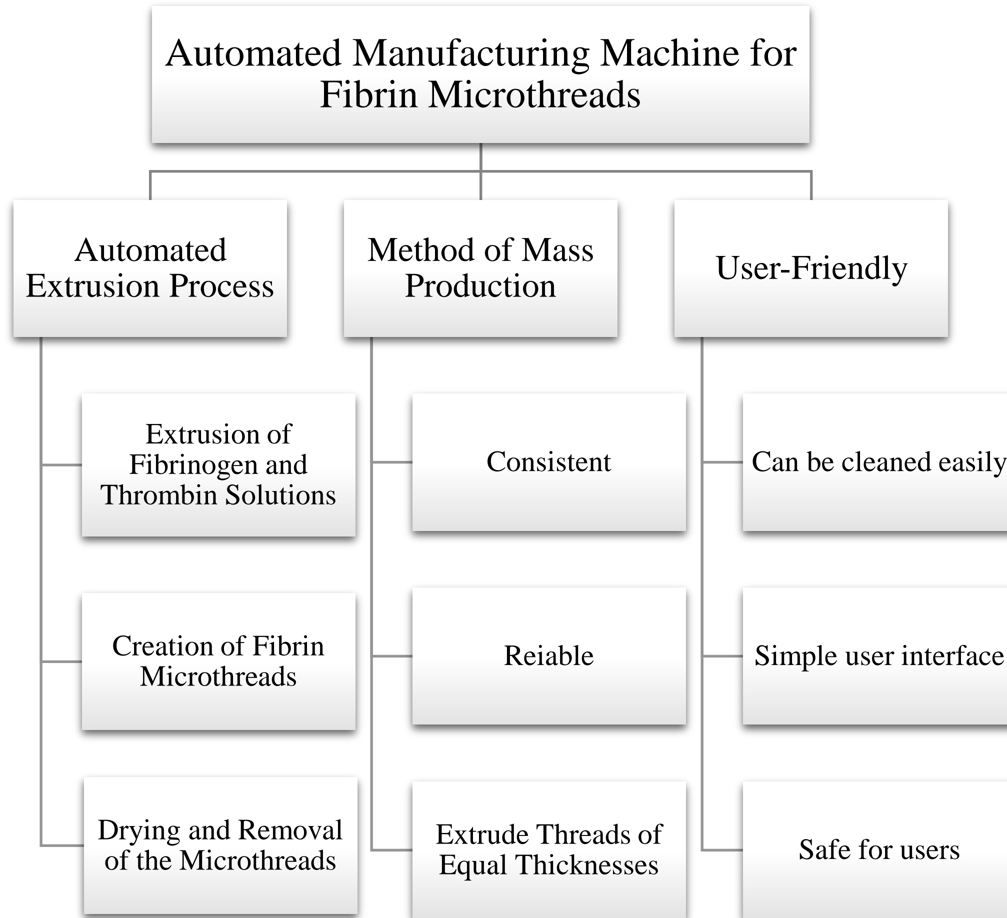


Figure 2: Objective Tree for the Automated Manufacturing Extrusion Device

The team broke down the major objectives for the automated extrusion machine in three separate categories: the automated extrusion process, the machine's effectiveness and its ability to produce a quality end product, and its usability.

3.3.1a Automated Extrusion Process

The team defined the first objective by breaking down the fibrin microthreads manual extrusion process into three separate steps: the extrusion of the fibrinogen and the thrombin, the creation of the fibrin microthreads, and the drying and removal of the fibrin microthreads. The team found it necessary to break down the extrusion process into different steps in order to efficiently organize the automation process. The purpose of automating each step was to reduce

the amount of manual labor needed for the extrusion process. This ensured more consistent threads of equal thickness, which is hard to achieve during the manual extrusion process.

3.3.1b Method of Mass Production

The second objective of this device was to produce fibrin threads in mass quantities. This means two things: the first is that the threads being produced by the machine are consistently identical (within a given tolerance). The second meaning is that the machine can reliably produce threads over and over again, in large batches. Fibrin microhreads should become a surplus resource material because of this device.

3.3.1c Machine User-Friendliness

The third objective of this device was to have a user friendly machine interface. A user should have minimal difficulty with controlling the device. A user should also be able to understand how the device works, ideally through a user manual, but also by looking at the device and understanding its basic actions. Overall, one should be able to recognize what the device does, and how to go about using it without difficulty.

3.3.2 Constraints

The constraints of the automated extrusion device are based on how the device runs and the setting of the device. Constraint factors such as complexity, software resources, powering resources, and safety affects how the device runs. Constraint factors pertaining to where the machine is placed (i.e. a laboratory setting) involves the machine's size. Cost was the ultimate limit for the design of the machine.

Powering and software resources were a major constraint for any automated. Based on the scope of the design, it was important to choose the correct motor or actuator. The control of these motors were dependent on the software decided upon by the team.

Safety was the constraint that takes priority for an automated system. The device must be safely operable under any conditions. These conditions can include, but are not limited to, starting the device, stopping the device, repairing the device, replacing any parts to the device, and cleaning the device. In summary, the device could not be a threat to the safety of a user.

The constraint of size affects the dimensions that the machine can be designed around. The machine prototype had to fit on a lab bench to allow the machine to be efficiently tested in a laboratory setting.

Considering the budget allocated for the project, the team also had to determine the type of materials and moving parts which will be used to develop this device. The team kept in mind that this budget was also used for any preliminary device testing is crucial. Reusing parts from preliminary and being smart about material purchases will help to maintain the cost constraint from devaluing the end result of this project. In thinking about the kinds of materials to use and their respective costs, the team must consider the machine's functions and specifications.

3.3.3 Functions and Specifications

The functions and specifications of the automated fibrin microthread extruder machine related to the machine's ability to output quality microthreads, as well as the machine's capabilities. The machine needed to perform the following actions as they pertained to the current manual process of making threads: 1. The machine had to extrude fibrin microthreads; 2. The machine needed to dry the fibrin microthreads.

The first main function for this machine was to extrude threads. Threads must be extruded into a HEPES bath in order for the threads to form properly. From there, either the HEPES bath or the threads themselves must be moved in order for the drying of the threads to

occur. Finally, threads produced by the machine must move to a stage where they can be stored for future use, or placed into a grinder or other form-changing machine.

Other minor existing functions included the need for the device to be cleaned prior to the next extrusion batch. This was important for removing excess fibrin that may be lying around the surface part of the machine where new threads would be formed via extrusion. The machine itself also needed to run at a set pace: given the multiple stages of extruding, drying, removing, and cleaning of the threads, if the device was to run in sync, then for efficiency's sake, each stage had to run in coordination with the others.

3.3.4 Standards and Regulations

The automated extrusion device consisted of programmed mechanisms that perform a general task without human intervention. To account for the standards and regulation for this manufacturing machine, ISO standards must be abided by. Under ISO 8373:2012, a manipulator is defined as, "machine in which the mechanism usually consists of a series of segments, jointed or sliding relative to one another, for the purpose of grasping and/or moving objects (pieces or tools) usually in several degrees of freedom" [87]. As this pertains particularly to the intended design of the mechanism, further consideration must be put into ISO 9283:1998, which outline testing and performance standards for manipulating industrial robots. Under this standard, methods for regulating pose accuracy and repeatability, distance accuracy and repeatability, path accuracy and repeatability, and static compliance is included, along with other regulations [88]. Finally, under ISO 3952-1:1981, in order to provide proficient description of the mechanism through technical writing, reports must contain kinematic diagrams. These diagrams are required to include motion of links, kinematic pairs, links and connections of components, and N-Bar linkages and components [89].

3.4 Conversion of Fibrin Microthreads into a Usable Form

An important aspect of the project was to tie Objectives 1 and 2 together. To accomplish this task, the team had to determine a method to convert fibrin from microthreads to a particle form.

3.4.1 Objectives

There was one major objectives for this part of the project: find a method to equally convert the fibrin microthreads. Within the two sub categories, the team identified potential conversion methods for transforming the fibrin from microthreads to particle form and the specifications for mechanism to allow for more equal conversion of the microthreads. In Figure 3 below, a breakdown for this objective is shown.

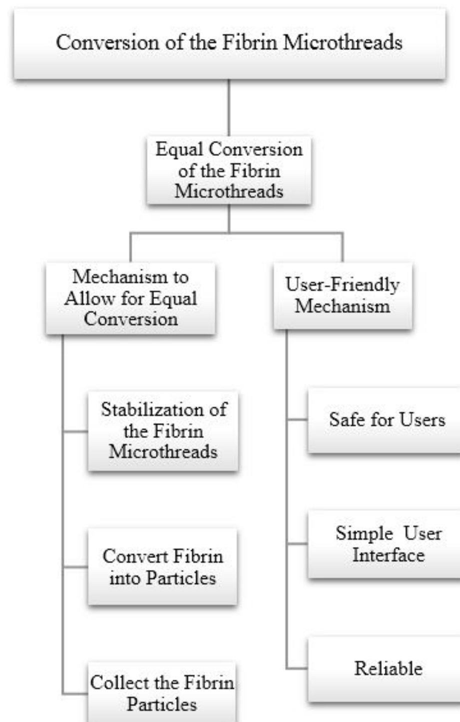


Figure 3: Objective Tree for the Conversion Method of Fibrin Microthreads

3.4.2 Constraints

Constraints of this objective included the type of conversion mechanism and materials used for producing fibrin microthreads within a certain particle size range. When considering the conversion mechanism and the materials/components used, the team looked into options that allowed the user to have good control over the conversion process and reduced static cling.

Producing fibrin particles that fall within a certain particle size range is important because the fibrin used for delivering actives in the skin cream must both be successful in its delivery and its 'feel' when mixed with the final skin cream composition. The integration of fibrin with the skin cream is important because in order to be a favorable product with consumers, the cream must 'feel good' when applied to the skin. Therefore, the conversion mechanism must convert the fibrin microthreads to particle sizes suited for delivering actives, while feeling good when applied topically.

Static cling is a phenomenon that occurs between dry materials and is caused by static electricity. An exchange of electrons causes the two surfaces to stick together [90]. An example of this is dry hair strands attaching to combs or brushes or dust particles on the floor. Dry fibrin microthreads or particles experience this phenomenon. A way to reduce static cling is by wetting the threads [90]. When looking into potential materials for the mechanism to allow for more equal fibrin microthread conversion, the team must consider materials that can be compatible with wet microthreads and reduce the potential of static cling. This limits the team's component options to items that are smaller and more compact to allow for more control over the fibrin particles' collection process.

3.4.3 Functions and Specifications

Based on previous research performed for agents used in active delivery, the size of the agents were around the range of 100 microns or less [91]. The active particle sizes delivered

usually range from 1 – 10 microns [92]. In order to maximize delivery, the team decided a range of 0.1 – 1mm for the fibrin particles appropriate for the use in skin cream. Even though this size range is larger than typical ranges, it allows for maximum active delivery to the skin.

The team formulated a list of functions and specifications based on the background knowledge and objectives formulated above:

1. The fibrin particles produced must be between 0.1 – 1 mm in length.
2. The method for conversion must be consistent and produce particles within the range stated in specification 1.
3. The mechanism for conversion must be safe to use.
4. The mechanism must convert and collect the fibrin particles.

3.4.4 Standards and Regulations

Similar to the machine standards explained in section 3.3.4, the ISO 9283:1998 and ISO 3952-1:1981 protocols were followed, allowing for correct execution of mechanism conversion decisions and designs.

3.5 Revised Client Statement

After the team analyzed the necessary quantitative and qualitative parameters of the project, a revised client statement was generated with the necessary detailed updates:

*"The **first main objective** of this Major Qualifying Project is to **validate fibrin's value** in cosmetic skin cream applications through **experiments with active ingredients found in cosmetic cream, and customer discovery on product value**. The **second main objective** is to create an **automated, reliable, and user-friendly** manufacturing device designed for **the mass production** of fibrin microthreads that **can extrude** fibrinogen and thrombin solutions, and **consistently create** fibrin microthreads. The **third main objective** is to **convert** the fibrin microthreads into **equal particle** sizes that range between **0.1 – 1 millimeter**"*

The team's plan to separate the cosmetic application objectives and goals, the machine objectives and goals, and the conversion objective and goals into three parts helped to produce organized and coherent results.

Chapter 4: Design Process and Approach

The team performed separate analyses on the application of fibrin in a novel cosmetic skin cream termed Fibrina, the fibrin microthread extrusion device and, the conversion of fibrin microthreads to particles. In order to determine the ideal components for the preliminary design, the team formulated three different full-scale machine designs and performed a design analysis on each individual component. This design approach also will help the team formulate a final design essential to the process. The team also took part in a Worcester Polytechnic Institute program that pivoted the scope and application of Fibrina in the cosmetics market.

4.1 Fibrina

The team wanted to explore the development of a fibrin-based skin cream product. This involved the creation of an initial value proposition. This decision led the team to take part in a program hosted at WPI, known as the Accelerate Program.

During the Accelerate Program, the team connected with various industry peoples and consumers of interest. Within the duration of this program, the team conducted over 30 interviews. Every ten interviews, the team revised its value proposition while making critical insights on steps for the team to take in the future.

During the process of conducting interviews, the team made a few substantial pivots which led to different concepts of a fibrin-based skin cream. By the end of the interviews, one path towards commercialization was established for the time being. Suggestions and requirements on how to prove the value of the cream were documented and subsequently planned out for experimentation.

4.1.1 Interviews

The interviews are broken down into separate categories. Each category is based on the type of individuals interviewed. The team conducted interviews with dermatologists, cosmetic/plastic surgeons, one cosmetics industry marketing specialist, several consumers and salespeople at low, middle, and high-end stores, and lastly two wholesale manufacturers/distributors. The chronology of the interviews discussed in the upcoming sections does not align with the order in which the team conducted the interviews.

4.1.1a Dermatologists

The team conducted several interviews with several dermatologists throughout the time of the program. The first interview ever conducted by the team centered on questions about treatment options for cleaning and healing wounds after skin surgery. The team also asked about the importance of minimal scarring for patients, post-operation. In order to remain concise of the interview insights and takeaways, all of the major questions/insights are organized in a table. Summaries of the takeaways from the interviews are shown in Tables 30-35 in Appendix II.

This first interview answered questions that were important in understanding the project's objective of creating a novel fibrin-based skin cream. Two main takeaways became apparent from the first interview: A substantial amount of time can be spent concocting various formulas or recipes for an ideal fibrin-based skin cream and the market ultimately sells the product.

The team's second interview was with a dermatologist with their own cosmetic line. The team looked to address three main questions from this interview:

1. What kind of treatment options would you recommend to patients after a dermatology procedure or appointment?
2. What do you look for in product labeling?
3. What key ingredients do you look for in a product?

The second dermatology interview revealed the truth behind skin cream products on the market today as well as highlighted the general practice behind producing and selling a skin cream. The interview introduced the notion of claims and described the process of making cosmetic claims using careful terminology.

4.1.1b Cosmetic/Plastic Surgeons

Cosmetic and plastic surgeons were interviewed within this program as the team sought benefit from learning more about their view of post-op products. As with some dermatologists, there are cosmetic and plastic surgeons who have their own cosmetic product lines. Interviews with surgeons were typically very brief, and many of the insights taken from surgeons were nearly identical.

Prior to the first interview with a cosmetic surgeon, it was assumed that cosmetic/plastic surgeons commonly recommended topical products to their patients for better post-surgery recovery. This was found to be mainly true for those surgeons which had their own cosmetic lines. Other than pointing out products to research and compare with, most surgeons followed one main principle: a good product that is recommendable has to work, and has to have scientific backing to prove it works.

4.1.1c Cosmetics Industry Marketers and Branders

The team interviewed one executive with major success in marketing/positioning and branding. Due to the nature of this interview, the main questions asked pertained to:

- What are the key considerations that go into marketing of a product?
- Where does one begin to market a product? How does that process evolve over time?
- How do we compete against existing competition? (Target Audience)
- What resources/techniques can we use to target initial key consumers?

The major takeaways received for the marketing aspect of cosmetics were substantially helpful. While the knowledge gathered from this interview applied more so to the pricing of a

product based on a number of costs made to ensure product success, the information itself proved vital for better product creation. Understanding the competitive market and establishing a unique answer to consumer problem(s) very much affects the final product. The advice/push to get out of the building and interview salespeople and consumers at different stores was also a major breakthrough and motivator for the team.

4.1.1d Salespeople from Low, Middle, High-End Stores

As a means of dividing and conquering research and customer discovery on various stores, the team split off to take the low, middle, and high-end stores. The resulting interviews with both salespeople and general consumers provided major insights into consumer behavior during shopping, as well as key insights into marketing of products via packaging and shelf placement. To concisely address each of the stores visited in an organized fashion, the following tables are being categorized by the type of store (low, middle, or high-end) which best describes them.

1: Low-End Stores

The low-end store interviews gave a varied glimpse into the consumer habits of skincare shoppers. A special note should be taken to look into the online market, which was noted during the team's interview with the cosmetics marketer/brander. With regards to product placement, it is obvious that certain brands are constantly paying a little more to be at eye-level in multiple stores. In the end, there is still a ton of products out there, and choosing just one seems overwhelming at first glance. Shoppers in these stores are variably picky with ingredients. Cost can be a major factor in some cases, and in others, a willing sacrifice.

2: Middle and High End Stores

For the middle and high end interviews, the team interviewed sales representatives at major department stores and high end stores. The Department Stores visited included

Bloomingdales, Neiman Marcus, and Nordstrom. Some of the counters visited in the department stores included Estee Lauder, Chanel, Lacome, Tris McEvoy, Space.NK, and Philosophy. In general, the major takeaways from these interviews surrounded branding and marketing through the representatives' sales pitches. For example, the branding and display for the products were marketed in such a way that had bold adjectives associated with each product ("dramatically different skin cream", "All natural, fresh feel skin cream"). The representatives would take these adjectives and, based on each customer's need, explain why the product is different and works the best for his or specific condition. Since many of the counters in the department stores were part of a high-end brand company, similar marketing tactics were used at the high-end stores visited (Sephora, Mac, Chanel, and Clinique).

4.1.1e Wholesale Manufacturers and Distributors

The team met with two wholesale marketers and distributors. One individual was the president of a group of companies, while the other owned a smaller business which focused on producing batches for sample development. Both interviews offered unique perspectives on manufacturing and distribution, and will be discussed separately.

The first wholesale manufacturer/distributor interview the team held was with the CEO of a conglomerate of companies, one of which was a major manufacturer and distributor of what appeared to be high-quality cosmetics products. Said high-quality cosmetics products openly claimed use of cellular growth factors, retinoids, and stem cells.

4.1.1f Arizona Conference

Two members of the team flew to Scottsdale, Arizona to attend a conference where many cosmetics-based companies were in attendance. Due to the scheduling nature of the conference, it was difficult to make time with anyone for an interview. In fact, the two team members were

only able to have one interview with the president and VP of a wholesale manufacturer/distributor company.

However, while in Scottsdale, it was noticed by the two team members that the Scottsdale area was riddled with salons, spas, and most notably, dermatology offices. Given the spare time the team members had while in Scottsdale, a number of interviews were conducted with salon owners, cosmetics specialists, and a number of dermatologists.

One interview conducted in Arizona was with a wholesale manufacturer and distributor who offered to educate the team further about the complexities of manufacturing cosmetic products. The main takeaways gathered from the interview were that the manufacturing of cosmetic products itself has its own set of regulations. If a group such as ourselves wants to begin mass producing, then the process by which our skin cream is developed must follow regulated manufacturing guidelines. Failure to do so prevents us from getting product out into the market.

Aside from the wholesale manufacturer and distributor interviews, the team's main takeaways from the salons, spas, and dermatology offices are as follows:

- The retail market is highly saturated with products. There's essentially a product for nearly every skincare problem out there.
- The retail market is becoming much more educated. Scientific backing is essential. Education of a product's efficacy to salon and spa specialists are extremely effective.
- Dermatologists are always on the look for better products, as their improved results with patients benefit their business. Again, scientific backing is a must. Marketing is also very important to any product sitting on a dermatologist's shelves.

4.1.1g Summary of Takeaways

Consumers today are trying to understand the ingredients used within their products. All-natural based products are on the rise. Dermatologists are in need of better products which can meet consumer demands (such as ingredient originations) while still addressing consumer

problems (healthier, better looking skin). It is easier to determine the types of experiments to conduct on a cream to prove its efficacy by asking those who readily perform such experiments. Finally, regardless of how good our product may become, marketing is what ultimately sells.

4.1.2 The Accelerate Program

In the beginning, the team created both a value proposition and customer segment based on underlying assumptions learned from a superficial view of the cosmetics skin cream industry. Table 2 below shows the team's first take on a value proposition and customer segment.

Table 2: Initial Value Proposition and Customer Segment

1st Value Proposition	1st Customer Segment
No new development in skin creams over the last 25 years.	Problem: Customers want whatever it is that they're buying to work.
We have a new all-natural ingredient (fibrin). We can integrate our new ingredient with other natural ingredients.	Consumer Target: Women in their 40s and 50s
Combining fibrin with other natural ingredients produces synergistic effects.	Distribution Channel: Sell through surgeons, physicians, and cosmetic companies.
Fibrin has a lot of potential to be the next big brand in today's cosmetics industry.	

This value proposition and customer segment was crafted before any interviews were conducted. Table 3 shows our final value proposition and customer segment, which was created after the 31 interviews conducted.

Table 3: Current Value Proposition and Customer Segment

Current Value Proposition	Current Customer Segment
There has been no new "special ingredient" introduced into skin creams in over two decades.	Problem: Customers want all-natural, scientifically backed products with proven results.
The team has fibrin: a novel ingredient found naturally in the body during wound healing.	Archetypes: The team is pitching to cosmetics companies looking for a competitive edge in the market.
The team has a process that allows us to combine fibrin with other ingredients that is unique	Channel: The team wants to license the technology to cosmetics skin cream manufacturers: The suppliers
The team can show synergistic properties between fibrin and other natural ingredients resulting from our processing and experimentation.	Relationships: The team wants to build awareness toward scientifically backed products in the cosmetics industry. The plan is to educate both the customer companies and general cosmetics consumers on the product.

The conclusions for what the team needs to do in order to prove fibrin-based skin cream as a valuable product in the cosmetics market all point toward one objective: show data on fibrin's usefulness within a skin cream. There is currently no known scientific research that has been done on fibrin-based skin cream. Through both literature and discussion with dermatologists, the team created a list of experiments to perform that would show fibrin's value as a cosmetic ingredient.

Aside from experimentation, the team needs to conduct more interviews with both dermatologists and manufacturers and distributors in order to build a larger network of dermatologists to contact, as they are currently the target distributors/marketers of our product. The team was only able to conduct two interviews with wholesale manufacturers/distributors of cosmetics products. In order to better understand the benefits and costs of mass producing product through these individuals and companies, the team needs to learn more about them.

A comparison of scientific data and testing on competing skincare products on the professional market is necessary. Determining the market value of these products and comparing their claims with ours will help to configure a price point for fibrin-based skin cream. With that, a more in-depth cost analysis of our product must be done. Considering larger scale production, the team must discover the various suppliers needed to mass produce.

4.2 Fibrin Microthread Extrusion Device

Utilizing the feedback from our advisor and reevaluation of our initial client statement, the team performed different analyses that helped to determine the necessary components of the final preliminary design. The team took the main three objectives and performed a needs analysis of the main and sub objectives to develop quality design ideas.

4.2.1 Machine Components and Needs Analysis

The team broke the major stages of the machine into different phases: Pumping Phase, Extrusion Phase, Stretching Phase, Drying Phase, and Removal Phase. Below in Table 4 is a summary and description of each phase.

Table 4: Definition of the Different Phases of the Machine

Phase	Definition
Phase 1: Pumping	The stage in which fibrinogen and thrombin are combined to form a fibrin-infused mixed
Phase 2: Extrusion	The stage in which the threads are extruded from an automated extrusion head onto the chain system, which is incorporated in our final design
Phase 3: Stretching	The stage in which the threads are moved and stretched uniaxially in opposite directions to increase the threads end-product viability
Phase 4: Drying	The stage in which the threads are dried with an external dryer source to speed up the drying process
Phase 5: Removal	The stage in which the threads are removed

Along with our machine objectives defined in Chapter 3, the phase approach helped the team determine the essential features needed for a successful preliminary and final design. The team also put together a chart of the important wants and needs of the system to determine the necessary components of the machine's final and preliminary design. The system needs are defined as the systematic components of the machine design that are essential to success. The system wants are defined as the components of the system that the team would like to include to the machine's preliminary design, but are not required for success. Below in Table 5 is an outline of the Needs and Wants of the machine design:

Table 5: Summary of the Needs and Wants of the Machine Design

Needs	**Wants**
Automated Extrusion and Stretching Process	Durable
Safe for Users	Simple, Modifiable User Interface
Option for Input of Additional Ingredients	Fits on a Lab Bench
Minimize Thread Failure	Precision
Mass Production of Threads	Reliable
Conversion of Threads into an Applicable Form	Sterilization

4.2.1a: System Needs

The needs of the machine were chosen based on objectives highlighted in Chapter 3 of the Report and the phases broken down in the previous section. A major objective of the machine was to create a safe and fully automated machine. Therefore, it was important to include an automated extrusion and stretching process to achieve this design objective. Two major objectives include the option for customization, which include the option for additional ingredient input, and the conversion of the threads into an applicable form. These need components are essential to the final design because the combination of other ingredients with fibrin is vital to our final project because it aids the success of the team's second main objective, which is the infusion of fibrin microthreads in a cosmetic skin care application.

In order to create these infused threads, it is also necessary to minimize thread failure in order to gain a mass production bulk of the threads. This need is essential to the machine's ability to mass produce fibrin microthreads, which is an important factor in ultimately creating a successful skin care product.

4.2.1b: System Wants

Similar to the system's needs, the system's wants were generated based on the objectives highlighted in Chapter 3 of the report and the phases broken down in the previous section. While not essential to the final success of the machine, formulating the wants of the machine was important in determining the essential components of the preliminary and final design since it directed the team toward a better and well defined design. The team formulated these wants based on the features that would enhance the machine's ability to produce fibrin microthreads. Even though a main objective of the machine was its user friendliness, the team decided it was essential that the machine was completely automated, meaning sub-objectives such as its simple user interface was not crucial to the final design.

Other components such as its durability, reliability, precision, size, and sterility are great components to have for the machine design, but are not essential to the ultimate goal of the machine, which surrounds the mass production of infused fibrin microthreads that can be converted for cosmetic applications. Therefore, the precision of the fibrin microthreads are not necessary because the threads will be converted into an alternative form used in the powder. The reliability, sterility and durability of the machine would be ideal, but the system can be altered, cleaned, and repaired in time (if needed) without the constraint of using expensive materials to ensure a longer lasting machine. While it is also important for the machine to be of a reasonable size that can fit on a lab bench and be safe for users, a bigger machine that has an intricate design and still outputs the necessary product is more desirable.

<u>4.2.1c: Design Needs and Wants Decision Matrix</u>

After defining the needs and wants of the machine, the team put together two separate design matrices to connect the needs and wants objectives to specific components in the machine design. These components are ones the team generated when discussing options for the alternatives and final preliminary design. Tables 6 and 7 below show these comparisons.

Table 6: Design Matrix for the System Needs

COMPONENT:	**NEEDS:**	**Automated Extrusion and Stretching Process**	**Safe for Users**	**Option for Input of Additional Ingredients**	**Minimize Thread Failure**	**Mass Production of Threads**	**Conversion of Threads into an Applicable Form**
Pumping System		X		X	X	X	X
Syringe Head System		X			X	X	
Channel System			X				
Chain System			X		X		
Oval Conveyer system							X
Clamping system			X		X		
Magnetic tracks			X		X		
Dryer					X		

Air Dry				X		
Collection Bin						X
Robotic Removal				X		X

Table 7: Design Matrix for the System Wants

COMPONENT: / **WANTS:**	**Durable**	**Simple, Modifiable User Interface**	**Fits on a Lab Bench**	**Precision**	**Reliable**	**Sterilization**
Pumping System				X	X	
Syringe Head System				X	X	X
Channel System	X		X		X	
Chain System	X				X	X
Oval Conveyer system	X	X			X	X
Clamping system					X	X
Magnetic tracks	X				X	
Dryer		X	X			
Air Dry					X	
Collection Bin			X			
Robotic Removal	X	X	X		X	X

This analysis helps the team make decisions on important components to include in the final preliminary design based on the wants and needs. For example, in Table 4, the Clamping system component can affect the automated stretching process and the minimization of thread failure, while, as seen in Table 5, it can affect the reliability of the machine. The purpose of this analysis is to draw conclusions based on the comparison between the potential components of the design and the needs and wants of the system.

4.2.2 Concept Maps/Conceptual Designs and Alternative Designs

In order to develop a novel, efficient method to automate the production of fibrin microthreads, the team developed several preliminary and alternative designs. The designs herein incorporate concepts for both the production of fibrin microthreads in an experimental, laboratory environment, and the large-scale manufacturing process of fibrin microthread infused skin cream. The team seriously considered three preliminary designs, and further considered

alterations to certain processes of these designs, and ultimately made a decision on a final preliminary design.

4.2.2a Concept Design 1

The team's first preliminary design can be seen in Figure 4 below. The design involves a single conveyor system that starts from extrusion of the fibrin microthreads and delivers the microthreads through different phases required for the microthreads to successfully form. It is important to note that aspects of this design may be adjusted based on the intended use of the production for microthreads. For example, the phase of removing threads from the conveyor may involve the eventual storage of the Fibrin microthreads for future experimental use, or may include an adaption of a grinder to create a powder.

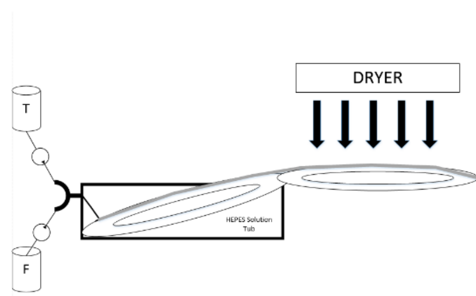


Figure 4: Design Concept 1

The first aspect of the design involves storing fibrinogen and thrombin with an incorporated pumping systems to deliver the protein solutions to the device. In an industrial application of producing fibrin microthread infused skin cream, there would be an extra storage unit depicted for the storage of an antioxidant ingredient, such as retinol or hyaluronic acid. An existing pumping system (Figure 5) would be incorporated.

Figure 5: Piston Rod Syringe Pump

This pumping system is designed to be used with syringes that store the protein. A piston rod is controlled to extrude the protein out of the syringes at a specified extrusion rate. In an industrial application, this concept would involve larger tanks for storing the protein that would have individual pumps that direct the fluid from the tanks to an extruder head, as depicted in the schematic of the design. For the sake of our preliminary design for a prototype, the piston controlled pump system would be used and be adapted with the rest of the conveyor system.

When the piston rod compresses the syringes located on a syringe holder, the protein would be in fluid communication with an extruder head. The extruder head is a two to one port system, such that fibrinogen and thrombin would be extruded out a single port as a protein mixture. Further, in the production of fibrin microthread infused skin cream, the antioxidant ingredient would be pumped into the same port as thrombin, where it would mix; then, the antioxidant thrombin mixture would mix with fibrinogen as one mixture and be extruded.

The system itself consists of a conveyor belt, controlled by an external motor, which would run continuously through a tub filled with HEPES solution, and external the tub through a dryer. The conveyor would move at a speed to give threads a minimum of three minutes in the

HEPES tub to allow the threads to formulate. Further, the speed of the conveyor may be directly adjusted based on the desired diameter of the threads, as a faster conveyor would produce thinner threads, and a slower conveyor would produce thicker threads.

The thread would be extruded directly from the extruder head through a stationary tube directly to the surface of the conveyor. This design would allow for a single, continuous thread of fibrin to be produced through the system. The conveyor would extend out of the upper corner of the HEPES bath and level out to enter the dryer phase. The dryer that may be incorporated in this design can be found in Figure 6 below.

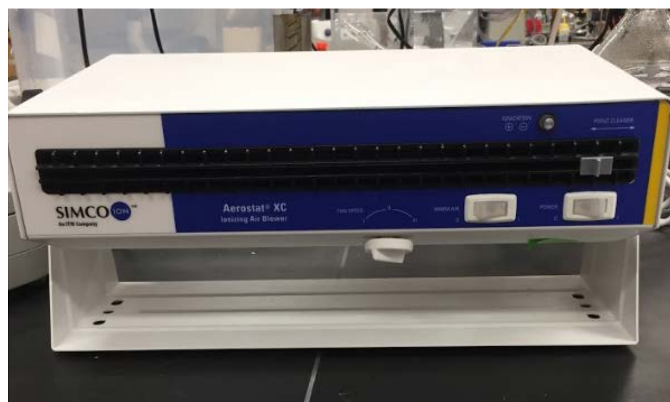


Figure 6: SIMCO Aerostat XC Air Blower

Once the continuous thread of fibrin leaves the tub of HEPES, the conveyor levels out and the threads enter a drying phase. To expedite the drying process, a dryer such as the SIMCO Aerostat XC Air Blower may be used. In this aspect, the dryer can be set to various settings, such as air blowing rate, to a standard that is tested to be most effective for successful production of fibrin threads. After this phase of the design, the threads must be removed from the system.

Removal of threads from the system in Design 1 may vary depending on the purpose of use of the machine. If the machine is being used for industrial application, or the production of fibrin microthread infused skin cream, the single thread may fall off the conveyor into a grinder in which the thread would be manipulated to a powder and collected. In a laboratory setting, the

thread may be cut by a user, once dried, and placed in a storage unit. In further embodiments, a robotic mechanism can be designed to cut the thread into piece and place them on a storage rack. However, for the preliminary design, it is intended to have a user control cutting the threads and removing them from the device.

Advantages of Design 1 include the ability to control the rate of extrusion and the movement of the conveyor to a custom rate in order to effectively create threads of a desired diameter. By incorporating the piston rod extrusion pump, there is an easy user-interface that allows the user to change the rate of the piston with a control module. Further, the system makes use of extruder heads that are currently used in the manual production of fibrin microthreads in a laboratory. Additionally, the conveyor system is completely linear, which is a design of minimum complexity. The simple chain that the conveyor would rely on in this linear system is mechanical in nature, and are readily available for reasonable prices by several manufacturers, making it a reliable design.

Disadvantages of Design 1 includes the limitations the linear design has on organization of the threads. Since the system is completely linear, and the extruder head is stationary, only one continuous thread can be produced. The production of threads by cutting one continuous thread into smaller pieced either requires a user that must be stationed after the drying stage of the machine, or an advanced system of robotic nature to cut the threads and place them to storage. In an industrial application, the single thread would be fed into a grinder; however, the end of the design is convenient for only this purpose. Finally, one aspect of fibrin microthread production that is not incorporated in design 1 is the stretching of threads. Stretching of fibrin microthreads have beneficial benefits for their formation and increase the amount of thread produced from a given amount of protein solution. Therefore, the lack of the stretching of

threads may drive the cost of production of microthreads up compared to other designs that stretch threads.

4.2.2b Concept Design 2

A schematic of a side view of the team's second design can be seen below. The design incorporates a conveyor system that operates on both the upper surface of the conveyor belt, and the bottom surface of the conveyor belt. The design incorporates a channel system that can separate the extruded mixture into separate threads of a desired length. Design 2 involves an intricate design that includes 8 main components: pumping, extruding, stretching, flipping and dumping of HEPES solution, drying, collecting, cleaning, and replenishment of HEPES solution. These aspects of the design can be found in Figure 7 and are labeled 1-8, respectfully.

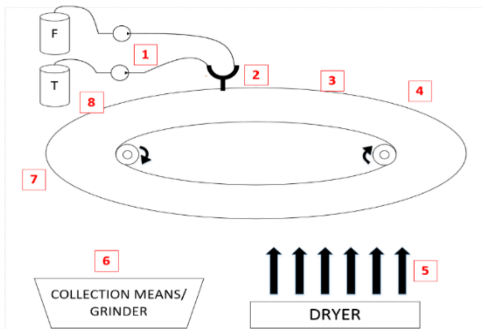


Figure 8: Design 2

The first aspect of the design includes pumping. Similar to design 1, the system may include storage tanks with a pump for each individual tank that would lead a 2-to-1 tube extruder head. Like the first design, another tank may me incorporated with an antioxidant solution with a separate pump that may be mixed with thrombin in one entry tube of the 2-to-1 tube extruder head. In another embodiment, the piston rod controlled syringe pump may be used with the system. However, with extrusion, unlike Design 2, the extruder head has one degree of freedom across the extruder. Specifically, the extruder head would move back and forth along the axis of movement along a grooved channel within the surface of the conveyor. This channel

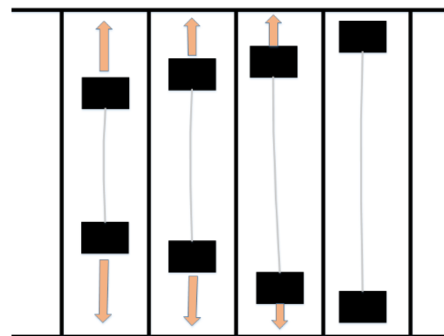


Figure 7: Design 2 channel system for organizing and stretching threads

system would allow for the system to extrude separate threads. The length of the channels would correspond to the desired length of the threads. Additionally, each individual channel would be filled with HEPES buffering solution to allow the threads to form. A top-view schematic of the channel system may be seen in Figure 8.

The channel system of Design 2 serves two purposes. The first purpose is to separate the extruded mixture into separate threads; and, the second purpose is to create individual systems to stretch the threads. As seen in the above schematic, each channel system includes two blocks that are stationed across from one another. In the schematic above, each block is in a “closed” position, in which a thread is clamped within the block and is able to be stretched. The block has two plates that are connected by a hinge. Once the extruder head moves across the channel and extrude the thread on the lower plate, the block closes. This movement of the block may either be controlled by a timed motor system, or by magnetic forces that exist between the block and a surface below the conveyor.

Once the blocks are in a closed position, they are able to move along the bottom surface of the conveyor. In the design, the bottom of the conveyor may include a track set-up in which a motor would allow the blocks to move away from one another on the track. This track can be a roller system, or a separate chain system within the channel may allow pull the blocks away from one another to allow the thread to stretch. In other embodiments, a magnetic system may be used to separate the blocks. By the end of section 3 of the design, the threads should be in a completely stretched position.

At section 4 of the design, the conveyor begins to turn over. At this point, the blocks still remain in the closed position, so the threads do not move despite the channel beginning to flip. As the conveyor begins to fall over, the HEPES solution is dumped out into a collection bin (not

depicted in the schematic). This HEPES can either be disposed of, or it can be collected and pumped back into a storage tank for re-use. Once the conveyor is almost upside down, one clamp may open through a step-motor or other means to allow the first plate to open with movement by the hinge joint. At this point, the thread may hang. In other embodiments, both clamps may remain shut to ensure the stability of the thread.

At section 5 of the design, the threads go through the dryer. Like design 1, an incorporated dryer may be the SIMCO Aerostat XC Air Blower. An aspect of this dryer is that it is on a swivel, and the angle of air flow may be adjusted to accommodate for the threads being above the dryer. Additionally, the rate of airflow may be adjusted to whatever desirable specification that will be most effective for the production of the fibrin microthreads.

After drying, the threads must be removed from the system. This can be accomplished in a few embodiments. In a first embodiment, the threads are already hanging by one clamp when approaching section 6 of the design. At this point, the clamp holding the thread may open and allow the thread to drop into either a collection bin, where a user must take the thread out, or a grinder, where the thread is grinded into a powder and collected. In another embodiment, both clamps are closed as the threads approach section 6 of the design. If this is the case, a first block will become open through a step-motor, and a second clamp will open after the first block is opened to allow the hanging thread to fall into the collecting bin or the grinder. In a third embodiment, similar to Design 1, a user may be stationed at section 6 of the machine and cut the threads off and place them in a storing unit.

At section 7 of the design, the channels must be cleaned. Since threads formulated on the surface of the conveyor and on the inner surface of each plate of the blocks that clamped the threads, residue of fibrin may still exist within the channels. Before HEPES is replenished in

each channel, the channels are cleaned with water. At section 7, a tank of water and hose system is stationary and sprays down each channel as the conveyor moves (not depicted in the schematic).

Finally, at section 8, HEPES solution is replenished in each channel from a HEPES storage tank (not depicted in the schematic) and a stationary extruder head. Each channel, that had just been cleaned, is replenished with HEPES solution before a new thread is extruded into the channel. The HEPES may be recycled HEPES, or may be a limited supply of HEPES solution if the HEPES is not recycled or continually replenished in the storage tank.

There are several advantages to preliminary Design 2. Like Design 1, the pumping system enables to flow rate of the protein mixture to be controlled to a desired value. If the piston rod syringe pump is used, there is an easy user-interface. The only true difference between this extrusion processes of the first preliminary design to the second preliminary design is that the extruder has a degree of freedom to move along each channel of the conveyor, so the piston rod syringe pump would be adapted with a tube to the extruder head from the 2-to-1 syringe head. This degree of freedom allows for separate threads to be formulated within each channel, which is a great advantage and less work for the user. Additionally, the channel system with incorporate blocks allow the threads to be stretched, which is more efficient use of the protein solutions. Further, the compactness of the system is an advantage of this design. Since the system is not completely linear, the specifications of the length of the device would not be as long. Finally, this design enables adaptability of its use as either an industrial machine or laboratory machine used for the production of the threads.

Although the design incorporates many aspects for efficient fibrin microthread production, some of its complexities have disadvantages. For example, the channel system with

moveable blocks is a costly design. Each individual channel may essentially need a motor or a driver to move the blocks, which is inefficient and costly. Further, the material in which the channels and block plates are made of is important. The material must not rust in the HEPES solution. Additionally, the fibrin threads must be easily removed from the surfaces, and there is risk that the threads may adhere too well to the plates when they are clamped, which would not enable them to fall when unclamped effectively. Further, the force in which the threads are clamped must be perfect that the threads are not torn, and timed well enough that the threads are formed enough to be clamped effectively when the blocks closed. This process includes very tedious complication, and excessive timing, as if one aspect of the stretching of threads is not timed correctly, the whole process can be thrown off time. Also, although cost may be saved through stretching the threads, more cost is lost through individual HEPES channels that are dumped. With this, especially if HEPES is tested to be unsanitary to be re-used from one thread to the next, a lot of HEPES solution will be wasted in this design. Finally, in this design, many different tanks would need to be used, which in the manufacturing of fibrin microthread infused skin cream could be five tanks (water, HEPES, fibrinogen, thrombin, antioxidant). Maintenance of all the tanks, and the efficiency of all individual pumps may be complicated.

4.2.2c Concept Design 3

The team's third design concept can be seen in Figure 9 below. The design incorporates a chain system in which chain links are coupled to small clamps. Threads are able to be extruded across the clamps within a HEPES buffering solution tub. From the tub, the clamps may close to allow the chain to pick up the threads and stretch them. Like the previous designs, a dryer is incorporated to expedite drying, and threads can be collected or grinded.

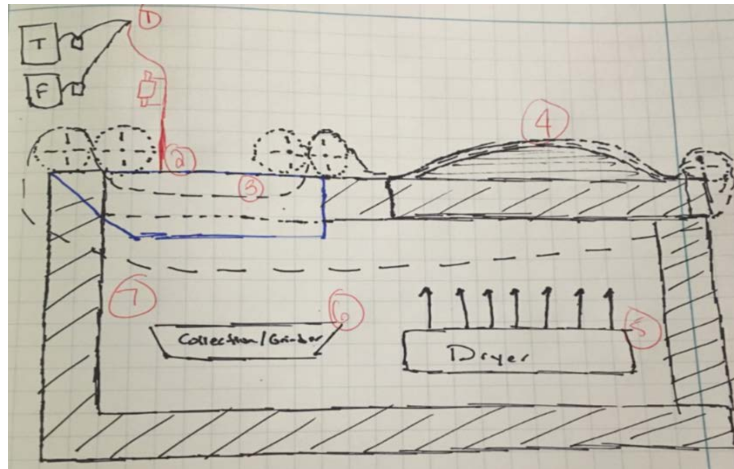


Figure 9: Design Concept 3

Design concept 3 consists of seven main components, including pumping, extruding, polymerization, stretching, drying, collecting or grinder, and cleaning. The design itself incorporates a chain link system that leads threads from extrusion to all these processes. The main chain of the design is run by a motor, such as a stepper motor. Sprockets are used to control movement of the chain throughout the system, such as the rising of the chain out of the HEPES tub (see label 3 in drawing), and to the stretching platform.

The first aspect of the design involves the pumping of the protein fluids to the extrusion portion of the design. Like previous designs, the syringe pump can be used to control the flow rate of fluid going to the extruder head. In this design, a larger needle apparatus is in fluid

communication, through tubes, with the 2-to-1 extruder head. The needle apparatus may move linearly to draw individual threads across a conveyor. Once a piston compresses the syringe and forces fluid through the tubing, the mixture will continue through the needle into the extrusion portion of the device. Additionally, similar to previous designs, in an industrial setting, large tanks can be used to store the protein fluid and any additive, such as antioxidants, that can be mixed with thrombin prior to mixture with fibrinogen.

The next aspect of the design is the polymerization of the threads. At this point, the threads are extruded onto a conveyor that is submerged within the HEPES tub. The conveyor is coupled to a hex shaft that is driven by the motor. A drawing of this concept can be seen in Figure 10 below.

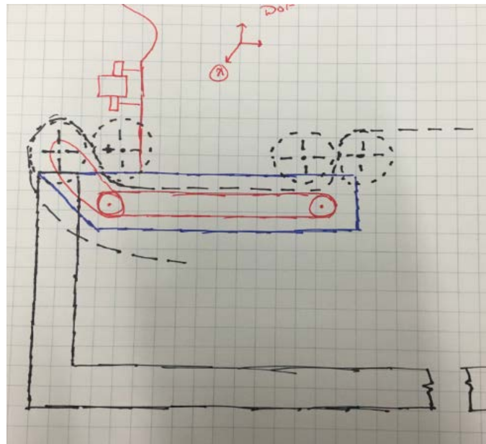


Figure 10: Conveyor submerged within HEPES solution concept

As seen in the drawing, the first sprocket that controls the direction of the chain is coupled through the hex shaft (not seen from side view in drawing), to the submerged conveyor. Considering this, the conveyor would move in uniform to the movement of the chain. Further, threads would be extruded directly onto the conveyor. Once threads are drawn, they can remain submerged in the HEPES solution to polymerize. The time in which the threads may be easily adjusted by a user by adjusting the location of the second set of sprockets along the frame of the device. The closer the second set of sprockets are to the first set of the sprockets, the shorter the threads will stay submerged within the HEPES solution. At the end of the conveyor, the threads are lifted by the chain that is controlled by the second set of sprockets, and the threads are brought to the stretching aspect of the device. However, in order to complete this transition, the threads must be clamped in place as they are lifted off of the conveyor. A top-view of Design 3 is shown in Figure 11 below.

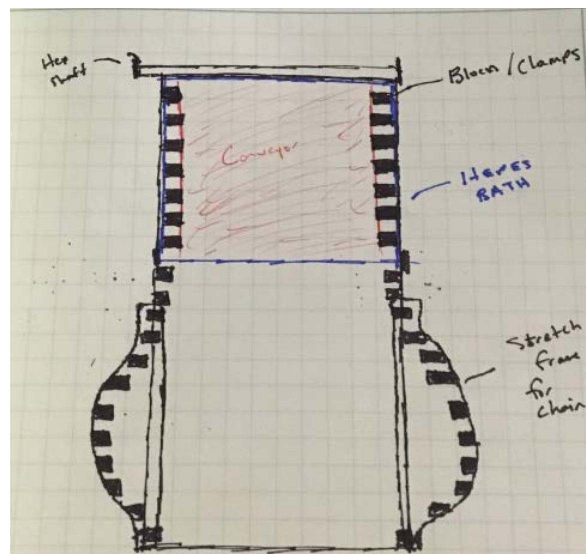


Figure 11: Top-view of design concept 3

There are blocks that are coupled in increments along the chain. These blocks act as clamp mechanisms that hold extruded threads in place. In the HEPES tub where the threads are

extruded, the blocks run along-side the conveyor. When the mixture is extruded, it is extruded along a surface of parallel blocks and the conveyor, so that the original length of the thread is equal to the width of two blocks plus the width of the conveyor. Each block has a method of opening and closing in order to clamp polymerized threads before that are lifted by the chain out of the HEPES tub toward the stretching component of the device (Figure 12).

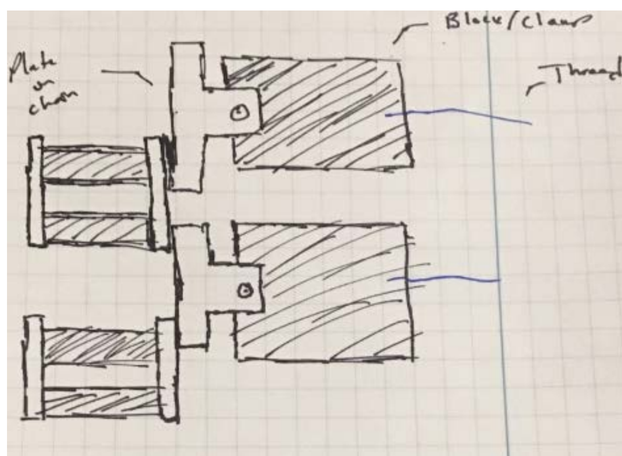


Figure 12: Blocks coupled to chain for clamping threads

Each block is coupled with a pin to the chain. The chain has small plates that extends toward the center of the device in between links. The blocks may simply be coupled to the plates on each chain, and follow the chain through its movement throughout the device. In the drawing above, a portion of thread is depicted to visualize that threads are extruded on these surfaces that extend from the chain. There is another identical block mirroring this block on the other side of the device. The second portion of the thread can be extruded to this second block. Once the

threads are polymerized and formed in the HEPES bath under a pre-set timeframe, the threads can be clamped by the blocks (Figure 13).

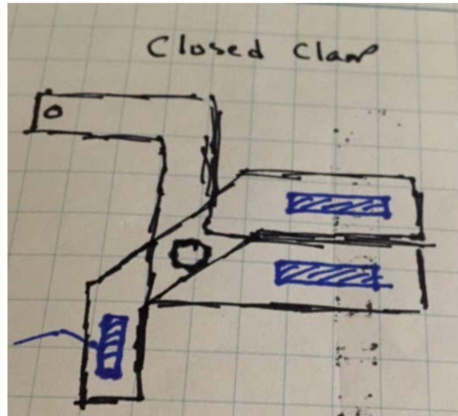


Figure 13: Drawing of closed clamping mechanism

The above drawing depicts a block that is in the clamped position. The clamp consists of two parts that are coupled with a hinge joint. The first part of the clamp is pinned to the chain at the top portion, while the second part of the clamp may move along the hinge joint to an open and closed position. A magnet is placed within the first part of the clamp where the thread is extruded on. Magnets are also placed within both surface of the second part of the clamp. In Figure 13, the top surface of the second part of the clamp is in contact with the surface of the block that the thread is extruded on due to magnetic force. In the actual machine, a servo may be used to provide force to separate the magnets, or force to the bottom surface magnet toward the magnet of the first part of the clamp to open the clamp (Figure 14).

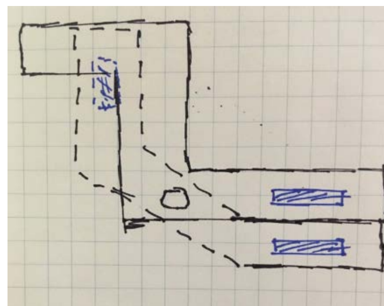


Figure 14: Drawing of opened clamp

The clamp is depicted to be in the open position. As previously described, an external force, such as from a servo or other means, may bump the bottom surface of the second part of the clamp to force the magnet from the top surface of the second part of the clamp away from the magnet in the first part of the clamp, and to force the magnet in the bottom surface of the second part of the clamp toward the magnet in the first part of the clamp.

Once magnetic forces close the clamp, the threads may be lifted out of the HEPES bath toward the stretching portion of the device. In this aspect of the device, a stretching platform is attached to the frame of the device to bend the chain to eventually double the distance between parallel clamps, allowing the threads to stretch. A depiction of this overall concept can be seen in the SolidWorks model below (Figure 15).

Figure 15: CAD assembly of Design 3 concept

The platform is coupled to the frame of the device and incrementally extends away from the center of the device in a parabolic like shape. On the device, and as can be seen in the top view drawing depicted in Figure 15, an identical platform is mirrored on the other side of the device coupled to the frame. The distance between the furthest points between the clamps is exactly double the distance of the clamps when the threads are first extruded in the HEPES tub

The stretching platform (Figure 16) is designed in a way to allow the threads the

incrementally stretch apart. A platform that stretched the thread too dramatically would risk the threads breaking. This allows the threads to be stretched gently. Since the frame of the machine is stationary, the stretching platform must return its shape to allow the chain to return to the end sprockets. Further edits to this design may include adjusting the frame to allow for the threads to remain taut at its widest point throughout the duration of the device.

Figure 16: CAD part of stretching platform

Next, threads remained clamped by the closed blocks and brought over a dryer. This can be seen at label 5 in Figure 9. This design, like the previous designs, allow the use of the SIMCO Aerostat XC Air Blower dryer. Like the second design, since the air blower can swivel, the dryer may direct air flow up toward the threads that would move above the dryer.

After the drying stage, threads may be released from the clamps to fall into a collecting unit or a grinder to allow for the creation of powder. Further, a pair of clippers may be implemented in the design to cut the threads. However, for cost efficiency, an ideal aspect of this design would be to allow the threads to simply fall when opening the clamps above the collector or grinder. Finally, at stage 7 of Figure 9, the blocks can be cleaned off. This can be done by placing a brush stationary with a small water hose to wash down individual blocks as the chain moves by this portion of the device.

Design three has several key advantages that fit the team's needs/want analysis. Several advantages of Design 3 include its complete automation of both the extrusion process and the stretching process. The design allows for minimum complexity to achieve complete automation. Like other designs, design 3 can add antioxidants to the threads, which is important to produce fibrin infused skin cream. Unlike previous designs, since the sprockets are adjustable along the frame, the timing of threads to be emerged in the HEPES solution may be easily adjusted. This is important, as when different ingredients are added to the fibrin microthreads, polymerization may take different times. Design 3 may accommodate for this variance in polymerization characteristics of different kinds of threads.

Disadvantages of this design include the cost of the customization of the clamps. These clamps need to either by produced/manufactured by the team, or printed three dimensionally. At least 100 of the parts would need to be created for the design, which may be costly. Also, the length of the device may be larger than what is convenient in a laboratory setting. Since the HEPES tub needs to be able to accommodate threads for as high as ten minutes, and the extrusion of fibrin needs to be continuous to avoid clotting the pump or tubing of the device, the device needs to be long to accommodate these threads. Further, some material may be lost if the surface areas of the clamps are not large enough, and if the surface areas of the clamps are made larger, it is more expensive for the team to produce them. Finally, the chain itself, given its unique plate design and ability to flex into the necessary flexing positions during stretching, may be rather costly.

4.2.3 Final Preliminary Design Selection

After generating the three design concepts in detail, the team put together two decision matrices: one for the design components used in the three designs and one for the overall designs themselves. The team formulated eight total design conditions, in which were each given a weight on a scale from 0-1 based on their importance in making the final and preliminary designs a success.

A score of 1 was viewed as being extremely important while a score of 0 indicated the condition was not as vital to the machine ultimate success. Specific Design Components (First described in the Design Matrices in Tables 4 and 5) were ranked on a scale from 0-10. A score of 0 indicated the component would have a large-scale effect on the specific condition of the machine, while a score of 10 indicated the component would not be affected by a specific design condition. The team performed this analysis to gain a quantitative ranking of the important design components and overall designs to consider for the final and final preliminary design. The design conditions (shown in the top row of Tables 8 and 9 below) were used to produce quantifying measurements of the design components, which were qualified in the wants and needs analysis described earlier in this chapter (Section 4.1.1). The tables below show the weights of the design conditions for the components and overall designs respectively.

The first four design conditions dealt with the major objective for the overall machine. The team decided to rank these objectives only for the overall final machine because certain components did not align with specific objectives (ex. Automation of the machine does not affect the function of the dryer).

Table 8: Decision Matrix for the Individual Machine Components

Design Component	Automation of the Machine	Mass Production of the Microthreads	User-Friendly	Customization	Cost	Size of the Machine	Complexity	Durability	Total
Weight	1	0.8	0.5	0.2	0.7	0.6	0.6	0.7	
Pumping System	N/A	N/A	N/A	N/A	6.5	6	9	9	19.85
Syringe Head System	N/A	N/A	N/A	N/A	9	9	8	3	18.6
Channel System	N/A	N/A	N/A	N/A	6.5	7	8	5	17.05
Chain System	N/A	N/A	N/A	N/A	8.5	6	8	4	17.15
Oval Conveyer system	N/A	N/A	N/A	N/A	6.5	8	5	6	16.55
Clamping system	N/A	N/A	N/A	N/A	6	6	6	6.5	15.95
Magnetic tracks	N/A	N/A	N/A	N/A	6	7	7	5	16.1
Dryer	N/A	N/A	N/A	N/A	7	8	8	8	20.1
Air Dry	N/A	N/A	N/A	N/A	2	0	10	10	14.4
Collection Bin	N/A	N/A	N/A	N/A	8	8	4	8	18.4
Robotic Removal	N/A	N/A	N/A	N/A	4	5	5	5	12.3

Table 9: Decision Matrix for the Overall Machine Designs

Design Component	Automation of the Machine	Mass Production of the Microthreads	User-Friendly	Customization	Cost	Size of the Machine	Complexity	Durability	Total
Weight	1	0.8	0.5	0.2	0.7	0.6	0.6	0.7	
Design 1	7.5	6	6	6	6	2	7	7	31
Design 2	9	8	4	5	2	9	1	3	27.9
Design 3	9	8	5	5	4	7	6	5	33

The results from the decision matrices indicated that Design 3 was the most optimal design to choose for the final preliminary design. As shown in Table 8, many of the components utilized in Design 3 (Pump system, chain system, dryer, collection bin, etc.) rank high as well, validating Design 3 is the best option for the team to choose for the Final Preliminary Design. Moving forward, the team separated the machine into the five different phases and determined

what types of tests were required to fully verify and create a functioning preliminary design concept and prototype.

4.3 Conversion of Fibrin Microthreads

The team utilized two types of analyses when determining the best conversion approach. The team split the conversion objective into a biomedical engineering analysis and a design analysis. Biomedical engineering analysis was used to determine the best method for converting the fibrin microthreads to particle form. Based on the preliminary results from the image analysis, preliminary designs were fabricated and design analysis was used to determine the best design mechanism for ensuring equally converted particles within the 0.1 – 1 mm range.

4.3.1 Conversion Method Approach

Before designing a mechanism to allow for more even and equal conversion of the fibrin microthreads, the team wanted to demonstrate fibrin's potential as a novel skin care ingredient by separating and exploring the methods of conversion into two different categories: grinding and trimming. Based on the specifications described in section 3.4.3, the team looked at commercial methods that convert bigger items (strands, threads, etc.) into a particle or powder size and specifically analyze their mechanisms of conversion. In Figure 17, the team describes specific conversion methods tested for this objective.

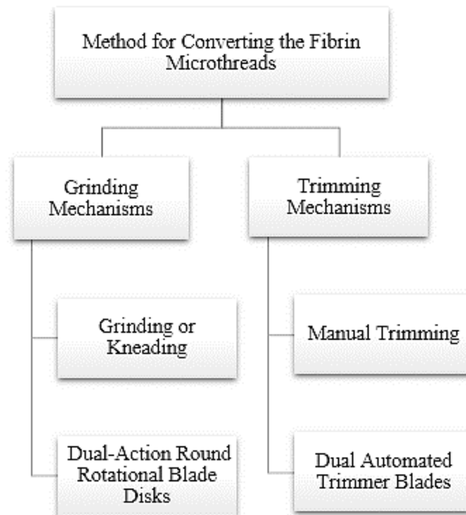


Figure 17: Objective Tree for the Specific Methods of Converting the Fibrin Microthreads

The team first looked at a mortar and pestle and an herb grinder and analyze their groinding or kneading and dual-action rotational flat blade disks methods respectively. The action of the mortar and pestle included groinding and mixing the microthreads until they were in particle form. The herb grinder uses stacked blades rotating in opposite directions to convert the threads into particle form. For the trimming methods, the team looked at commercial scissors and an automated trimmer in which an analysis of the manual trimming and dual automated blade trimmer actions, respectively, were analyzed. When analyzing these methods, the team created a list of advantages and disadvantages for each mechanism. Tables 10 to 13 below display the results.

Table 10: Advantages and Disadvantages of the Grinding or Kneading Method

Advantages	Disadvantages
Easy to execute the groinding and kneading method	Groinding and kneading method is tedious and time consuming
Low cost method	The microthreads must be cut beforehand, leading to more tedious manual labor

Table 11: Advantages and Disadvantages of the Dual-Action Flat Blade Disks Method

Advantages	Disadvantages
Easy to execute the dual-action flat blade disk method	The microthreads must be cut beforehand in order to fit within the mechanisms dimensions, causing more tedious manual labor
Commercially available in a range of sizes	Range of size needed for ideal effectiveness is not commercially available, so team would need to replicate design to fit our standards.

Table 12: Advantages and Disadvantages of the Manual Trimming Method

Advantages	Disadvantages
Easy to execute the trimming method	Uneven particle sizes (human error)
Manual trimming devices readily available	Tedious manual labor

Table 13: Advantages and Disadvantages of the Dual-Action Automated Trimmer Blades Method

Advantages	Disadvantages
Requires little manual labor	Automated trimmer more expensive than other methods
The threads do not need to be cut to smaller lengths	

4.3.2 Conversion Mechanism Design Approach

After conducting preliminary image testing from mechanisms described in section 4.3.1, the team designed mechanisms surrounding the conversion methods that produced the best results. Ideally the team wanted to create a design that could incorporate different conversion mechanisms. However, based on the cost, time, and materials available, the team developed design options based on the results of initial method testing.

The team first developed a list of needs and wants of the machine to further understand what was needed for the final design option. Table 14 below displays a summary of the mechanisms needs and wants.

Table 14: Needs and Wants of the Conversion Mechanism

Needs	Wants
Filter unwanted particle sizes (>1mm) and debris	Adaptable for different conversion methods
Stabilize fibrin microthreads	Simple Interface
Bundle fibrin microthreads	Automated Process
Collect the fibrin particles needed for Fibrina	
Safe for Users	

After determining the needs and wants, the team formulated the list of design requirements and objectives for the mechanism. In Table 15 below, a list of these recommendations is described.

Table 15: Objective Descriptions

Objectives	**Description**
Filter System	Filters out the debris and particles larger than 1 mm
Stabilize Threads	Allows for equal conversion of the threads
Bundle Threads	Automated process
Collect Particles	Collects the desired converted fibrin particles
Safe	Mechanism is safe use
Simple	Mechanism has a simple user interface
Adaptable	Mechanism is adaptable for different conversion methods
Automated	The mechanism’s process of bundling and conversion is automated

The team will use these objectives to design a device that implements the successful conversion methods into the device based on the preliminary image analysis results.

Chapter 5: Preliminary Design Verification

In order to generate a successful final design, it was essential to verify the success of a preliminary design and its components. This verification helped the team identify the aspects of the design that worked and did not work. This chapter consists of three major sections: analysis needed to verify fibrin's use in a cosmetic skin care product, machine testing for the final preliminary design and methods, and methodology for determining the best conversion mechanism.

5.1 Fibrina Testing

As a result of the insight gained during the interviews conducted with dermatologists, as well from the knowledge gathered in the literature review, the following experiments were created to test the value of a fibrin-based skin cream: cream composition testing, additive-infusion testing, fibrin degradation assaying, and fibrin surface roughness measuring. Additional experiments for UV absorbance measuring and diffusion testing were established. However, given several constraints, they were not completed.

5.1.1 Cream Composition Testing

There are numerous base formulas for creating a skin cream. Different formulations are based on the percentages of ingredients used in an emulsion/cream, and the manner in which they are mixed together. Using a baseline formula found in literature, the team began to test variations in compositions. In Table 16 below, one can see the breakdown for a basic composition of an oil-in-water cream.

Table 16: Basic Composition of Oil-in-Water Cream [93]

Ingredients	% (weight/weight)
Water Phase	
Deionized water	60.0 - 90.0
Humectant	2.00 - 7.00
Preservative	0.05 - 0.5
Water soluble emulsifier	0.25 - 2.5
Thickener(s)	0.1 - 1.0
Water soluble emollient	0.5 - 2.0
Chelating agent	0.05 - 0.20
Oil Phase	
Emollient System – oils, esters, silicones, etc.	3.0 - 15.0
Oil soluble emulsifiers	2.0 - 5.0
"Active ingredients"	As required by regulations of the actives being used
Oil soluble antioxidants	0.05 - 0.5
Fragrance/essential oil etc.	0.1 - 2.0
Color	As needed/required
Preservative	0.05 - 1.0
pH Adjustments	As required for stabilization of emulsion

The goal to test for the right composition was primarily to have the ability to make a cream which could be inexpensively used to test fibrin's capabilities within a cream. Based on the composition table's various ingredient options the team aimed to simplify down to a set of essential ingredients. The decided upon composition would contain grape seed oil, emulsifying wax, and water as the main ingredients. Smaller quantities of glycerin, essential oils, preservative, and vitamin E were added as well. This recipe is based upon a DIY website/blog for a basic moisturizer [94].

The team decided upon observational goals for the outcome of the cream composition which would deem the experiment a success. Said observations of the outcome of the cream included ideal texture properties, an appealing viscosity upon use, and an overall pleasant aroma (if any aroma was observable). The experiment was prepped to target the different weight percentages of the key ingredients within the base cream. The first batch, based on the online DIY site, had a water content of 50% in relation to the rest of the cream composition. The team

used this as a starting point, and created modifications for the water content to increase by 10% per batch attempt until optimal properties were found.

5.1.2 Additive-Infused Fibrin Testing

In attachment to the cream composition testing, there exists the incorporation of additive-infused fibrin into the base composition. The additive-infused threads had to go through experimental procedures of their own. Said experiments highlighted the amount of additive mixed into each batch. They also produced a protocol for the whether the additive could be pre-mixed with both the fibrinogen and thrombin solutions, or with just one.

For the sake of the time and resources set for the project, the team only focused primarily on retinol. With respect to FDA regulatory limits of retinol use within a cream, standards were made on the number of additive infused fibrin batches required to contain enough retinol to match those FDA standards. Up to 1% of a cream can be comprised of retinol.

Results of the cream composition and the additive-infused fibrin experiments helped to design the rest of the experiments performed within this project. With particular respect to degradation, the additive-infused fibrin experiment results helped make the decision on how to explore releasing the additives from fibrin through degradative means.

5.1.3 Fibrin Degradation Properties Testing

In order to better qualify fibrin's ability to act as a vehicle for actives within a skin cream, its degradation properties must be well understood. In natural wound healing, fibrin is typically broken down by plasmin via a process known as fibrinolysis. The resulting enzymatic degradation of fibrin produce fibrin degradation products (FDPs) which normally compete with thrombin during wound healing to prevent blood clots from growing and becoming problematic [95]. Because this process does not occur in a non in-vivo environment, the team had to find a way to replicate the process of fibrin degradation outside the body. Through literary research on

fibrinolysis, the team identified several proteases which could be purchased over-the-counter that have already been shown to break down fibrin.

The team identified three possible proteases for use. These proteases were bromelain, serrapeptase, and nattokinase. Bromelain is derived from pineapples. Serrapeptase is produced by non-pathogenic bacteria found in silkworms. Nattokinase is extracted and purified from a popular Japanese food called Nattō. Each of the three proteases have shown to promote fibrinolysis when ingested [96] [97] [98]. The team wanted to observe whether the same degradation effects could be recreated within a neutral solution comprising only fibrin and protease. After looking into each of the acquisition means for each of the three identified proteases, the team chose to use bromelain.

The bromelain used for experimentation was purchased over-the counter. Figure 18 below shows an image of the purchased bottle. The bromelain existed as a lyophilized powder held within capsules composed of rice flour, gelatin, maltodextrin, and magnesium stearate. The capsules themselves were not used in any part of the experimentation process.

Figure 18: Over-the-Counter Bromelain Capsules (500mg per Capsule)

To fully comprehend the manner of controlling fibrin's degradation rate through the proteases, an exploratory experiment was created to see what quantities of protease were required to fully breakdown a specific quantity of fibrin. 1, 2.5, 5, and 10 milligrams of protease were placed into separate petri dishes, each containing 1 milligram of fibrin within 5 milliliters of phosphate buffered saline solution (PBS). Images were taken of the threads from under a microscope at 0, 1, 3, and 5 hours to see the degradation that occurred. The dishes were left in an orbital shaker throughout the time points. A final round of imaging was done after the dishes were left overnight to see if continued movement would eventually degrade the fibrin across each of the different quantities of protease.

From the observations made in this exploratory experiment, another experiment would be conducted. This follow-up experiment would take advantage of UV spectroscopy. Retinol's molecular structure reveals a pi bond that makes it a viable candidate for UV spectroscopy. Upon further literature research, retinol's UV absorbance spectrum was confirmed at 325um [99].

The team proposed degrading retinol-infused fibrin threads to assess fibrin's infused-active release capabilities. Said threads would be degraded over time using calculated amounts of protease. Samples from key time points would be collected for UV spec analysis. Comparing the data from the threads as they released retinol over time, the team would be more knowledgeable of fibrin's release kinetics of additives.

5.1.4 Fibrin Surface Topography Testing

It may be required for the fibrin-based skin cream to stay on the skin for an extent period of time. During this time, it is important that the fibrin (and the actives it may carry) stay adhered to the skin long enough to perform their function. In addition to adherence, there is a possibility

for fibrin's natural texture to be beneficial for fibrin to act as both a delivery method for actives, as well as an exfoliant.

Atomic force spectroscopy can be used to determine the adhesive forces of fibrin cream against a skin sample. An experiment measuring the adhesion, friction, and wear characterization of skin and skin cream using atomic force microscopy can be mimicked to obtain the desired data collection from this experiment [100].

Upon learning of the required time it would take to reach a mastery of atomic force microscopy in order to perform adhesion tests, the team opted for more basic surface property imaging. As an aside to degradation, understanding microscale topography changes could potentially provide insight into characteristic surface properties of fibrin powder.

The results of the degradation properties as well as the composition, additive-infused fibrin thread, and surface roughness experiments would become the main focus of this project. However, keeping in mind the objectives, the team identified two additional experiments that could be performed. One focus was on UV absorbance potential. Another focus was on fibrin's diffusion into skin.

5.1.5 Fibrin UV Absorbance Properties Testing

There has already existed published work on the changes in fibrin microthread strength and stiffness as a result of crosslinking under UV light [100]. This study indicates that fibrin does have UV absorbing properties, to a degree. This could mean changes in degradation properties, as well other influential changes of the fibrin-based skin cream composition. On a related note, there exists an entire industry within the world of cosmetics that relies upon key ingredients which block UV light from causing skin damage: sunscreen.

The objective of measuring the absorbance properties of fibrin for this project is two-fold. The first part of the objective is to learn of any changes in composition or degradation which may occur as a result of fibrin absorbing UV light. The second part of the objective is to uncover any potential for fibrin to not only act as a vehicle for cosmetic actives, but also act an additional ingredient which can be used for UV absorbance.

In order to determine whether fibrin that has absorbed enough UV light to start crosslinking will show degradative or other physical/chemical changes, a separate degradation assay can be performed alongside the degradation assay already proposed. The independent variable to be introduced is UV light. As a sample is observed for fibrin degradation under UV, the team will monitor differences in degradation rate, while also measuring promises

In order to test for fibrin's UV absorbance properties, a batch of fibrin powder can be created and dissolved in organic solvent. One possible organic solvent to use is isopropyl alcohol. Water, ethanol, hexane, and cyclohexane are also commonly used. Once fully dissolved, samples can be pipetted into a cuvette. The cuvette can then be placed in UV spectrophotometer. A UV spectrophotometer will diffract a beam of light into the sample cuvette and a reference cuvette (standard curve). The result will be a graph measuring the absorbance level of the fibrin at different wavelengths. Based upon whether fibrin absorbs any wavelengths within the UVA region (400-320 nm) or UVB region (320-290 nm) of the light spectrum, it can be determined whether fibrin has any significant absorption properties.

5.1.6 Fibrin Diffusion Properties Testing

There needs to be evidence of fibrin's effectiveness in diffusing through skin. Both the literature and interviews conducted show a common practice done to test for diffusion with skin cream formulations: Franz Diffusion Cell Assays. Given monetary constraints to purchasing a

Franz Diffusion Cell chamber, the team proposed a simple alternative design for a Franz Cell apparatus, which can be seen in Figure 19.

The makeshift apparatus would be comprised of two media bottles (of different size) which would be cut to even the surface and allow for a skin membrane (porcine) to be placed over the smaller bottle. The larger media bottle would act as a water jacket, maintaining the inner chamber at 37° C. If needed, to equalize the height of the smaller bottle, a supporting block would be placed at the bottom and glued. A donor chamber would be fashioned from any suitable non-readily absorbing material. A clamping mechanism would be applied through pressure of a heavy object pressing down on the donor chamber. The rest of the device follows in precisely the same manner as an actual Franz Cell apparatus.

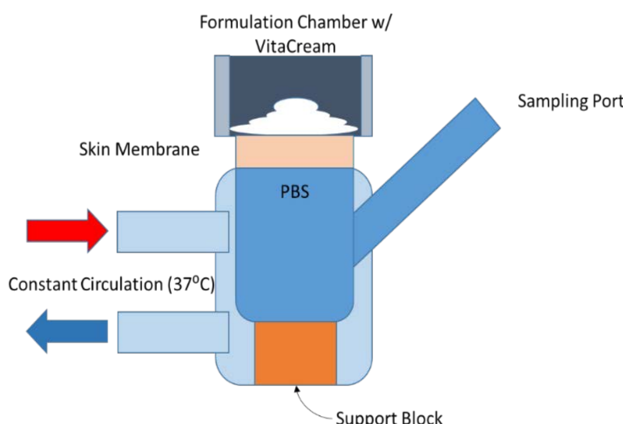


Figure 19: A simple design of a Franz Diffusion Cell Apparatus. Produced by means of cutting two media bottles of different sizes and appropriately creating an inner chamber separately surrounded by a water jacket

The objective of this experiment was to determine the concentration actives that pass through the skin membrane and dissolve into the PBS bath within the inner chamber. The team hypothesizes that due to fibrin's large molecular size, it would not be found diffusing through the samples. This would be a positive result, considering that fibrin's passage through a skin sample would no longer define it as a cosmetic. This would result in FDA requirements which the team has identified as a major barrier to entry for fibrin as commercial product.

Due to the small percentage of actives typically used in formulations (e.g. with retinol the percentage averages out to less than 1%) it was favorable to acquire or build a small diffusion

chamber. Although this experiment was not defined as one of the main experiments to be accomplished by the team, an attempt was still made at creating a makeshift Franz Cell apparatus.

5.2 Preliminary Design Machine Testing

Based on the phases described in Table 2, the team formulated what tests were required to verify the design components. The team discussed the aspects of each phase in conjunction with the Final Preliminary Design. In total, the team perform three tests to determine and verify the best conditions needs for successful extrusion of threads for the preliminary design prototype.

5.2.1 Phase 1: Pumping

When discussing the first phase of the design, the team considered the budget and decided to spend a bigger portion of the funding towards the extrusion and stretching process, as these phases are important in delivering a complete automated machine. The team decided to utilize laboratory resources and determine the pumping speed of the machine based on previous extrusion logs. The team thought this was the best approach to take for the final preliminary design because it did not cost extra budget money and could perform the job needed to extrude threads successfully for the preliminary design. Below is the list of conditions, based on laboratory extrusion logs [101], for the pump system and its components:

Flow Rate: 0.657 mL/min
Inner Diameter of mix tubing: 1/16 cm
Length of Mix Tubing: 15 cm
18 Gauge Cannula for the tip velocity: 0.5 cm/s

Based on the values listed above, the team estimated the general size of the prototype that would allow use to produce consistent threads without the allowance for clogging or backup in the pump system, which were within 165 cm x 60 cm lab bench space.

5.2.2: Phase 2: Extrusion

In order to prevent clogging of the extruder head, the team needed to determine how many threads could be drawn and polymerized without interrupting the extrusion process and speed. To determine the amount of threads that could be made in conjunction with the movement of the machine, the team performed a test to figure out how long it would take for threads to be viably removed following extrusion into a water bath. The purpose of this test was to establish the required length of the HEPES bath used to hold the extruded threads. Threads that take a longer time to polymerize would require a longer bath size, while threads that take less time to polymerize would require a smaller bath size.

Test 1: Thread Removal Time

This experiment was performed to test if the threads could be removed successfully at different time points following the extrusion process. In order to determine if the extrusion phase could be shortened, which saves material and money for the final and preliminary design, the team wanted to verify if the threads were viable and strong enough to be removed earlier than the typical 10-minute sitting period following the extrusion process (See Appendix I for the normal extrusion processed used currently in the lab). After the threads are drawn, the team removed threads after one minute, three minutes, five minutes, seven minutes, and ten minutes (control). The team compared the threads removed at time points one, three, five, and seven minutes to the threads removed after the normal setting time of ten minutes or the control. The results of the test are shown in Table 17 below.

Table 17: Results from Testing the Removal of Threads at Different Time Points

Time (min)	Were they viable after removal from the water bath?
1	Yes, but the thread was not fully formed and weak in structure
3	Yes
5	Yes
7	Yes
10 (control)	Yes

Based on the results of the test, the team concluded that the threads are viable after removal at earlier time points, therein reducing the size of the HEPES bath used in the final design. The team observed that the threads removed after one minute were a reach in terms of stretching viability since they were not completely polymerized. The threads at three minutes, however, were easy to remove and were fully polymerized and formed. Moving forward, the team decided to quantify the viability of the threads with mechanical tests of the threads removed at smaller, incremental time points.

5.2.3: Phase 3: Stretching

Quantifying the threads' mechanical properties at different time points helped the team determine the length to which the threads could stretch and remain in one piece and viable. This test aided the team in defining the length the threads would be stretched to on the machine.

Test 2: Mechanical Testing of the Threads

This experiment was performed to determine the mechanical properties of threads at different time points following the extrusion process. The purpose of this experiment was to determine how long the threads could be stretched in order to determine the quantitative parameter of the machine dimensions. The team wanted to verify the strength of the threads following the extrusion process following the extrusion process (See Appendix I for the normal extrusion process used currently in the lab). Based on the results and conclusions of the "Removal of Threads at Different Time Points" experiment, the team decided to test the mechanical properties of threads at time increments closer to three minutes. The time points the threads were removed were at two minutes, three minutes, five minutes, and ten minutes (control). The team compared the mechanical properties of the threads removed at the specific time points.

Based on the results of the mechanical testing, the results were inconclusive in telling whether the threads formed at two minutes, three minutes, five minutes, and ten minutes had significantly different mechanical properties. There are a couple explanations as to why this was the case. As a primary explanation, human error is a major issue. Based on the performance of the individual drawing the threads, the threads may have had better or worse mechanical properties, regardless of how long they sat in the bath. As an example, both the two minute and the ten minute threads were drawn from the same batch of fibrinogen and thrombin. Looking at the results of the mechanical testing, there is insignificant data showing the two minute threads having less (or more) tensile strength and subsequent elastic modulus as compared to the ten minute threads.

Other explanations include the relatively small sample size of threads tested, which was in part by the team's choice to produce only 10 samples for measure. During the process of wetting the samples for measurement, two of the two-minute threads had broken from their test strips and became unusable. Based on what could be gathered from this experiment, it may be recommended to have threads being produced under the automated machine to sit for at least three minutes as a safety measure.

5.2.4: Phase 4: Drying

The team defined a dry thread as one that does not have water droplets and extra residue hanging off. Utilizing the SIMCO Aerostat XC Air Blower in the laboratory, the team tested different settings and placement of the dryer to determine the best placement of the dryer in conjunction with the main stretch frame component of the machine. The team used threads that were manufactured following the normal protocol (See Appendix I). The team focused on distance of the dryer from the threads and the settings on the ionized blower/dryer in the lab.

Test 3 Drying Tests for the Threads

The team considered many different variables when designing the drying experiment. The team used threads drawn at regular dimensions used in the lab. The team used two 40cm long boxes (one for cold air and one for warm air from the blower) and each was marked with fifteen 2.7 cm length sections on the side of the boxes. The purpose of sectioning off the length of the boxes was to determine which distance was most ideal for drying the threads. The boxes were place five centimeters from the dryer while the dryer was placed at a 90-degree angle facing the box. The sections were then split into three different groups, with a total section length of 13.3cm. The variable groups are shown in Table 18. Figure 20 shows a set-up of Box 1 with the blower at medium fan speed.

Table 18: Variables for the Drying Test

Box 1 (Warm Air)	Group 1: Low Fan Speed, close to the dryer (first 13.3 cm section)	Group 2: Medium Fan Speed, 18.3 cm away from the dryer (second 13.3 cm section)	Group 3: High Fan Speed, 31.6 cm away from the dryer (third 13.3 cm section)
Box 2 (Cold Air)	Group 4: Low Fan Speed, close to the dryer (first 13.3 cm section)	Group 5: Medium Fan Speed, 18.3 cm away from the dryer (second 13.3 cm section)	Group 6: High Fan Speed, 31.6 cm away from the dryer (third 13.3 cm section)

Figure 20: Drying of the Microthreads with warm air at a medium fan speed

Based on the results of the test, it was determined that the blower did speed up drying time of the threads. However, the warm vs. cold air setting does not change the drying quality of the threads as the threads ended up drying at similar times for both boxes. The blowing speeds also did not impact the speed of the drying process. It did, however, show that the threads were strong enough to withstand high blowing speeds. The variable that did show changes between thread groups was distance. The distance of the threads from the blower displayed variable changes in drying speed. The threads that were placed closer to the blower dried quicker than ones placed at a further distance from the blower. The results from the tests helped the team determine that a closer distance of the dryer to the stretching frame (where the threads are stretch) is the most optimal position for faster drying.

5.2.5: Phase 5: Removal

Since the final microthreads do not require exact uniformity and precision, the team decided the best way to remove the thread was to cut them and have them drop into a collection bin. This section of the machine will be replaced in the final design with a system that can convert the threads into an alternative form for use in the Fibrina skincare product. Figure 21 below is an image of the final preliminary design prototype.

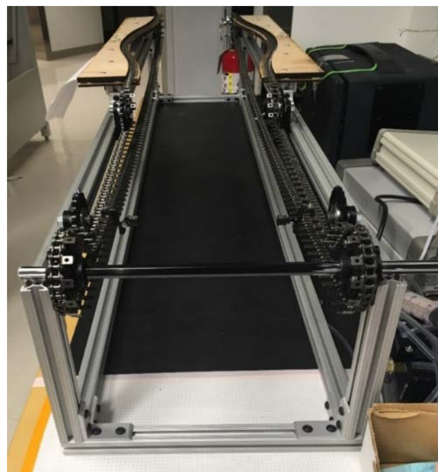


Figure 21: Machine Prototype

5.3 Conversion of Fibrin Microthreads

After performing initial image analysis on the converted fibrin using the four methods, the team determined which methods were suitable for the designs of the final conversion mechanism.

5.3.1 Conversion Method Application Preliminary Imaging Results

Initial testing involved four experimental groups, one group for each of the methods. There were 28 fibrin microthreads total with average lengths of 16 cm. 7 threads were used per method group. After the fibrin was converted to its particle form, images were taken and analyzed in ImageJ. In Figures 22 to 25 below are some of the image results of the initial testing phase.

Figure 22: Grinding or Kneading Results at 10x Magnification, Scale bar = 0.1 mm

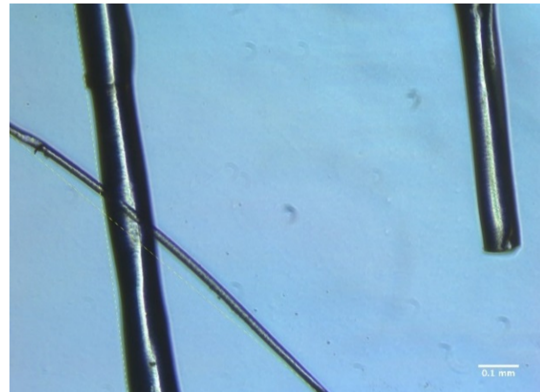


Figure 23: Rotational Disks Image Results at 10x Magnification, Scale bar = 0.1 mm

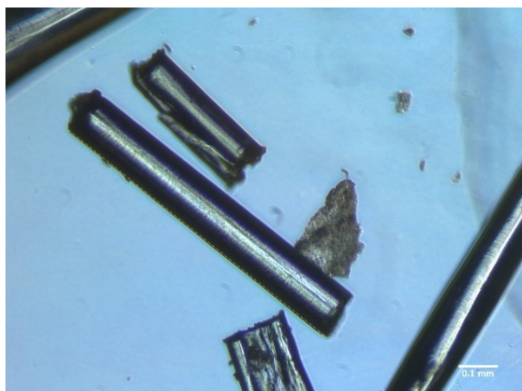


Figure 24: Manual Trimming Image Results at 10x Magnification, Scale bar = 0.1 mm

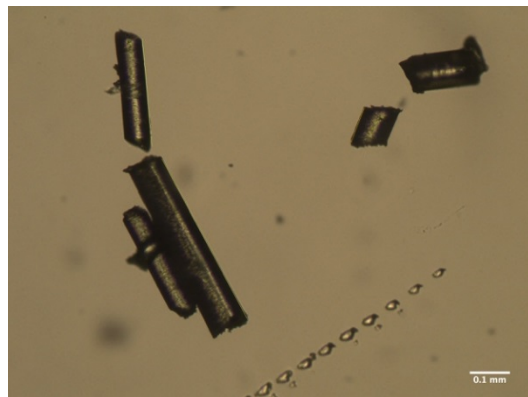


Figure 25: Automated Dual-Blade Disks Image Results at 10x Magnification, Scale bar = 0.1 mm

Table 19 below summarizes the results of the average lengths of the threads taken for the four methods. 5 fibrin particles per method group were measured and the averages were calculated using Microsoft Excel (See Table 41, Appendix VI for the fibrin particle measurements).

Table 19: Fibrin Particle Average Lengths for Each Thread

Method	Average Length (mm)
Grinding and Kneading	0.42
Dual-Action Rotational Disks	1.76
Manual Trimming	0.32
Dual Automated Trimmer Blades	0.24

The initial results demonstrated that all methods were successful except the dual-action round rotational disks, as no threads were converted to particle form using this method. The kneading method was also not chosen as a desired final method because it required more tedious and manual labor than the trimming methods.

5.3.2 Conversion Mechanism Preliminary Analysis

The team aimed to create a mechanism that allowed for a more stable and cleaner conversion. Considering the needs and wants of the conversion mechanism, the team came up with 3 preliminary designs that are displayed on the next page.

Design 1

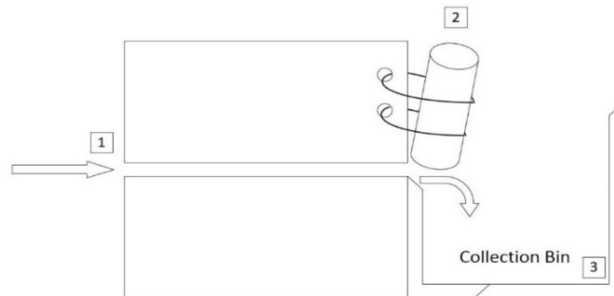


Figure 26: Design 1 Conceptual Diagram

Design 1 (Figure 26) included the use of a 25 mm diameter tube around 80 mm long. Through this tube, the threads would be pushed manually, allowing the tube's small diameter to bundle the threads together. At the end of the tube, represented by a cylinder in Figure 26, the conversion method would be placed and fastened by zip ties. A collection bin would be below to collect the particles. While this design is simple, organizes, and bundles the fibrin microthreads, it does not contain a filter system, so any particle size and debris would fall into the bin below. It also contains a collection mechanism.

Design 2

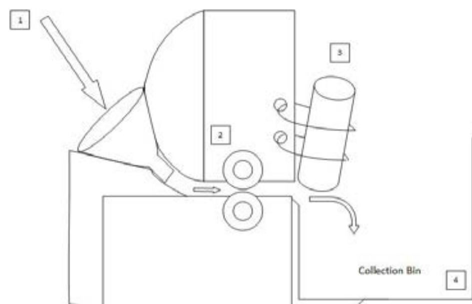


Figure 27: Design 2 Conceptual Diagram

Like Design 1, Design 2 (Figure 27) bundles the threads together, fastens the automated mechanism at the end of the track, and has a collection bin for the particles at the end of the track. However, using a servo motor and two motors, this design process automates the bundling

process. However, this method does not have a filter system as well, so even though it is easier to bundle the fibrin, it is harder to filter out the particles.

Design 3

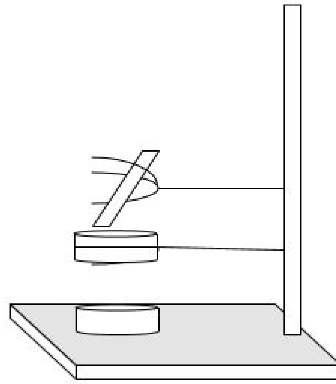


Figure 28: Design 3 Conceptual Diagram

Design 3 (Figure 28) is different from Designs 1 and 2 because it utilizes a filter system (a mesh that filters out particles larger than 1 mm) and gravity to collect the particles in a 60mm petri dish below the filter. A ring stand with two beaker clamps are used to hold the filter system and stabilize the fibrin microthreads. Figure 28above represents the system while the parallelogram represents the stabilized fibrin microthreads. The advantages of this method are that it can filter out unwanted particle sizes and more than one conversion method can be utilized. The disadvantages of this design include the difficulty of stabilizing and bundling the threads because the threads are not confined to a smaller diameter area.

Design Analysis

In order to determine the best design, the team performed a pairwise comparison based on the needs and wants formulated in Section 4.3.2. Using scores of 0, 0.5, and 1, the team determined the importance of each objective as they related to one another. A score of 0 was given if the objective in the row was less important than the objective in the respective column.

A score of 1 was given if the objective in the row was more important than the objective in the respective column. A score of 0.5 was given if the objectives in the row and column were of equal importance. These weights would determine the importance each objective would have in the design matrix. Table 20 shows the pairwise comparison chart from one of the team members while Table 21 shows the total weights of importance for each objective.

Table 20: Example Pairwise Comparison Chart

Pairwise Comparison	Filter System	Stabilize Threads	Bundle Threads	Collect Particles	Safe	Simple	Adaptable	Automated	**Total**
Filter System		0.5	1	1	0	1	1	1	5.5
Stabilize Threads	0.5		0.5	0	0	1	1	1	4
Bundle Threads	0	0.5		0.5	0	1	0	1	3
Collect Particles	0	1	0.5		0	1	1	1	4.5
Safe	1	1	1	1		1	1	1	7
Simple	0	0	0	0	0		0	1	1
Adaptable	0	0	1	0	0	1		1	3
Automated	0	0	0	0	0	0	0		0

Table 21: Total Pairwise Comparison Results

Objective	**Pairwise Comparison Scores**
Filter System	12
Stabilize Threads	12
Bundle Threads	11.5
Collect Particles	16
Safe	21
Simple	5
Adaptable	6
Automated	0

The team ranked safety, particle collection, a filter system, and the stabilization of threads as high importance. The team did not rank the automation objective of high important because for the purposes of this project and the time constraints the team was under, it was more important to stabilize and filter the fibrin.

After determining the important objectives, a decision matrix was performed on the most feasible design. The team ranked each objective on a scale of 1-5. The numbered values are as follows:

1 = Does not fulfill the objective
2 = Somewhat fulfills the objective
3 = Moderately fulfills the objective
4 = Mostly fulfills the objective
5 = Fulfills the objective

Table 22 and 23 are an example of a team member's design matrix and the final results respectively. See Tables 42 and 43 in Appendix VI for the results of the pairwise comparison and design matrix analyses from each group member.

Table 22: Example Design Matrix Used in Calculating the Total Design Matrix Score for each Design

	Filter System	**Stabilize Threads**	**Bundle Threads**	**Collect Particles**	**Safe**	**Simple**	**Adaptable**	**Automated**	**Total**
Weight	*12*	*12*	*11.5*	*16*	*21*	*5*	*6*	*0*	
Design 1	1	3	4	3	5	4	2	2	279
Design 2	1	4	5	4	4	2	2	4	287.5
Design 3	5	4	4	5	5	4	4	2	383

Table 23: Total Design Matrix Score

Design	**Total Score**
Design 1	829.5
Design 2	874
Design 3	1107

Based on the design matrix analysis, the team decided that Design 3 was the most feasible design. The team utilized the trimming methods when testing the design and performed further image analysis after the mechanism's assembly.

Chapter 6: Final Design Validation

This chapter describes how the team further validated Fibrin's use in cosmetic skin cream, modified the preliminary machine design prototype, and tested the conversion mechanism that creates more equal and cleaner conversion. From the construction of the machine and the experiments conducted in the lab, the team could determine and formulate conclusions that will help the future development of Fibrina. This section is separated into two sections: The final machine design and the Fibrina testing results.

6.1 Fibrina Testing

The results of the Fibrina testing were organized by each of the varying experiments. Again, the experimental approaches were based off of findings on validating fibrin's value, which were highlighted in Chapter 4. Complete protocols, data, and images for each of the experiments can be found at the end of the report, within Appendix III and IV.

6.1.1 Composition Results

The team formulated three creams based on the ingredients decided upon earlier in the project. The first cream made followed the quantities found in the DIY site, which corresponded to a cream comprising of about 50% water. The rest of the quantities for this first cream can be seen in Table 24 below.

Table 24: Composition Experiment, 50% Water-Based Cream

Ingredient	Unit	Original Measurement	Unit of Measure	in mLs	% to total
Grape Seed Oil	Cup	0.50	cup	118	31.50%
Emulsifying Wax	Cup	0.25	cup	59.1	15.75%
Water	**Cup**	**0.75**	**cup**	**177**	**47.25%**
Glycerin	Tbsp	1	tbsp	17.8	4.73%
Vitamin E	Tsp	0.25	tsp	1.48	0.39%
Essential Oil	Drops	12 -15 drops	drop	0.700	0.19%
Germall Plus	tsp	0.13	tsp	0.740	0.20%

The resulting 50% water-based cream failed to meet physical requirements indicated by the team. The cream was found to be too viscous, and unpleasant to apply. However, it did prove to have a pleasant, non-overwhelming aroma. Recognizing that the viscosity could be lowered by increasing the amount of water within the cream, a 60% water-based cream was made. The quantities used for the second cream can be found in Table 25 below.

Table 25: Composition Experiment, 60% Water-Based Cream

Ingredient	Unit	Original Measurement	Unit of Measure	in mls	% to total
Grape Seed Oil	Cup	0.13	cup	29.6	23.08%
Emulsifying Wax	Cup	0.0625	cup	14.8	11.54%
Water	**Cup**	**0.33**	**cup**	**78.1**	**60.93%**
Glycerin	Tbsp	0.25	tbsp	4.44	3.46%
Vitamin E	Tsp	0.0625	tsp	0.370	0.29%
Essential oil	Drops	3.25	drop	0.700	0.55%
Germall Plus	tsp	0.03	tsp	0.185	0.14%

The 60% water-based cream was ideal for a baseline cream. It had a smooth texture, without feeling entirely liquid. The aroma was pleasant, and the cream itself was physically appealing to put on skin. As a precaution, one 70% water-based cream was created. The third cream was found to be overly water-based. After a day of sitting a container, the oil and water mixtures had already separated out. Figure 29 below showcases the 70% water-base cream's separating states.

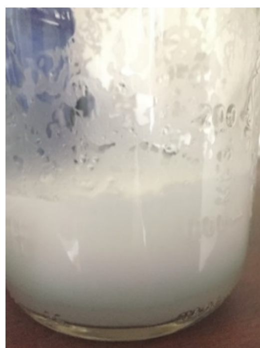


Figure 29: 70% Water-Based Cream, water separating from oil base at bottom of jar

With knowledge of FDA regulations on the maximum retinol concentrations, a ratio for fibrin-to-additive was determined which ultimately affected the final cream composition. Table 26 seen below highlights the optimal percent compositions with additive-infused fibrin particles. An explanation of the particle-to-additive ratio is mentioned in the results of the additive-infused thread testing.

Table 26: Weight % calculations for a baseline moisturizer with additive-infused fibrin particles

Ingredient	% to Total
Grape Seed Oil	23.%
Emulsifying Wax	12%
Water	60%
Glycerin	3.5%
Vitamin E	0.3%
Essential Oil	0.55%
Germall Plus	0.15%
Fibrin Particles	10X % of Active

6.1.2 Additive-Infused Fibrin Experiment

Before moving forward with mixing additive-infused fibrin into the baseline cream that was decided upon, tests on introducing additives into fibrin microthreads in the most optimal way possible were performed. By learning what the ideal concentrations of additive-to-thread were, the amount of fibrin required for a cream could be identified.

The team focused on retinol, in the form of retinoic acid. Retinoic acid comes in the form of a lyophilized yellow powder. This form of retinol was only dissolvable within ethanol. Two different ratios for dissolution were used. The first ratio was 25 mg of retinoic acid per ml of ethanol. The rate of dissolution for 25mg of retinoic acid in 1 ml of ethanol was rather slow. The 25mg were not completely dissolved until a number of days passed.

With a ratio of 25mg of retinoic acid per 2ml of ethanol, the retinoic acid dissolved in solution much more rapidly, although it still took a significant amount of time (several days) for complete dissolution. The team opted to use the 25mg/ml retinoic acid in ethanol for the remainder of the experiments despite its slower dissolution time. This was due to the objective to deliver as much active within a singular batch as possible.

When the retinol in ethanol solution is pre-mixed with the thrombin solution, the resulting mixture is a white, opaque solution. Figure 30 below is an image of that solution just described. The result of pre-mixing with fibrinogen was immediate clotting, and was thus impossible to use for extrusion.

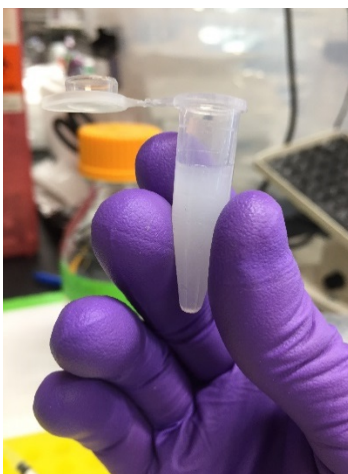


Figure 30: 100ul Retinol in Ethanol (25ug/ml) pre-mixed into 900ul Thrombin/CaCl2 Solution.

With the retinol in ethanol solution only pre-mixing with the thrombin solution, experimentation with the ideal amount of retinol in ethanol solution that could be pre-mixed without causing difficulties with thread formation and extraction occurred. The first attempt at making retinol threads used 200ul of retinol in ethanol solution pre-mixed into thrombin solution. The observations that came from using the 200ul amount were as follows:

1. Threads formed well on the bottom of the pan.
2. Threads were difficult to remove from the pan if not drawn near perfectly.

3. Any threads drawn too thin were irretrievable. Threads drawn larger than average were a 50/50 success.
4. The consistency at which a thread was drawn well, taken off the plate, and successfully placed on a box for drying was low.

Impediments with making these batches ultimately led to the use of 100ul for pre-mixing. The resulting batches were much easier to deal with, at the expense of having less retinol concentration per batch of threads. As a result of only being able to mix 10% of retinol into a single batch of fibrin threads, multiple batches are required to achieve the desired maximum allowable concentration of retinol for a cream, within fibrin-based particles. As an example, for a 100ml cream, a maximum of 1ml of the cream is allowed to be retinol given FDA regulations. Given that only 100ul of retinol can be infused into a single batch of fibrin microthreads, ten batches would have to be produced in order to attain 1ml of retinol-infused fibrin particles.

6.1.3 Fibrin Degradation Experiment Results

Two experiments were performed to better understand fibrin-based particle degradation properties. These experiments were the exploratory enzyme degradation experiment and the UV spectroscopy analysis experiment. The first experiment addressed the capabilities of a degrading fibrin using a natural fibrinolytic enzyme, bromelain. The second experiment addressed the need for quantitative data on active release performance.

Five quantities of bromelain were used for this experiment: 1mg, 2.5mg, 5mg, 10mg, and a control (0mg). The entire protocol for this experiment can be found in Appendix III. Figure 31 below shows the performance results of the 10mg bromelain sample in its ability to degrade fibrin particles over only two hours within a neutral buffer.

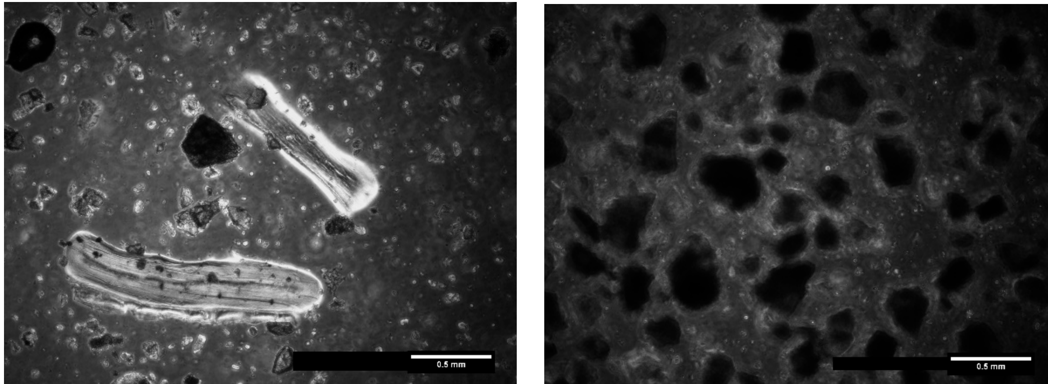


Figure 31: Left: 1mg fibrin particle degradation via 10mg bromelain exposure after 1 hour. Right: 1mg fibrin particle degradation via 10mg bromelain exposure after 3 hours.

Observations of the amount of fibrin left in each of the dishes were done with the naked eye, then through a microscope. Microscopic images for each of the time points and quantities can be found in Figures 54-84 in Appendix IVa. The 5mg and 10mg bromelain dishes were the only dishes to completely dissolve the fibrin completely. It can't be determined through sole observations if the fibrin particles within each of the samples were degrading at different rates. However, with the results of the first experiment providing a baseline for the amount of enzyme to use on fibrin-based particles, a quantifiable UV spectroscopy experiment could be conducted.

Retinol-infused threads were drawn and weighed. Six batches of 1mg each were created. Five sample plates of 6, 7, 8, 9, and 10mg of bromelain were created alongside a control. These quantities were chosen based on the results of the exploratory degradation experiment, which highlighted the complete degradation of 1mg of fibrin particles using 5 and 10mg of bromelain. By looking at the differences in the release of retinol with the different quantities of bromelain, a release profile could theoretically be identified. Each of the plates were filled with 5ml of PBS. 10mg of fibrin were then added to each of the sample plates. Supernatant sample from each dish was taken at the zero hour, and then again every other hour starting from the first hour. The full protocol for this experiment can be found in Appendix III. Figure 32 below shows graphed results of the UV spec values obtained from each of the samples.

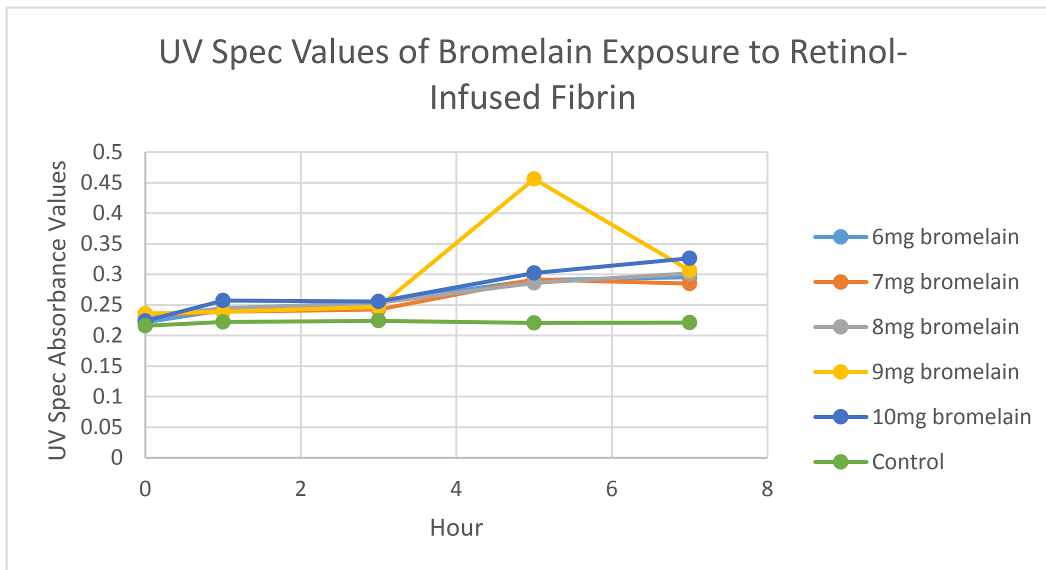


Figure 32 Results of UV Spec Analysis for Bromelain Enzyme. Significant Outlier for the 9mg dish at hour 5.

No significant change in the readings from the first to third hour occurred. The most notable change occurred between hours three and five, with the exception of the 9mg reading at hour five, which was likely an outlier resulting from a mishap. The 6, 7, 8 and 9mg dishes all ended at fairly similar quantities of released retinol, while the 10mg dish showed an averaged

0.02 higher release of retinol. A two-way ANOVA test was calculated with MATLAB coding to determine if there was statistical significance between each of the samples. As shown by the results of the run MATLAB code (see Appendix IVc for the complete code), which can be seen in Figure 33 below, there was no statistical significance.

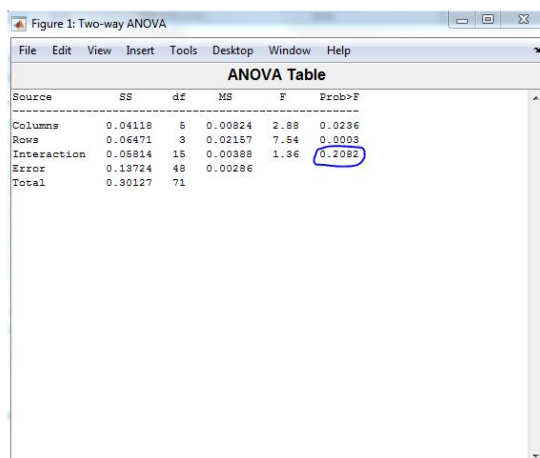


Figure 1: Two-way ANOVA

File Edit View Insert Tools Desktop Window Help

ANOVA Table

Source	SS	df	MS	F	Prob>F
Columns	0.04118	5	0.00824	2.88	0.0236
Rows	0.06471	3	0.02157	7.54	0.0003
Interaction	0.05814	15	0.00388	1.36	0.2082
Error	0.13724	48	0.00286		
Total	0.30127	71			

Figure 33: Results from running the MATLAB code for the Two-way ANOVA. Circled is the calculation indicating no statistical significance

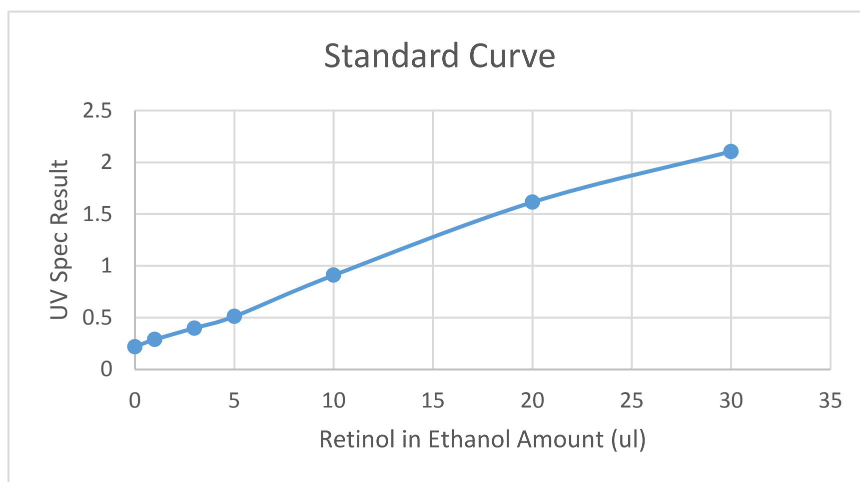


Figure 34 Standard Curve of retinol in ethanol mixed with PBS. The retinol in ethanol amounts represent the amount of retinol in ethanol within a 500ml PBS solution

The standard curve seen above in Figure 34 shows the expected readings from the UV Spec analysis in relation to the amount of retinol in solution. Based on the readings from the SpectraMax 250, not a single sample read showed a retinol concentration higher than 3ul of

retinol in ethanol solution. Due to the results of this experiment, it was speculated that some of the retinol might be escaping at some point during the experimental process. In an attempt to identify if this was the case, a sub-experiment was performed using a special 3D printed multi-channel tub. Figure 35 below is an image of this tub prior to extrusion.

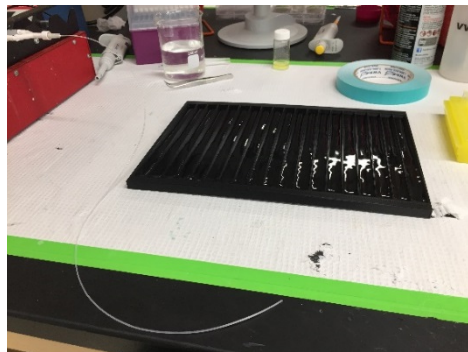


Figure 35: 3D printed multi-channel tub. 5ml HEPES within each channel.

Individual threads were drawn into each channel. Once a complete batch was produced, the 5ml HEPES from each of the channels were extracted and analyzed through UV spectroscopy to see if retinol would be found within the threads. This would indicate retinol loss in the thread, to the bath. No statistical significance could be identified to indicate or not indicate retinol loss. In-depth results from this sub-experiment can be found in Appendix IVd.

6.1.4 Surface Roughness Results

Fibrin particles were created and transferred over to a sample plate, where they could be imaged under an atomic force microscopy machine. The type of AFM machine used was a NaioAFM. Figure 36 below shows images taken of a single fibrin particle.

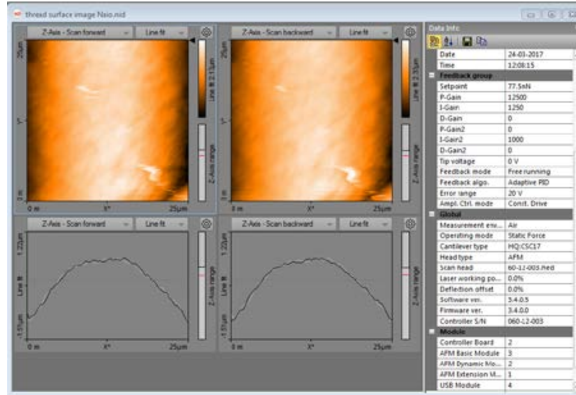


Figure 36: Surface imaging of Fibrin Particle under AFM

Surface roughness values of the particles varied heavily by the location along the particle. Due to the mechanical means by which the AFM cantilever travels across the particle, surface roughness values change more dramatically around the edges of the particle.

Quantified data of the surface roughness at different points from within the above image were taken. Figures 37 and 38 below show two separate sections of the image being calculated for their area roughness values.

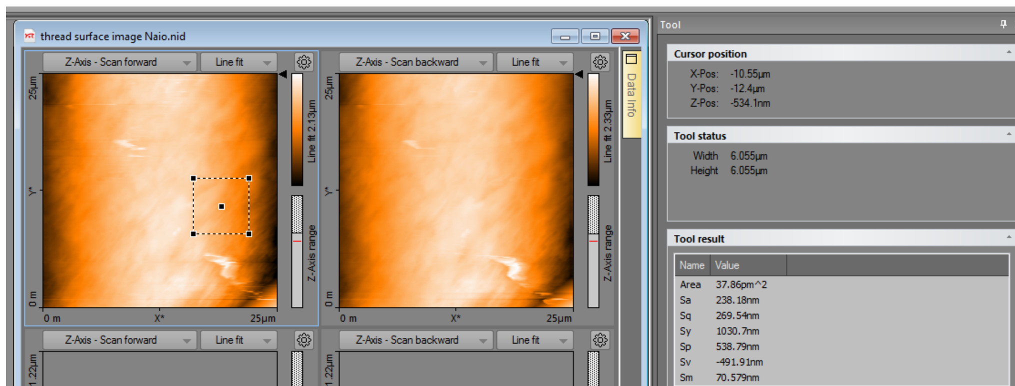


Figure 37 Thread Surface image with Area Roughness Calculations

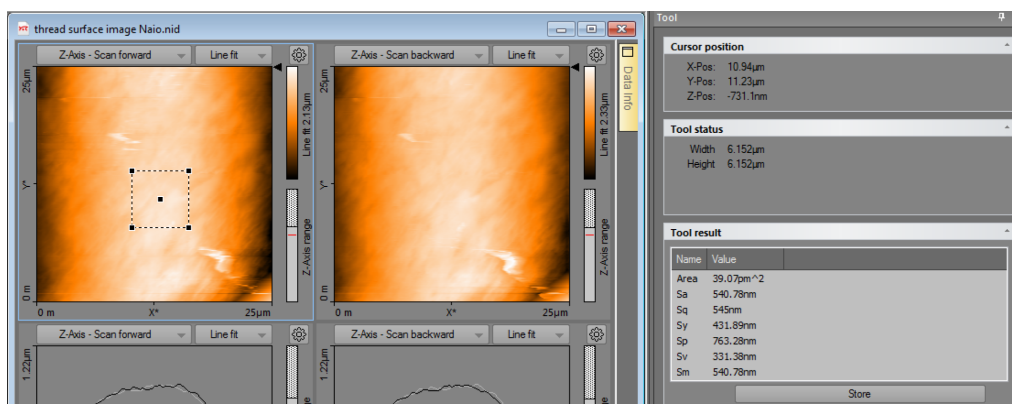


Figure 38 Thread Surface image with Area Roughness Calculations 2

In-software analyses for the cross-sections seen in Figures 37 and 38 produce six different roughness values. The values are shown in Table 27 below.

Table 27: Surface Roughness Values for the cross-sections in Figures 37 and 38

	Surface Roughness Calculations (nm)					
	Sa (Average Roughness)	Sq (Root Mean Square)	Sp (Peak Height)	Sv (Valley Depth)	Sy (Peak-Valley Height)	Sm (Mean Roughness)
Figure 37	238	270	539	-492	1030	70.6
Figure 38	541	545	763	331	432	541

Only imaging on the uncut surface of a fibrin particle could be conducted due to the difficulty in "standing" a particle on a cut face. Figures 97 and 98 found in Appendix IVc show AFM imaging of a "stood up" cut face image. The image was of unreadable quality, given issues with the movement caused by brushing the cantilever head against the unsupported particle.

6.2 Final Machine Design

Due to the structural quality of the preliminary prototype and time constraints of the project, the team decided to add components necessary for a complete final design (Figure 46) (See Appendix V: Figures 104 to 129 for specific SolidWorks models of individual components of the machine). The team took into consideration the results from the experiments conducted when designing the initial prototype and implemented the necessary changes to the final design. The major components that were added to the final design were the automated extruder head, the tub and the base it sits on, the conveyor, and the magnetic clamps.

Figure 39: Final Machine Assembly

6.2.1 The Extruder Head

As described in Chapter 5, the team used the syringe pump in the lab to combine the fibrinogen and thrombin. In order to begin the automated extrusion process, the team assembled an extruder head using a stepper motor to automate the extrusion process. 80/20 was used as the structural base for the motor, while an Arduino was programmed to automate the uniaxial movement of the extruder head. A long strip of 80/20 was cut to the width of the machine (13”) while two 80/20 stands were cut to 3” in length. The stands and the strip were then mounted to the base of the machine.

The team set a flow rate of the fibrinogen and thrombin mix at 0.5 cm/s and used this speed to extrude the fibrin microthreads onto the clamps. This speed was chosen based on previous manual microthread extrusion batches [94].

6.2.2 The Tub

The tub component of the design is comprised of 0.100 inch clear, rectangular. The tub was assembled and sealed with Kwik seal ultra-clear and Loctite fast cure marine sealant. The following dimensions were used for the tub:

Base: 23.5" x 8.125"
Short Side Panel (x 2): 8.125" x 3"
Long Side Panel (x 2): 23.5" x 3"

The team cut two base pieces with the laser cutter machine in Washburn Shops and used the sealant to glue the pieces together, forming a double layer. The long side panel pieces were then glued to the base and held in place overnight. The short side panel pieces were then glued into place.

In order to keep the tub as steady as possible, a portable wooden base was constructed. A vertical band-saw was used to cut the pieces to their respective dimensions. The dimensions of the wooden stand are as follows:

Base: 24.5" x 10.5"
Short Side Panel (x 2): 9.5" x 6.5"
Long Side Panel (x 2): 24.5" x 9.5"

Since the lab is a dynamic environment, components of the machine may be moved around the lab space often. The team considered this when designing the tub and its stand. While the two components are not attached to one another, they will be easy to handle by users because they can be moved around easily. Figures 40 and 41 show Top and Isometric views of the tub.

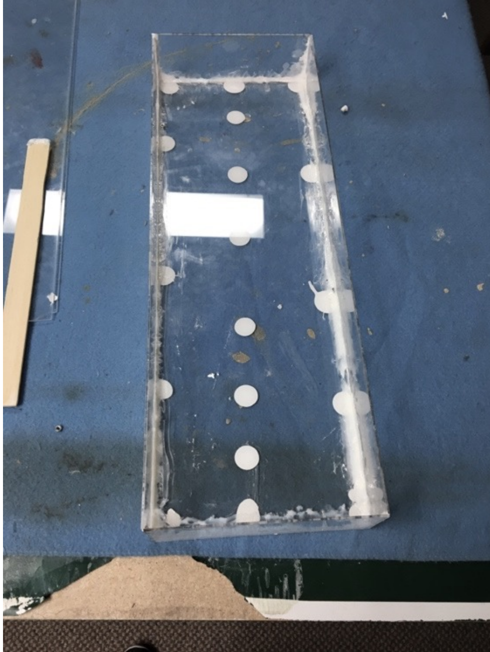


Figure 40: Top View of the Assembled Tub

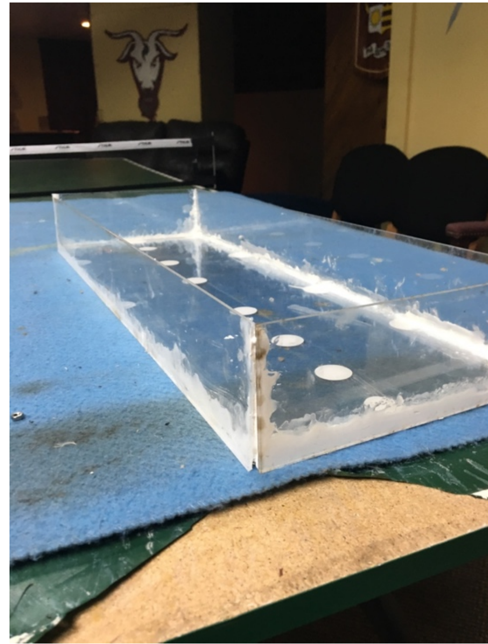


Figure 41: Isometric View of the Tub

Testing

The team ensured the bath is tightly sealed through leakage tests. The team filled the assembled tub with 1.2 L of water. Water was poured into the bath using a 2000 mL beaker then the bath was placed on white paper towels and monitored for potential leakages. If there were spots of water on the paper towels, the team would pour the water in the tub back into the 2000 mL beaker to check the amount of water that was left. If the team discovered a leakage, the source would be identified and sealed with more sealant. The team observed the bath at 30 minutes, 60 minutes, 720 minutes (12 hours), and 1,440 minutes (24 hours). At each time interval, the team indicated if the tub passed or failed the leakage test, recording the values and results in Table 28 below.

Table 28: First Test Tub Leakage Results

Time (minutes)	Amount Measured (L)	Leakage Amount (L)	Pass/Fail
30	1.13	0.07	Fail
60	N/A	N/A	Fail
720	N/A	N/A	Fail
1,440	N/A	N/A	Fail

Since leakage was discovered during the first 30 minutes of the test, the team ceased the first tub test. The leak occurred at the side edges of the bath and went in between the double base layer and onto the paper towels. The team sealed the edges of the bath and retested it using the same protocol as used for the first leakage test. The results are shown in Table 29 below.

Table 29: Second Test Tub Leakage Results

Time (minutes)	Amount Measured (L)	Leakage Amount (L)	Pass/Fail
30	1.2	0	Pass
60	1.2	0	Pass
720	1.2	0	Pass
1,440	1.2	0	Pass

The team was successful in fixing the sources of the leaks in the tub system and the testing for this portion of the machine was complete.

6.2.3 The Conveyor

In addition to the extruder head, the conveyor was also automated by the same step motor using a string. In order to get the material at the dimensions needed for the conveyor, the team utilized the vertical band-saw and the plasma cutter in Washburn Shops to cut the pieces to the necessary shapes and sizes. The conveyor was assembled with the following materials and dimensions:

Zinc Plated 1.5” 90 º Steel L-Bar with 3/8” holes and 5/16” slots (x 2): 20”
3/8” Threaded Rod (x 1): 8”
ABS Polyflex PVC Tube (x 6): 6”
Acetyl Sleeve Bearing for the Conveyor Roller: 3/8”
Acetyl Bearing for 5/16” Axle
Washer, Nuts, Bolts: 3/8” and 5/16”
Rubber Bung
Non-Slip Stick Tape and Orange Conveyor Tape

The team is planning on automating the conveyor by attaching a thin, circular sleeve to the stepper motor used to extrude the fibrin, allowing it to move simultaneously with the extruder head. Figure 42 below is a picture of the structure of the conveyor. Additions will need to be implemented within the first two weeks of D term.

Figure 42: Conveyor Base

6.2.4 The Magnetic Clamps

In addition to the test clamps used in preliminary design, the added a total of 44 clamps, making up 22 pairs of clamps. The magnets were 3/16" in diameter with a 1/16" thickness. Using screws and hex nuts, the clamps were attached to the machine's chains and placed in their starting position above the tub. Figure 43 below shows the assembled clamps.

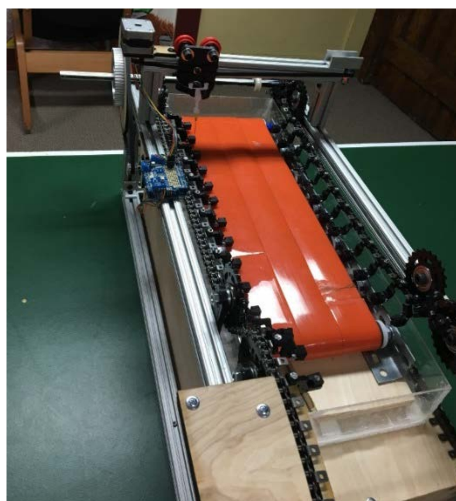


Figure 43: Assembled Clamps and Conveyor Incorporated in Final Design

6.3 Conversion of the Fibrin Microthreads

The final mechanism included the following materials:

Two clamps with PVC coated prongs
1 ring stand
50 mm circular filter mesh
60x15 mm petri dish

The first clamp contained taped fibrin microthreads, while the second clamp is holding a 50 mm circular piece with mesh to filter out debris and particles larger than 1 mm. A petri dish collects the desired particles. A funnel can also be used and placed in the first clamp to help with the grouping of the fibrin mcirothreads as well, making this mechanism adaptable for different method applications and bundling options. This mechanism can incorporate the two trimming methods. Figures 44 and 45 displays the set-up of the mechanism with the funnel bundle system in use and the bundled threads mounted respectively.

Figure 45: Final Mechanism with the Funnel to Bundle the Fibrin

Figure 45: Set-up of the Mechanism without the Funnel

Using image analysis, the team wanted to see if the particle mixes using the mechanism improved over initial image analysis. Figures 46 and 47 show the results of these experiments. The images include threads that are within the desired particle range, while the image itself is cleaner due to the removal of the extraneous debris and large particle sizes.

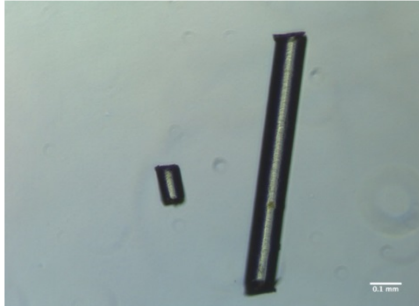


Figure 47: Image Results Using the Manual Trimming Method with the Mechanism, Scale bar = 0.1 mm

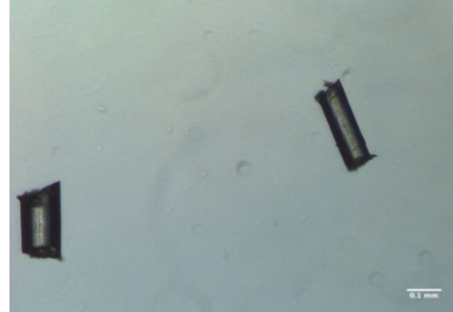


Figure 47: Image Results Using the Automated Dual-Action Trimmer Blades with the Mechanism, Scale bar = 0.1 mm

Using the manual trimming method, the particle sizes in the picture shown above was 0.1 and 0.8 mm in length respectively. Using the automated dual action blades method, the particle sizes were around 0.1 and 0.2 mm in length. The mechanism was successful in both removed extraneous debris and converted particle sizes within the desired range.

6.4 Project Impact

6.4.1 Economics

When looking to market and promote Fibrina, the team targeted the high-end commercial market, which include store brand names, such as Philosophy, that contribute to the overall growth of the store brand market economy. Fibrina is a niche product being introduced to cosmetics and can have an impact in increasing economic growth if implemented. The protein itself does not cost much to manufacture and should not have a major effect on economy.

6.4.2 Environmental Impact

The production process of Fibrina is an environmentally friendly process. The designed mechanism that will be used for the production of Fibrin-based particles for our product does not require any environmentally damaging components.

To obtain solutions for our product, livestock may be used. If the process of obtaining materials for our product caused the killing of livestock, these are less wild-life animals that positively contribute to the environmental world.

6.4.3 Societal Influence

Through sales and marketing, our product has the potential to disrupt existing products and their performance on the market. These disruptions include causing consumers to be more alert in avidly fact checking their products. This knowledge will help customers understand if the ingredients used are safe, effective, and of an all-natural source. Within certain cultures and nations, bovine-sourced products are deemed immoral or unsafe. Therefore, Fibrina would have to switch over to human-sourced materials.

6.4.4 Political Ramifications

Due to the nature of this project, the only political ramifications could be in relation to adhering to FDA labeling standard regulations. As a governing body, the FDA does not have jurisdiction over the manufacturing of cosmetic products. However, it can regulate the labels and packaging of cosmetic products in order to ensure transparency for the customer.

6.4.5 Ethical Concerns

The ethical concerns behind using foreign body proteins as ingredients within topical creams seem minimal if at all existing. This product, however, is a problem for customers who are actively against the use of livestock as a resource for creating the product. Animal rights activists are very firm in their beliefs against the use of animals for cosmetic experiments. While

the team does not directly work with the animals, the team uses animals' drained blood as a source of the fibrin, which could be deemed cruel by some consumers.

6.4.6 Health and Safety Issues

As a product made for cosmetic use, Fibrina is currently being testing to maintain the safety and healthiness of any users of the product is of the upmost importance. Given the nature of our product and where it comes from, guidelines must be followed to ensure contamination/disease free product. There have existed outbreaks of disease which originated from bovine sources. As long as safety precautions are followed, and quality inspections are performed, compromises in consumer health through the result of delivering unsafe product can be easily averted. Due to the history of incidences with contaminated biological materials finding their way to ordinary people, there are many regulations out there which now prevent such things from reoccurring. The team plans to follow those regulations accordingly to ensure good health and safety of future consumers.

6.4.7 Manufacturability

For designers and engineers with the knowledge of manufacturability and machining, assembling a machine that can extrude fibrin microthreads would not be a difficult task. In addition, proficient programmers can alter the machine's automation capabilities, allowing it to extrude at different speeds. This will allow the machine to produce the desired amount of threads depending on the application and need of the user.

6.4.8 Sustainability

The machine will save time and money through its use to produce fibrin microthreads in a faster amount of time. While the amount of resources utilized will still be the same, the reduction in time spent making threads will help speed up the time it takes to plan and execute

laboratory experiments and produce Fibrina itself. It will also reduce the need for continuous manual labor, eliminating the need for additional laboratory assistants.

Chapter 7: Discussion

7.1 Fibrina

7.1.1 The Fibrina Business Model

The team gathered plenty of insight regarding the commercial prospect of Fibrina through the interviews conducted back toward the beginning of the year. The team also gathered insights through a number of presentations and contacts made later on in the second half of the year. Fibrina as a business stands to take two possible positions. One position would be as a licensor for the intellectual property that is fibrin-based particles being used in cosmetic compositions. The other position is as a main manufacturer/distributor of the product itself: additive-infused fibrin particles.

Regardless of what position is taken, marketing is going to be an incredibly large part of the success of Fibrina. Marketing can be approached in a number of ways. One recommendation that was brought up was the idea of partnering with tier-two cosmetics companies looking for an edge in the cosmetics market. By tier-two, the team means a company that is not one of the cosmetics giants (Olay, L'Oréal, etc.), but a company that does have some existing reputation with good brands and an ambition to grow. Teaming up with such a company could benefit Fibrina by allowing Fibrina to be labelled in concurrence with an already reputable name.

The concept of working with multiple companies becomes problematic as it would make sense that a single company would want to exclusively have access to Fibrina. That being said, fibrin within the cosmetics world would likely not last long without continuous innovation through the form of research and development. This is where the existence of a company around Fibrina would be beneficial, given that continued academic research through WPI cannot continue if its purposes are directed toward commercial prospect.

More interviews can always be done, especially follow-up interviews with many of the subject matter experts in the marketing and wholesale manufacturer/distributor fields. At this point, interview objectives would likely lead more in the direction of understanding costs relating to marketing and distribution.

It was fairly surprising to see the number of pivots made by the team on what Fibrina would exist as and how it would enter the cosmetics market. With the original intent of creating the entire cream, then jumping back and forth between dermatology and general consumer, the team had to consider many ideas and perspectives. This was done without having a complete base understanding of fibrin-based particles and their capabilities with containment and subsequent release of additives. Ultimately, the easy part of all these pivots was the change in perspective on how fibrin could be seen in cosmetics. The difficult part of making the pivots became understanding how to prove fibrin's value with each pivot.

The team quickly picked up on the importance of scientific validation and proven effectiveness. It was discussed at nearly every professional interview conducted. At more than one point, the team witnessed several general consumers searching product ingredients on their smart phones to learn more about what ingredients had positive backing. Those search results significantly affected their decision making. From the professional world, proven effectiveness came down to two major things: the objective finding, and the sample size of the conducted study. Without question, scientific validation and proven effectiveness remained a well understood need for the future of Fibrina.

Through all the interviews conducted, one understanding failed to make it to the team: how does one show the science behind a product. It would make sense that the answer to this question lies within the research and development of a cosmetics company. Such information

was not easy to come by, and even when trying to make it the primary objective to learn such information, the team was stumped. Literature provided some insight, but not a lot. The team was limited in its capabilities given the small budget for the project. Because of this difficulty, many improvisations were made. Such improvisations become more visibly clear in the discussion on experimentation and learning.

7.1.2 Fibrina Experimentation and Learning

As a team with zero prior experience or knowledge within the cosmetics realm, many if not all of the experiments and learnings gathered throughout this project were a result of trial and error. Assessing the experiments done for the validity was in itself a challenge. Information and knowledge which the team lacked could've significantly helped the process of experimentation overall.

The core triumph made by the team was the success in devising concepts for experiments to show scientific validity. Each of the proposed experiment types, starting from cream composition testing all the way to diffusion, were designed to help the team learn something in benefit to Fibrina. Each experiment added value to Fibrina. Ultimately, much more was learned from failure on the way to accomplishing the experiments.

7.1.3 Composition

Beginning with the cream composition experimentation, a major success came in building a baseline skin cream that was both cheap and easily producible. From there, the next step was throwing fibrin particles within the cream. That's where the team ran into trouble. There was no way of testing how fibrin reacted to the oil and/or water phases of the cream, if it was reacting at all. From an isolation standpoint, it appeared difficult to separate fibrin particles out of a cream for testing purposes. Outside of the testing obstacle that existed with this experiment, the process of tinkering with the base cream was simple.

7.1.4 Additive-Infused Threads

Moving on with the additive-infused fibrin experiments, the many successes and setbacks came from mostly trial and error. When it came to using any kind of additive in any kind of form, the initial tests always go through the same two questions:

1. Does this mix with fibrinogen and/or thrombin solution?
2. Can threads form under the extrusion process and be extracted successfully?

Through many of these attempts, a number of aliquots of fibrinogen and thrombin were used. Many drawn threads were not extractable. Syringe tips and tubing had to be thrown out when pre-clotting occurred during extrusion. In the worst case scenario, the pre-clotting could occur within the blending applicator. The team considered all these results a cost of figuring out what works.

The team successfully managed to make threads using retinol, and is confident that more types can be extruded. With practice, and luck, the team was even able to produce some higher percent-concentration additive-infused threads. However, it is clear that the need for consistently well drawn threads should not be solely relied upon by skilled hands.

One seemingly unanswerable question that always came up: how much of the additive is actually getting trapped within the threads? After all, the extrusion process is not so much an encapsulating process in itself, where the additives are in the center of the "fibrin molecule mesh". How much of the additive simply falls into the HEPES solution upon entering the tray? A sub-experiment was performed last-minute in an attempt to solve this. Unfortunately, the results of said experiment turned to be inconclusive. Above all this, there was the question of what interactions between additives and fibrinogen molecules were occurring. This was somewhat

being addressed by the results of the degradation experiment, however much was left unanswered.

Because of curiosity over the interaction between the fibrinogen molecules and the introduced additives, the team sought the opinion of an expert in the biochemistry field. Protein binding sites are nearly always hydrophobic in nature. This makes sense considering the largely aqueous environment that is the human body. Individual fibrinogen molecules, once cleaved by thrombin, will naturally look to bind with the hydrophobic sites of other fibrinogen molecules. With this knowledge, the team developed a yet untested theory: small hydrophobic molecules such as retinol may find themselves trapped between the hydrophobic binding sites of the fibrinogen molecules. This can result in poor binding between fibrinogen molecules, which leads to more difficulty in extracting/removing threads from the HEPES solution. The team spent, and sometimes wasted, hours trying to produce quality batches with high amounts of retinol (20% concentration), with much failure. Should this theory be correct, then any inability to extract those threads was not a result of their extracting approach, but of the more poorly bound threads.

7.1.5 Degradation

The results of the exploratory degradation experiment positively highlighted the capabilities of degrading fibrin using natural enzymes. However, when it came to performing the UV spectroscopy analysis experiment, the results failed to explain whether it is possible to control the degradation and release of fibrin and its infused additives respectively. Looking at the data from the standard curve and comparing it to the values obtained from the degradation of the retinol-infused fibrin, the team goes back to the question presented in the previous section: where are the additives going? Throughout every step of the manufacturing process, as well as throughout the experiment, there was a chance for loss of additive.

From the experimental process, the team noted a lot of difficulty in stabilizing and moving fibrin-based particles, mainly because of static cling. The team also with fairly small quantities of fibrin particles at a time, to avoid having to produce excessive amount of thread batches. The result was a constant struggle in handling small amounts of particles that needed to be transferred from one container to another. Within that struggle, there was chance for loss.

7.1.6 Surface Topography

With regards to using atomic force microscopy for quantifying surface adhesion, the team was initially disappointed upon learning the time, practice, and education needed to use AFM to acquire such data. However, it was understood that reaching the capabilities to perform surface adhesion measurements would become future work. Meanwhile, there was much inclination to at least start with basic surface imaging for fibrin-based particles. The reason why goes back to the application of those particles. Within the skin cream industry, there exists a variety of products and treatments which use abrasion, by either mechanical or chemical means, to improve the absorption of active ingredients through the skin. Mechanically speaking, such methods of abrasion came in the form of brushes/abrasive tools, and microbeads, which became infamous for their impact on the environment. The team theorized that given the right method of grinding, it would be possible to create fibrin-based particles that would have beneficial abrasive properties. Such properties would aid in removing the top layer of skin, the stratum corneum, which would benefit a user via exfoliation and an increased penetration of actives through to the dermis.

7.1.9 Assessing/Validating the Experimental Processes

Seeing as the team had no major experience in the science behind validating a skin cream prior to this year, there was a lot of discussion on the validity of the experiments performed. Needless to say, many of the experiment proposed were adapted from background information

learned, alongside a simple willingness to test many of the team's assumptions about what fibrin could do for skin cream. Ultimately, the team felt satisfied with the experimental processes that were decided upon, but it wouldn't hurt to have an expert opinion on the choices. There was a lot of attempt at finding contacts both during the customer discovery phase of this project, as well as through personal relationships among the team members, to find knowledge about some of the testing that goes on in the cosmetics industry. With much digression, the team was not very successful in finding such a professional.

7.1.10 Future Considerations

A more extensive cosmetology background could have aided the team's approach to the experiments for this project. Existing insight into biochemistry could have shed light on the complications that occurred during fibrin microthread production. This project, from the experimental perspective, turned out to be much less of a biomedical or mechanical engineering endeavor, and more of a biochemical one. Nevertheless, the team's biomedical engineering background proved to be quite useful in solving the day-to-day complications and failures that came and went with experimentation.

With the commercial, mechanical, and biomedical/biochemical approaches all tackled together in succession by the same team, a lot was learned. With the multidisciplinary experiences propagating question after question within each of the approaches, the team was both satisfied yet eager to see the project come to close.

7.2 The Machine Design

The team developed and assembled a machine that could automate the extrusion and stretching phases of the fibrin microthread extrusion process. However, due to time constraints, the final design requires the manual removal of the threads.

7.2.1 Design and Assembly of the Machine

When developing concepts for the machine, the team had many ideas. The team developed concepts such as a long, continuous conveyor that can extrude threads and a condensed conveyor that can extrude threads into a bath. The idea was to automate the entire extrusion process. However, due to time constraints, the team decided to focus on automating the first four phases of the extrusion process: mixing of the fibrinogen and thrombin, extrusion of the fibrin, stretching of the microthreads, and the drying of the threads during the automation process. The last phase, removal, will need to be furthered researched, but the team decided that utilizing a cutting mechanism to remove the threads as they are collected in a bin below the machine is the best way of going about this process.

Due to time constraints, the team could not design and build devices for all parts of the machine. For the pumping phase, the team utilized the pump in the lab instead of building a new device that could be integrated into our design. While the pump performed the task of mixing the fibrinogen and thrombin, the team could have designed a smaller device that would perform the same task and be better integrated into the design, saving lab bench space and laboratory resources.

When designing the components of the tub, conveyor, and clamps, the team ran into issues that slowed down production of the machine. As the team began to assemble the clamps, it was discovered that original measurements of the tub and conveyor design needed to be altered. Both the tub and conveyor were originally measured to a length of 13 inches. After assembling the 44 clamps, the team decided to extend the length of the bath to 24.5 inches to allow the clamps enough time to close and the threads to crosslink in the HEPES solution. For the tub, the team decided to use strong glue and sealant to put the acrylic pieces together. Another option

was cutting slots to fit the pieces of the bath together, but the team found the glue and sealant to be strong alternatives.

The conveyor, however, required more alterations. The base of the conveyor was assembled and fit in the bath space, with the respective PVC tubs in place. However, finding a way to connect the drive shaft the stepper motor was an issue. One design that the team tried to alleviate this issue was to create pulleys to be placed on the chain drive shaft and the drive roller for the conveyor belt. A XL timing belt could then be placed within these pulleys, such that when a stepper motor drives the chain, the drive roller would move in tandem. Unfortunately, this design did not work due to a clearance issue with the tub. In the future, the tub would need to be redesigned to accommodate the timing belt, or there would need to be a deeper angle between the conveyor and the chain draft shaft to alleviate the clearance issue.

The current final design, due to the issue with coupling the conveyor and chain drive shaft, does not have a conveyor belt that is functional. In the future, the clearance issue would need to be alleviated, or a third stepper motor could be mounted on the mechanism to drive the conveyor separately from the stepper motor that drives the chain, and the stepper motor that drives the extruder head.

Altogether, despite the lack of functionality of the conveyor belt, the mechanics of the device operate as was designed. The Arduino for the stepper motor allows for the chain and extruder to move to the exact specifications that are needed for Fibrin Microthread production. In order to make the machine completely functional, a few steps moving forward would be to make the conveyor functional, improve the quality of the ABS plastic clamps, and to finalize a method of guiding the clamps closed upon lift from the tub. Once these further goals are met, the team

will be able to efficiently test the machine for its ability to produce Fibrin Microthreads to the specifications that are desirable for our Fibrina product.

7.3 Conversion of Fibrin Microthreads

The team found methods to convert the fibrin microthreads to particle form and created a mechanism to make the conversion process more accurate. The team ran into issues when finding materials to convert the fibrin microthreads because when converted to a particle form, the fibrin goes through the phenomenon of static cling. When coming up with methods for conversion, the team ran into problems because methods with large surface areas made it harder to collect the fibrin particles due to their tendency to attach to the surfaces of the object. Due to time constraints and budget, the team was limited in terms of the complexity and materials used to make the mechanism. Therefore, when coming up with designs and weighing the advantages and disadvantages of each, the team had to consider the weighed objectives when making a final decision in terms of which design was most feasible to manufacture in a short period of time.

Chapter 8: Conclusions and Recommendations

8.1 Conclusion

The purpose of the team's project was to provide a means for incorporating fibrin as an ingredient into cosmetic cream, and to develop a unique manufacturing process to automate the production of fibrin-based particles. Through involvement in the WPI Accelerate Program, the team assessed the cosmetic industry to create a business model that would include delivering a cosmetic cream that incorporates fibrin as a time-release mechanism for already industry popular active ingredients, including retinol and glycolic acid. Customer discovery interviews and market research determined that the cosmetic industry has a continual need for new, scientifically tested cream compositions, and that a novel major ingredient has not been introduced into the market since the emergence of Collagen in the market over a decade ago. From these discoveries, the team developed a preliminary prototype of a fibrin-based skin cream which the team named Fibrina.

Further, the team did extensive research into validating fibrin's value as an ingredient in topical creams. Laboratory research conducted included diffusion, adhesion, and degradation assays of fibrin. Through these experiments, the percentages of ingredients in the cream may be determined, including the percentages of additive ingredients and fibrin, in order to enable the cream to formulate well, and have intended effect on its application surface. Further, the experiments educated the team about the properties of fibrin, which assisted in determining methods of incorporating active ingredients within fibrin. For instance, the degradation experiment proved that fibrin may degrade in bulk on a surface when activated with an enzyme.

In order to create fibrin particles for skin cream composition, the team developed a novel manufacturing process that enabled incorporation additives within fibrin through an automated

process. By utilizing previous WPI and VitaThreads intellectual property protection on the production of fibrin microthreads, the team was able to develop an automated process of producing infused fibrin microthreads. From this production process, the threads may then be grinded and incorporated into the topical cream. The team had to test mechanical properties of fibrin microthreads to determine how to allow the threads to effectively polymerize, stretch, and be grinded into smaller particles. The design process was followed in determining different preliminary designs, a final preliminary design, and an operating prototype that utilizes a chain based system run by a programmed stepper motor. Effectively automating the manufacturing process of fibrin-based particles for the skin cream was essential in alleviating the timely burden of manually formulating threads and grinding them into small particles.

The team's various experiments, Fibrina prototype, and extrusion mechanism prototype had promising results for the future of the product. Although the working prototypes and experiments met the team's original project objectives, there are further considerations that need to be made in order to progress the development of Fibrina and its manufacturing process.

With respect to the manufacturing process, the mechanism needs to be further worked with to improve aspects of the process, such as clamping the threads and a novel means for collecting the threads from the mechanism. Further, the mechanism, if used for manufacturing and mass producing the fibrin-base particles for the cosmetic cream, will need to be designed with materials and pumps for large scale application. Finally, the process of grinding will need to be eventually completely automated, or incorporated within the design of the machine.

In the realm of the cream composition, degradation and surface topography experiments were successful in their completion and acquirement of useful data. However, they have far more potential in data acquisition if they were to be explored further. The diffusion and UV

absorbance experiments present great opportunities for representing industry standard testing and added beneficial effects for Fibrina, respectively.

There is a lot to gain in expanding the knowledge fields surrounding the Fibrina experiments. A more in-depth understanding of biochemistry, atomic force microscopy, and cosmetology are but examples of subjects that would be greatly suited to improve upon this project's work. The resulting data from the Fibrina experiments presents a positive step in the future of Fibrina's use in the cosmetics skin cream industry. However, such experiments need to be repeated for further validation. Future experiments and opportunities are organized in the following recommendations chapter.

8.2 Recommendations

8.1.1 Future of Fibrina

8.1.1a Composition

As mentioned in the discussion of the composition experiment, the team would like to see future endeavors with the composition experiment as they pertain to studying the interactions between fibrin-based particles and the cream itself. Other potential future work is testing the effects of different types of skin cream ingredients with fibrin-based particles. Lastly, opportunities with introducing additive-infused fibrin-based particles within the composition differently should be explored.

Questions surrounding fibrin-based particles being used in an oil/water cream can be addressed better if the interactions between the two can be observed and understood. Both the oil and water phases of the cream are likely to have different types of interactions with the fibrin-based particles. The team recommends looking into ways of conducting those observations. One idea that was proposed involves using chemical markers within the fibrin that can be seen with

the naked eye. Think dyes. With markers entrapped within the fibrin, one could create a batch of cream with the marked fibrin particles, and squish the cream between two plates. The "sample" should be thin enough to see the marked fibrin particles, which can then be observed over time to see what interactions are happening. Note that this proposal addresses the oil and water phases together, but not separately.

Another proposed experiment to see the interactions of the oil and water phase separately as they interact with fibrin simply involve creating the two phases separately and conducting an observation experiment using markers which clearly identify the fibrin particles within. For the oil phase alone, the team already proposed the marker experiment in the previous paragraph. For the water phase, the team recommends running a process similar to the exploratory degradation experiment.

There a plenty of skin creams available. Each cream has its own list of ingredients and manner in which it is produced. With regards to the different types of ingredients, it may be interesting to explore the types of interactions fibrin-based particles can have with say various oils, preservatives, emollient systems, and so on. There may be certain ingredients which fibrin works even better with, or not at all. When it comes down to working with a partner who expertizes in making skin cream, such information may be vital to having the cream and this technology combine successfully.

Advancements in the manufacturing of moisturizers and other creams have shown that there are various methods by which creams can be made. Such advancements go so far as to create oil-in-water-in-oil systems, vice-versa systems, and more. The process by which the team created a basic moisturizer made use of a lot of heat. It is well known that a high enough temperature will cause proteins to denature. Nevertheless, there may be ways to encapsulate

additive-infused fibrin in these phases within phases. The team acknowledges that this kind of experimenting would be far out of the comfort zone of those who aren't experts in creating emollient systems of such complexity, but it may prove to be worthwhile in the far future.

8.1.1b Additives in Fibrin-Based Particles

The team wants to see further experimentation of the various additives which can be introduced into fibrin threads. Said additives need to be experimented in all the various applicable forms. Experimentation with using multiple additives within a single fibrin batch is imperative. Finally, different ways of encapsulating or binding additives to fibrin-based threads or particles should be considered.

The team never experimented outside of using solutions/liquids which contained the additives that ideally needed to be incorporated into threads. Initial presumptions ran that lyophilized powder or other solid-like particles being pre-mixed into the fibrinogen and/or thrombin solutions would be in inactive states, and therefore useless to integrate fibrin-based particles in those forms. The team did not have the in-depth knowledge to say whether this was true or not. Nevertheless, it is being recommended to try. Oil-based additives are another opportunity outside of powders. With either approach, issues relating to pre-clotting and clotting mid-extrusion should be kept in mind.

Seeing as only a small concentration of a single additive has been successfully infused into a single fibrin batch, the team is curious to see how more additive, and how multiple additives, can be successfully infused. The team expects that well automated design can account for the imperfect manual drawings of threads, which is presumed to be a major cause of thread extraction issues. Outside of automation, other opportunities exist within redesigning the manner in which additives are introduced into threads.

One such opportunity would be to incorporate the additives at a different time then during the pre-mixing. When fibrin threads were drawn particularly slow, it was consistently noted that threads would sink to the bottom of the pan and then spread out, forming these “flat threads”. When these threads are lifted from the pan, the outer edges of the flat threads would converge toward the center of the thread, forming that familiar cylindrical shape that ultimately becomes a dried thread. The team recommends looking into the possibility of introducing additives just before lifting those flat threads. If the outer edges of the thread are able to wrap over the additives, then it is likely that the additive will be encapsulated.

Such a process gets around issues with additives interfering with the fibrinogen monomers binding to each other, as most of the fibrinogen monomers will have already formed those bonds. From a negative outlook, this introduction of additives at a later step in the thread formation process becomes an additional step in manufacturing, resulting in another complexity added to automation. Pros and cons should be weighed on the capacity for each thread to hold more additives versus the addition of another manufacturing step.

8.1.1c Degradation

The controlled degradation of fibrin particles needs to be understood further for the sake of commercial application. The experiments conducted by the team during this project should be repeated to determine accuracy in the team’s initial results. From there, improvements in degradation control should be explored. Finally, opportunities in releasing additives from fibrin particles outside of degradation should be considered.

Repeated results of the release outcomes of degraded fibrin-based particles are essential to validating the commercial capabilities for fibrin in the skin cream industry as the team currently sees it. Retinol and other additives which can be quantified through UV spectroscopy

due to their molecular structure should be quantified using UV spectroscopy so long as that works for the degradation experiments being conducted. However, for other additives which don’t work as easily with UV spectroscopy, the team recommends finding ways of tracking their release.

It could be argued that rather than tracking the release, one could track the degradation of fibrin within an experiment, and base the overall degradation of fibrin to the overall release of additives. This could theoretically work if the assumption of uniform distribution of additives across the fibrin particles can be proven as true. However, the tracking of degradative fibrin assumes that the only way for additives to be released is via degradation.

One theory that was discussed with the biochemistry expert was the concept of using polarity to egress additives from fibrin particles. This concept completely delineates from using degradation, although the team is unsure of how that would work for the commercial application of fibrin particles. The team was unable to explore this concept any farther than that short conversation. With that, a recommendation has been set to explore the capabilities of playing with polarity and the release of actives within fibrin.

Going back to quantifying fibrin degradation, one way of measuring the degradation rate of fibrin itself within a skin cream would be to use antibodies. When fibrin is proteolytically degraded within the body by plasmin, it is cut up into smaller protein pieces that together are called fibrin degradation products (FDPs). FDP-specific antibodies exist and can be purchased. Over time, as fibrin-based particles are degraded through the application of an enzyme, samples can be taken. Said samples could be mixed with antibody receptors. The resulting antibody receptor attachment to the FDPs could be quantified to show the rate at which fibrin is degrading

within the cream. The team does want to note that such an approach with antibodies would be costly.

8.1.1d Surface Adhesion using AFM

With the results of the surface topography images done on the fibrin particles with AFM, the team wants to ultimately see the surface adhesion measurements that were originally sought after. Atomic Force Microscopy represents a unique dive into the properties of fibrin overall. Within the application of fibrin-based particles, there is definitely potential in tying adhesion properties with proving fibrin's ability to stay on skin, and even exfoliate it. While it would take some considerable amount of effort to reach the level of proficiency with AFM required to perform such an experiment, the team places this recommendation with hope that it will be followed through.

8.1.1e Diffusion

It is expected that with time, the core understandings of fibrin-based particles and their role in cosmetics skin cream can become prominent enough for there to be testing done that is equivalent to industry standards. Among those industry standards in testing is diffusion through a quality Franz Diffusion Chamber. Although such an experiment is costly, the team recommends performing a diffusion assay with the proper equipment and materials, as that would showcase fibrin's capabilities of making a major impact in the skin cream industry.

Such an experiment would likely aim to show fibrin's inability to pass through skin, but at the same time show that the actives previously trapped within the fibrin make their way through the skin sample. This would highlight the claims made by the team, that fibrin itself is acting solely as cosmetics agent that promptly carries actives to the skin in a controlled release manner.

8.1.1f Testing Fibrina on Human Subjects

All of the experiments and their future recommendations are likely to be excellent opportunities to showcase fibrin's potential in skin care. However, the final hurdle for fibrin-based particles is going to be actual testing on human subjects. This study is absolutely vital, according to nearly every dermatologist the team spoke with during the project's customer discovery phase. A study such as this will prove to be difficult to conduct, given the intensive requirements of sample size, safety measures, a control over potential variable factors, and the overall time which the study would take.

Thankfully, a lot of the procedures on how to go about performing a study of this nature can be found in the book which was used by the team for background section on cosmetology. The focus of this study would primarily target the comparison between fibrin-based skin cream and a control group. The team recommends having the control group be applied to one of the subject's arm, and the fibrin-based cream on the other, for each of the subjects. This would provide an ideal side-by-side comparison. The book provides testing procedures that can be used to quantify the deliverance of actives into the skin on a living subject. The most notable of these testing procedures is Raman Spectroscopy.

8.1.1g Moving Forward with Fibrina

Looking at Fibrina as a future business, there are several action items which the team suggests be accomplished first. These actions are to gather more experimental data, find a key partner, learn more about Fibrina's supplier and the supplier network, and finally raise capital through finding funds.

1: Identifying Key Partners

It would take years for Fibrina to develop its own competitive line of skin cream. Everything from the manufacturing process to the lack of brand reputation becomes a barrier to

entry within the market. Rather than work through these barriers to entry, the team suggests finding a key partner, an up and coming skin cream company that would be interested in introducing additive infused fibrin-based particles into their existing products. This partnership benefits both parties very well. For Fibrina, the benefits of selling through an existing brand with a positive reputation helps get Fibrina out into the market much more quickly. For the partnering company, Fibrina presents itself as a new ingredient that can be mixed into existing products, and new products. The marketing potential with Fibrina is quite substantial.

2: Understanding partner network

As of right now, fibrinogen and thrombin solutions are purchased through MP Biomedical. MP Biomedical produces their fibrinogen and thrombin solutions through blood collection from a closed herd that exists in New Zealand. Should Fibrina grow over time, their demand for fibrinogen and thrombin will likely grow. With that, close relationships with MP Biomedical will help ensure that supplier connection. Other supplier network possibilities should be explored as well to prevent issues resulting in a lack of supplier competition.

3: Finding Funds

As a startup, Fibrina needs to find funds. There exists a number of opportunities within the area. These opportunities range from pitch competitions to local accelerator programs. The team was recommended to look into the I-Corp Program. The WPI accelerator program which the team participated in B term of 2016 was considered to be the miniature version of the I-Corp Program. Ultimately capital raising may end up coming from investors, which again would be found through programs, competitions, and overall networking.

8.2.2 Machine Recommendations

Based on the prototype of the final design, the team identified areas in which the machine must be further tested and adjusted. One aspect that will need to be tested is the extrusion rate of

the thread on a moving conveyor. Since the conveyor will be moving at an incremental speed, the rate of extrusion of the thread, and the location of extrusion may need to account for the movement of the conveyor to ensure threads are drawn completely linearly. The importance of drawing the threads completely linearly is to allow for the maximum amount of threads to fit on the conveyor. The clamping system was a big part in allowing for linear extrusion. Figures 48 and 49 below show the prototype version of one clamp in an open and closed position. In order to maximize thread production, the surface of the clamps on the machine will need to be changed in future alterations and models of the machine.

Figure 48: Clamp made through rapid prototyping in the open position

Figure 49: Clamp made through rapid prototyping in the closed position

In order to maximize mass production of the threads, the clamps will need to hold multiple threads. Since only so many chain plates can fit within the length of the device (assuming we continue to have the device fit on a lab bench), the surface of the clamps will need to be maximized. It should be tested if the bottom surface of the first part of the clamps will be able to hold up to three threads when clamped. Further, a means will need to be developed to force the magnets toward and away from one another. A servo may be stationed in the device at a certain point in the HEPES tub to close the clamps before the threads are lifted out of the tub toward the stretching plate. Finally, the material of the clamps will need to be tested. The ABS plastic is a fragile material to drill and pin the parts together. The material selected will need to

be strong enough to not have the clamps break during assembly and offer a reliable material for threads to be extruded on.

Figure 50: Stretching platform prototype made of birch plywood

Future considerations for the stretching plate (Figure 50) will be to replace the wood with a material that will be both durable and smooth. Additionally, the concept of the stretching plate will need to be tested to ensure that threads are spread apart at an incremental rate that avoids breaking. Further, the current design leads the chain back to a sprocket mounted on the frame of the machine. As the threads dry, the threads are placed closer together to the original length. However, if the threads are too brittle after drying, there is a risk of the threads breaking instead of hanging. The design may need to be adjusted to allow the threads to stay at their stretched length throughout the remainder of the device. The final Sprockets at the end of the chain cycle near the head of the tub should also be replaced with larger sprockets, so the magnets from the clamps do not attract each other, breaking the automated cycle.

Finally, the means for collecting the threads after they are dried will need to be tested. The current options are to either cut the threads from the clamps, or open the clamps and allow them to fall into a collection bin or a grinder. We will need to test how the threads will fall, and need to make sure that threads do not adhere to the surface of the clamps in a way to prevent the

threads from falling. Additionally, options on how to automate grinding the threads would be a good addition to the machine.

8.2.3 Recommendations for the Conversion of Fibrin Microthreads

To allow for better conversion of the fibrin particles, the team recommends to automate the conversion mechanism, while addressing the issues in relation to static cling of the fibrin microthreads to surface of the funnel before conversion via the automated dual action trimming blades. The team also recommends to add the fibrin microthread conversion process and mechanism to the automated machine, so there is continuous production of fibrin for its application in a skin cream.

References

[1] D. Goodsell, “Fibrin”, Protein Database, 2006.

[2] Z. Tao, J. Favreau, J. Guyette, K. Hansen, J. Lessard, E. Burford, G. Pins and G. Gaudette, "Delivering stem cells to the healthy heart on biological sutures: effects on regional mechanical function", Journal of Tissue Engineering and Regenerative Medicine, p. n/a-n/a, 2014.

[3] Eye of Science/Photo Researchers, Inc., Red blood cells (erythrocytes) trapped in a mesh of fibrin threads. Fibrin, a tough, insoluble …. 2017.

[4] E. Ryan, L. Mockros, J. Weisel and L. Lorand, "Structural Origins of Fibrin Clot Rheology", *Biophysical Journal*, vol. 77, no. 5, pp. 2813-2826, 1999.

[5] T. Ahmed, E. Dare and M. Hincke, "Fibrin: A Versatile Scaffold for Tissue Engineering Applications", *Tissue Engineering Part B: Reviews*, p. 110306231744007, 2008.

[6] W. Spotnitz, "Fibrin Sealant: The Only Approved Hemostat, Sealant, and Adhesive—a Laboratory and Clinical Perspective", *ISRN Surgery*, vol. 2014, pp. 1-28, 2014.

[7] P. Janmey, J. Winer and J. Weisel, "Fibrin gels and their clinical and bioengineering applications", *Journal of The Royal Society Interface*, vol. 6, no. 30, pp. 1-10, 2009.

[8] Food & Drug Administration, "FDA approves Raplixa to help control bleeding during surgery", 2016.

[9] M. Proulx, S. Carey, L. DiTroia, C. Jones, M. Fakharzadeh, J. Guyette, A. Clement, R. Orr, M. Rolle, G. Pins and G. Gaudette, "Fibrin microthreads support mesenchymal stem cell growth while maintaining differentiation potential", *Journal of Biomedical Materials Research Part A*, vol. 96, no. 2, pp. 301-312, 2010.

[10] M. O'Brien, M. Carnes, R. Page, G. Gaudette and G. Pins, "Designing Biopolymer Microthreads for Tissue Engineering and Regenerative Medicine", Curr Stem Cell Rep, vol. 2, no. 2, pp. 147-157, 2016.

[11] "VitaThreads | Fibrin Microthread Sutures - The Future of Wound Closure", *Vitathreads.us*, 2016. [Online]. Available: http://www.vitathreads.us/. [Accessed: 13- Oct-2016].

[12] K. Cornwell and G. Pins, "Discrete crosslinked fibrin microthread scaffolds for tissue regeneration", Journal of Biomedical Materials Research Part A, vol. 82, no. 1, pp. 104-112, 2007.

[13] K. G. Cornwell, G. D. Pins and K. Billiar, "Fibrin microthreads". World Patent Organization Patent WO 2007109137A1, 27 September 2007.

[14] G. Pins and S. Carey, "Biopolymer Microthreads with Microscale Surface Topographies". United States of America Patent US2013/0096610, 18 April 2013.

[15] G. Gaudette, G. D. Pins, M. Rolle, J. P. Guyette and K. Cornwall, "Microthread delivery system". United States of America Patent US 20150005739 A1, 1 January 2015.

[16] G. Pins, G. Gaudette, A. Collette and W. Edelman, "Absorbable fibrin microthread sutures in tissue litigation". World Patent Organization Patent WO 2015148993 A1, 1 October 2015.

[17] W. H. Kitchens, "FDA Regulatory Approval Process for Medical Products," Technological Innovation: Generating Economic Results, pp. 201-229, 2016.

[18] U.S. Food and Drug Administration (FDA), "Assessing User Fees: PMA Supplement Definitions, Modular PMA Fees, BLA and Efficacy Supplement Definitions, Bundling

Multiple Devices in a Single Application, and Fees for Combination Products," U.S. Food and Drug Administration, Silver Spring, MD, 2015.

[19] Food and Drug Administration, "HR.3590 Patient Protection and Affordable Care Act (Enrolled Bill [Final as Passed Both House and Senate] - ENR) Sec. 7002 Approval Pathway for Biosimilar Biological Products," 2009. [Online]. Available: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/ucm216146.pdf.

[20] Business Reference Services, “The Cosmetics Industry Today”, Business & Economics Research Advisor, Issue 17-18, 2010.

[21] FDA Office of Combination Products, "Guidance for Industry and FDA Staff: Current Good Manufacturing Practice Requirements for Combination Products," 2015. [Online]. Available: http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM429304.pdf. [Accessed October 2016].

[22] Business Reference Services, “The Cosmetics Industry Today”, Business & Economics Research Advisor, Issue 17-18, 2010.

[23] Food and Drug Administration, “Food Drug and Cosmetic Act (FD&C Act)”, Silver Spring, MD, 1938.

[24] CTPA, “Definition of a cosmetic”, EU Cosmetics Regulation, 2009 [Online]. Available: http://www.ctpa.org.uk/content.aspx?pageid=304 [Accessed Oct. 2016].

[25] Transparency Market Research, “Cosmetic Skin Care Market is projected to rise to US$200 bn by 2024; Anti-aging Products to Remain Most Valuable CaCategory: TMR Albany, NY, 2016.

[26] "Cosmetic Tubes Market - Global Industry Analysis, Size, Share, Growth, Trends, And Forecast 2017 – 2025 | Aotearoa Indymedia". *Indymedia.org.nz*. N.p., 2017. Web. Oct. 2016.

[27] "Skin Care and Cosmetics", Cleveland Clinic, Mar, 2015 [Online]. Available: http://my.clevelandclinic.org/health/healthy_living/hic_An_Overview_ofYour_Skin/hic_Understanding_the_Ingredients_in_Skin_Care_Products [Accessed Oc. 2016].

[28] V. Ronfard, A. Tuck and L. Wilkins, "Skin care compositions and treatments". United States of America Patent US 11761893, 18 10 2007.

[29] G. P. Martyn and J. Koopman, "Dry powder fibrin sealant". United States of America Patent US 9119897 B2, 1 September 2015.

[30] P. K. Farris, Cosmeceuticals and Cosmetic Practice, New Orleans: John Wiley & Sons, 2013.

[31] P. Elias, "Epidermal Lipids, Barrier Function, and Desquamation", *Journal of Investigative Dermatology*, vol. 80, no. 1, pp. 44s-49s, 1983.

[32] R. Potts, "Skin Barrier: Principles of Percutaneous Absorption", *Archives of Dermatology*, vol. 133, no. 7, p. 924, 1997.

[33] M. Robinson, M. Visscher, A. Laruffa and R. Wickett, "Abstracts: Natural moisturizing factors in the stratum corneum I. Effects of lipid extraction and soaking", International Journal of Cosmetic Science, vol. 32, no. 5, pp. 394-394, 2010.

[34] A. Rawlings and P. Matts, "Stratum Corneum Moisturization at the Molecular Level: An Update in Relation to the Dry Skin Cycle", *Journal of Investigative Dermatology*, vol. 124, no. 6, pp. 1099-1110, 2005.

[35] M. Lodén, "Effect of moisturizers on epidermal barrier function", *Clinics in Dermatology*, vol. 30, no. 3, pp. 286-296, 2012.

[36] R. Tyrrell, "Antioxidants in protection from UV-induced damage", *Pathophysiology*, vol. 5, p. 268, 1998.

[37] S. Pinnell, "Cutaneous photodamage, oxidative stress, and topical antioxidant protection", *Journal of the American Academy of Dermatology*, vol. 48, no. 1, pp. 1-22, 2003.

[38] Y. Suzuki, J. Koyama, J. Nomura, J. Sato and I. Horii, "The role of proteases in stratum corneum: Involvement in stratum corneum desquamation", *Journal of Dermatological Science*, vol. 6, no. 1, p. 58, 1993.

[39] "Mechanisms of natural moisturizing factors for skin hydration", *Journal of the American Academy of Dermatology*, vol. 60, no. 3, p. AB37, 2009.

[40] J. Arezzo, H. Schaumburg and P. Spencer, "Structure and Function of the Somatosensory System: A Neurotoxicological Perspective", *Environmental Health Perspectives*, vol. 44, p. 23, 1982.

[41] S. Ebner, "Expression of C-type lectin receptors by subsets of dendritic cells in human skin", *International Immunology*, vol. 16, no. 6, pp. 877-887, 2004.

[42] M. Nordin, "Low-threshold mechanoreceptive and nociceptive units with unmyelinated (C) fibres in the human supraorbital nerve.", *The Journal of Physiology*, vol. 426, no. 1, pp. 229-240, 1990.

[43] R. Johansson, M. Trulsson, K. Olsson and K. Westberg, "Mechanoreceptor activity from the human face and oral mucosa", *Experimental Brain Research*, vol. 72, no. 1, pp. 204-208, 1988.

[44] E. Jackson, "Natural Ingredients in Cosmetics", *American Journal of Contact Dermatitis*, vol. 5, no. 2, pp. 106-109, 1994.

[45] D. Cohen and J. Kaufmann, "Hypersensitivity reactions to products and devices in plastic surgery", *Facial Plastic Surgery Clinics of North America*, vol. 11, no. 2, pp. 253-265, 2003.

[46] H. Smith, D. Basketter and J. McFadden, "Irritant dermatitis, irritancy and its role in allergic contact dermatitis", *Clinical and Experimental Dermatology*, vol. 27, no. 2, pp. 138-146, 2002.

[47] A. Verma, C. Tancharoen, M. Tam and R. Nixon, "Pustular allergic contact dermatitis caused by fragrances", *Contact Dermatitis*, vol. 72, no. 4, pp. 245-248, 2015.

[48] D. Wetter, J. Yiannias, A. Prakash, M. Davis, S. Farmer and R. el-Azhary, "Results of patch testing to personal care product allergens in a standard series and a supplemental cosmetic series: An analysis of 945 patients from the Mayo Clinic Contact Dermatitis Group, 2000-2007", *Journal of the American Academy of Dermatology*, vol. 63, no. 5, pp. 789-798, 2010.

[49] E. Howard A., "Anatomy of a Moisturizer", *SKINmed*, vol. 3, no. 5, pp. 279-280, 2004.

[50] U. Kappes, "Skin ageing and wrinkles: clinical and photographic scoring", *Journal of Cosmetic Dermatology*, vol. 3, no. 1, pp. 23-25, 2004.

[51] H. Benson, "Transdermal Drug Delivery: Penetration Enhancement Techniques", *Current Drug Delivery*, vol. 2, no. 1, pp. 23-33, 2005.

[52] K. Moser, "Passive skin penetration enhancement and its quantification in vitro", *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 52, no. 2, pp. 103-112, 2001.

[53] M. Kirjavainen, A. Urtti, R. Valjakka-Koskela, J. Kiesvaara and J. Mönkkönen, "Liposome–skin interactions and their effects on the skin permeation of drugs", *European Journal of Pharmaceutical Sciences*, vol. 7, no. 4, pp. 279-286, 1999.

[54] G. Cevc, "Transfersomes, Liposomes and Other Lipid Suspensions on the Skin: Permeation Enhancement, Vesicle Penetration, and Transdermal Drug Delivery", *Critical Reviews™ in Therapeutic Drug Carrier Systems*, vol. 13, no. 3-4, pp. 257-388, 1996.

[55] A. BAILLIE, A. FLORENCE, L. HUME, G. MUIRHEAD and A. ROGERSON, "The preparation and properties of niosomes-non-ionic surfactant vesicles", *Journal of Pharmacy and Pharmacology*, vol. 37, no. 12, pp. 863-868, 1985.

[56] C. Schwarz, W. Mehnert, J. Lucks and R. Müller, "Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization", *Journal of Controlled Release*, vol. 30, no. 1, pp. 83-96, 1994.

[57] J. Whelan, "Nanocapsules for controlled drug delivery", *Drug Discovery Today*, vol. 6, no. 23, pp. 1183-1184, 2001.

[58] M. Dinno, L. Crum and J. Wu, "The effect of therapeutic ultrasound on electrophysiological parameters of frog skin", *Ultrasound in Medicine & Biology*, vol. 15, no. 5, pp. 461-470, 1989.

[59] K. De Paepe, A. Wibaux, C. Ward and V. Rogiers, "Skin Efficacy and Biophysical Assessment of Glycerol-Containing Hydrocolloid Patches", *Skin Pharmacology and Physiology*, vol. 22, no. 5, pp. 258-265, 2009.

[60] D. Roberts, "Transdermal Drug Delivery Using Iontophoresis and Phonophoresis", *Orthopaedic Nursing*, vol. 18, no. 3, pp. 50???54, 1999.

[61] Particle Sciences, *Franz Diffusion Cell Labeled Diagram*. 2009.

[62] S. Ng, J. Rouse, F. Sanderson, V. Meidan and G. Eccleston, "Validation of a Static Franz Diffusion Cell System for in Vitro Permeation Studies", *AAPS PharmSciTech*, vol. 11, no. 3, pp. 1432-1441, 2010.

[63] Emulsions and emulsion stability. (1996). *Choice Reviews Online*, *34*(03), 34-1554-34-1554. http://dx.doi.org/10.5860/choice.34-1554

[64] S. Hoeller, A. Sperger and C. Valenta, "Lecithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation", *International Journal of Pharmaceutics*, vol. 370, no. 1-2, pp. 181-186, 2009.

[65] Curry, H. (2016). The beauty of botanicals. *Science*, *351*(6271), 346-346. http://dx.doi.org/10.1126/science.aad9074

[66] Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses. (1999). *Choice Reviews Online*, *36*(07), 36-3905-36-3905. http://dx.doi.org/10.5860/choice.36-3905

[67] Reactive oxygen species and antioxidants — An overview. (2002). *Atherosclerosis Supplements*, *3*(2), 238. http://dx.doi.org/10.1016/s1567-5688(02)80793-0

[68] Inflammation and Antiinflammatories. (1979). *Annals of Internal Medicine*, *90*(4), 735. http://dx.doi.org/10.7326/0003-4819-90-4-735_2

[69] Vezzani, A., Bartfai, T., Bianchi, M., Rossetti, C., & French, J. (2011). Therapeutic potential of new antiinflammatory drugs. *Epilepsia*, *52*, 67-69. http://dx.doi.org/10.1111/j.1528-1167.2011.03242.x

[70] Peptides. (1984). *Peptides*, *5*(4), 845. http://dx.doi.org/10.1016/0196-9781(84)90035-4

[71] Elias, P. (1988). Structure and function of the stratum corneum permeability barrier. *Drug Development Research*, *13*(2-3), 97-105. http://dx.doi.org/10.1002/ddr.430130203

[72] Heng, M. (2011). Wound healing in adult skin: aiming for perfect regeneration. *International Journal of Dermatology*, *50*(9), 1058-1066. http://dx.doi.org/10.1111/j.1365-4632.2011.04940.x

[73] Human growth factor and cytokine containing skin cream for treatment of phototoxic skin reaction associated with photodynamic therapy. (2007). *Journal of The American Academy of Dermatology*, *56*(2), AB205. http://dx.doi.org/10.1016/j.jaad.2006.10.932

[74] Retinol. (2012). *Reactions Weekly*, *&NA;*(1405), 31-32. http://dx.doi.org/10.2165/00128415-201214050-00108

[75] Babamiri, K. & Nassab, R. (2010). Cosmeceuticals: The Evidence Behind the Retinoids. *Aesthetic Surgery Journal*, *30*(1), 74-77. http://dx.doi.org/10.1177/1090820x09360704

[76] WENDLING, P. (2005). Retinoid-Hyaluronate Connection Evaluated. *Skin & Allergy News*, *36*(11), 23. http://dx.doi.org/10.1016/s0037-6337(05)70804-4

[77] Maréchal, Y., Milas, M., & Rinaudo, M. (2003). Hydration of hyaluronan polysaccharide observed by IR spectrometry. III. Structure and mechanism of hydration. *Biopolymers*, *72*(3), 162-173. http://dx.doi.org/10.1002/bip.10343

[78] Creidi, P. & Humbert, P. (1999). Clinical Use of Topical Retinaldehyde on Photoaged Skin. *Dermatology*, *199*(1), 49-52. http://dx.doi.org/10.1159/000051379

[79] YOSHIMURA, K., MOMOSAWA, A., AIBA, E., SATO, K., MATSUMOTO, D., & MITOMA, Y. et al. (2003). Clinical Trial of Bleaching Treatment with 10% All-Trans Retinol Gel. *Dermatologic Surgery*, *29*(2), 155-160. http://dx.doi.org/10.1097/00042728-200302000-00009

[80] Segall, A. & Moyano, M. (2008). Stability of vitamin C derivatives in topical formulations containing lipoic acid, vitamins A and E. *International Journal of Cosmetic Science*, *30*(6), 453-458. http://dx.doi.org/10.1111/j.1468-2494.2008.00473.x

[81] Green, B. (2005). After 30 years … the future of hydroxyacids. *Journal of Cosmetic Dermatology*, *4*(1), 44-45. http://dx.doi.org/10.1111/j.1473-2165.2005.00159.x

[82] Van Scott, E., Ditre, C., & Yu, R. (1996). Alpha-hydroxyacids in the treatment of signs of photoaging. *Clinics in Dermatology*, *14*(2), 217-226. http://dx.doi.org/10.1016/0738-081x(95)00157-b

[83] A. Birch, S. Coffin, J. Perez-Rogers, H. Peruffo, "Design of an Automated Microthread Processing System", Worcester Polytechnic Institute, Worcester, MA, Rep. GXP-1201, Apr, 2012.

[84] C. Ellis, C. Serafin, P. Vasiliadis, C. Wood, "Design of a Multifunctional Semi-Automated Production System for Biopolymer Microthreads", Worcester Polytechnic Institute, Worcester, MA, Rep. GXP-1015, Apr, 2010.

[85] United States Food and Drug Administration, "New FDA Evaluates Regulated Products: Cosmetics", Silver Spring, MD, Sept, 2016.

[86] P. D. Kemp, R. M. Carr, Jr., J. G. Maresh, J. Cavallaro and J. Gross, "Collagen Threads". United States of America Patent US 5378469, 3 January 1995.

[87] International Standards Organization, ISO 8373:2012, ISO, 2012.

[88] International Standards Organization, ISO 9283:1998, ISO, 2012.

[89] International Standards Organization, ISO 3952-1:1981, ISO, 1981.

[90] "What is Static Cling? (with pictures)", *wiseGEEK*, 2017. [Online]. Available: http://www.wisegeek.org/what-is-static-cling.htm. [Accessed: 05- Mar- 2017].

[91] ("Patent US20060141046 - Particles for the delivery of active agents", *Google Books*, 2017. [Online]. Available: http://www.google.com/patents/US20060141046. [Accessed: 09- Mar- 2017])

[92] "Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability", *Sciencedirect.com*, 2017. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S1818087614000348. [Accessed: 09- Mar- 2017].

[93] Draelos, Z. (2016). *Cosmetic Dermatology* (2nd ed., p. 76). Wiley, Blackwell.

[94] "Basic moisturizer", *Instructables.com*, 2017. [Online]. Available: http://www.instructables.com/id/Basic-moisturizer/?ALLSTEPS. [Accessed: 03- Mar- 2017].

[95] P. Gaffney, "Fibrin Degradation Products", *Annals of the New York Academy of Sciences*, vol. 936, no. 1, 2001.

[96] M. Errasti, A. Prospitti, C. Viana, M. Gonzalez, M. Ramos, A. Rotelli and N. Caffini, "Effects on fibrinogen, fibrin, and blood coagulation of proteolytic extracts from fruits of Pseudananas macrodontes, Bromelia balansae, and B. hieronymi (Bromeliaceae) in comparison with bromelain", *Blood Coagulation & Fibrinolysis*, vol. 27, no. 4, pp. 441-449, 2016.

[97] "Effects of Nattokinase fibrinol supplementation on Fibrinolysis and Atherogenesis", *Journal of Life Science*, vol. 19, no. 2, pp. 289-298, 2009.

[98] E. Kotb, *Fibrinolytic Bacterial Enzymes with Thrombolytic Activity*, 1st ed. Berlin: Springer Berlin Heidelberg, 2012, pp. 1-74.

[99] C. Chen, E. Tsina, M. Carter Cornwall, R. Crouch, S. Vijayaraghavan and Y. Koutalos, "Reduction of All-trans Retinal to All-trans Retinol in the Outer Segments of Frog and Mouse Rod Photoreceptors", *Biophysical Journal*, vol. 88, no. 3, pp. 2278-2287, 2005.

[100] Tang, W. & Bhushan, B. (2010). Adhesion, friction and wear characterization of skin and skin cream using atomic force microscope. *Colloids and Surfaces B: Biointerfaces*, *76*(1), 1-15. http://dx.doi.org/10.1016/j.colsurfb.2009.09.039

[101] VitaThreads, “Fibrin Microthread Extrusion Logs”. Worcester, MA, 2012.

Appendices

Appendix I: Additive-Infused/Regular Fibrin Microthread Protocol

How to Make Threads

Materials:

- Baking Pan
- Syringe pump
- 1 aliquot fibrinogen - 70mg/ml
- 1 aliquot thrombin – 8U/200µl
- 10mM HEPES solution
- 2 - 1mL syringes
- 1 – 3ml syringe
- 1000mL graduated cylinder
- Clear cup of room temperature water
- 40mM $CaCl_2$
- 1000µl Micropipetter
- 2 sets of forceps
- Cardboard drying box
- Kimwipes
- Extruding tube
- Blending connector
- Polyethylene Tubing

Preparation:

1. Rinse graduated cylinder with DI water
2. Fill Graduated cylinder with ~350mL of 10mM HEPES solution. Transfer HEPES into silver baking pan
3. Obtain 1 aliquot of Fibrinogen and 1 aliquot of Thrombin from -30C freezer
4. Obtain 40mM $CaCl_2$ from fridge.
5. Defrost frozen aliquots by placing them into room temperature water
6. Once completely defrosted, add 850µL of 40mM $CaCl_2$ into the thrombin, mix thoroughly
7. Label two 1ml syringes, one as T and one as F
8. For a 5% special thread solution:
    a. Transfer 950ul of Thrombin and Fibrinogen into new microcentrifuge tubes
    b. Add 50ul of additive solution (ECM, retinol etc.) into microcentrifuge tube (Total V=1000 ul) and mix thoroughly

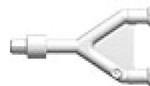
Percentage	Volume of Thrombin	Volume of Fibrinogen	Volume of ECM in T and F
5%	950 ul	950 ul	50 ul
10%	900 ul	900 ul	100 ul

15%	850 ul	850 ul	150 ul
20%	800 ul	800 ul	200 ul

9. Using 20 G syringe appropriate withdraw thrombin and fibrinogen into separate labeled syringes
10. Remove all air bubbles from syringe
11. Balance the two syringes so that they contain the same volume of solution

<u>Setting up Machine:</u>

1. Plug in syringe pump and turn the power on (Power switch is in the back of the machine)
2. Make sure **DIAMETER** is set to 4.699mm and the **RATE** is set to 0.23 mL/min.
3. Plug the Fibrinogen and Thrombin syringes into the blending connector. The Fibrinogen syringe should ALWAYS be placed into the side with the small circle



4. Place syringes into syringe pump.
   - Lift black knob and slide the ends of the syringes under black bar, splitting the two syringes with the knob. Make sure syringe body flanges are inserted in to clamp. Release black knob.
   - Tighten syringe clamp using the silver nuts.

5. Make syringe pump block flush with the syringe connector
   - Push down black button and pinch white piece
6. Attach polyethylene tubing to the free side of the blending connector

<u>Making Threads:</u>

1. Press start on the machine.
2. Wait for <u>ALL</u> the air bubbles to pass through the PE tubing. Use a Kimwipe to collect anything that comes out while air bubbles are passing through.

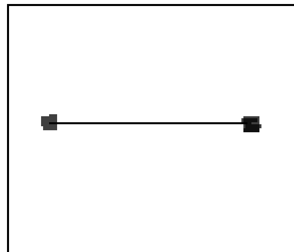
3. Once there is a constant flow, start making the threads.

Hold extruding tube ≈ 4 inches from the end. Drag end of tube horizontally along the bottom of the silver pan filled with 10mM HEPES. Move the tube from the left side of the pan to right side at a constant rate. At the end of one thread move quickly bring the end of the tube back to the left side of the pan, move ≈ 1 inch down and begin a new thread. Repeat until you cannot make anymore. *Make sure the end of the tube is clean and free of fibrin debris, wipe clean if needed

4. Do Not Overlap Threads!
5. Turn off machine once you are done.

Clean Up Part 1:
1. Immediately clean tube. Fill a 3ml syringes with water and flush extruding tube with water. Remove syringe from extruding tube, withdraw syringe with just air and blow air through extruding tube with syringe. Repeat with white co-extruder.
2. Throw away any used materials into the proper trash receptacle.

Transferring Threads:
1. Use the two sets of forceps to take the threads out of the pan.
   - Grab each end of the thread with the forceps
   - Move right hand counterclockwise and left hand clockwise so you can pick up the thread.
   - Make sure not to pull too fast and to keep threads in the HEPES as long as possible.



2. Transfer the threads to the cardboard box. Secure one side to the box and stretch the thread. Secure the middle of the stretched out thread to the box. Cut the thread in half then stretch the remaining half over the box. One extruded thread should produce two threads.
3. Repeat this for all threads. Leave threads out to dry overnight.

Clean Up Part 2:
1. Pour HEPES down the drain and rinse off pan. Dry the pan with paper towel.
2. Put all items away.
3. Dispose of any other used items in the appropriate receptacle.

Appendix II: Fibrina Interview Insights and Takeaways

Table 30: First Dermatologist Interview Insights and Takeaways

Major Questions/Insights	Team Takeaways
Many “Buzzword” ingredients labelled at the front of a product are typically just a minute percentage of the product itself (e.g. Glycolic Acid)	Fibrin may likely be a very small percentage of our actual product, so what is the rest of our product going to be of? Also, if we are mixing ingredients with fibrin, what percentage of those ingredients will consist of our final product? Is that percentage effective in doing anything?
Is our product cosmetically elegant? • Is it appealing to buyers aesthetically? • Does the product feel good	Consumers won’t buy our product if it doesn’t feel good to them or look appealing. There are ingredients we can use which aid in both of those aspects, and they should be incorporated.
The market is strict because of people’s personal preferences (e.g. some people like Vaseline because it is thick while others don’t.)	Whomever we are appealing to, we can’t please everyone, unless we make different renditions of the same cream. Is that worthwhile? Maybe, maybe not.
Are your ingredients safe?	Is fibrin safe? We need to test for that. How do we go about doing that? Is sterilization enough?
Be cautious about the claims you make on a product	This is where the gray area where cosmetic meets pharmaceutical. Should our claims be pharmaceutical in nature, the FDA will want to ensure safety before commercialization (which means we can’t sell tomorrow). What do want to claim about our product?
Cosmetics Companies (Branding and Marketing) • Choose consumer group and feed into their needs • Smart marketing ploys (feed into the masses demands) • Consumer Reports (price/ounce, minimize moisture loss, etc.) • Packaging of the product (ironically this can be more expensive than the actual product sometimes)	Marketing is invaluable, especially in the retail end of the cosmetics world. Do we have to think of product packaging and cost right now? Perhaps not, but it would come up eventually. Who is our consumer group? What needs are we addressing for them?

Table 31: Second Dermatologist Interview Insights and Takeaways

Major Questions/Insights/Comments	Team Takeaways
There are a lot of products out on the market. Few make money.	This is in part the nature of marketing as well as the point that a good product speaks for itself by addressing the needs of its consumers. If consumers can address their needs by using a product, then that product will sell.
In truth, some products work, and some don't.	There are a ton of products out there. Again, new products come out every month, claiming to be the next big. The hoax products actually make it more difficult to legitimize
Sell without regulations	The team took this as "don't claim anything". We were not exactly sure what this meant and want to follow up on it later.
Don't claim structural evidence.	This is again what was mentioned from the first interview. However, this comment related more towards the idea of "don't claim anything that the FDA will want data for".
Cosmeceutical: Relatively lax regulations - You don't even need to show that a product works, only that it doesn't have any toxic agents.	FDA guidelines on cosmetics products only address regulation of products. This is something the team understood through research on the FDA. However, the extent at which
Hyaluronic acid and glycolic acid are commonly used ingredients, because they work! Glycolic acid can be difficult to blend in emulsions.	Our original expectation was to use these ingredients in our formulation. Our research on these ingredients already show the positive use of HA and Glycolic. There will definitely be more research needed into the challenge of creating formulations with all these ingredients.
Cosmetics Companies bring marketing material: they don't do science.	Again, marketing is huge, but those who are in charge of marketing rely on the scientists to make product and explain the novelty to some degree.
Look carefully at some of the claims you see on products. You'll never read "Makes wrinkles go away" or "makes skin younger".	This goes back to the FDA claim gray area. Be careful with claims, but also be smart about claims. The right claims say nothing, but also play a beneficial role in marketing.

Retinol is another ingredient that works well, but there are side effects. Different products sell retinol in different forms and different percentages.	Retinol as we've seen it is the last big discovery in the skin cream world. There are tons of variations, and each have unique advantages and disadvantages. Finding the right type for our product will likely take some research and trial & error.
There's a surprising number of products that don't go through any form of rigorous testing.	This goes back to the lack of regulations on products. A company can produce a cream and sell it the next day without putting any heavy time or resource into proving its safety or effectiveness. This may be what leads to products that don't work, as well as products that aren't necessarily safe.

Table 32: Cosmetic/Plastic Surgeon Interview Insights and Takeaways

Major Questions/Insights/Comments	Team Takeaways
"I'm not a big believer in things that are out there". Not much data to support topical creams	In the professional realm, especially for surgeons, evidence of a cream being beneficial is vital.
For scarring, we've got topical silicone tapes.	There are other forms of competition to consider, such as medical products which currently exist to solve the same needs we are trying to address. Depending on our niche, it should be important to determine all possible competitors, including those outside of cosmetics.
Ideally, I'd like a skin cream that could increase the rate of healing, and reduce the rate of scarring.	While it wasn't recommended to us to ask, "what would be the most ideal thing for you to have?", mainly because the response we'd get would be something typically not practical, this response did show us insight that there isn't any product out there that really shines in the wound healing department, or in scar prevention. Are the two problems one in the same?
Think about what our product is beneficial for. Is it wound healing, or scarring?	Not to be seen as an either/or. Same question was asked by dermatologists. Finding our product niche is very important. It helps to determine our consumer base, and also helps define the experiments needed to prove efficacy.
Moisturizers - good for skin. Look at Lucederm™ (skin brightening cream)	We'd already been looking into moisturizers because of their usefulness
Look into Tretinoin, and TNS essential serum (collagen and elastin selling point)	Good leads for products to research and determine/compare benefits and examine literature.
Look into active serum by IS Clinical • Popular product that people seek out • Gentle exfoliant, brightens skin, reduces pore size	This was marked as a sought-after product, as in consumers would readily ask for it. Might be useful to understand why.

Table 33: Cosmetic Marketing and Branding Interview Insights and Takeaways

Major Questions/Insights/Comments	Team Takeaways
Key considerations of product marketing: • Target Audience • Distribution Strategy • Selling Proposition	See below notes for breakdown of each key consideration takeaways.
Target Audience: • Know who they are • Learn how they shop (online, stores, etc.) figure out their habits	Much like researching the product, one has to research the consumer. Learning about shopping habits is critical, as placing product in line of where the target audience shops is key to getting them to buy
Distribution Strategy • Where do you market the product? • Make it easy for audience to access	This ties in with the last takeaway. Knowing where to distribute to is based on where your shoppers are. Making it easy for shoppers to buy is the next big task. The more seamless, the better the experience for new consumers.
Selling Proposition: • What does our product address? • How does it address the people's needs? • Fills a need that isn't already met (optional) • Path of least resistance • Best product with most understandable story, succinct	Selling Prop = Value Prop • Our product addresses wound healing/scarring (as we claim right now) • Unmet need? Novel + quality product for this type of problem The path of least resistance asks us "what's the easiest way to get this product out the door". We don't know the answer to this yet. Fibrin needs a story that authenticates its approach into skin cream.
Online Consumers • Surveys for online audience • ID demographic, age group, location in the country • Offer incentive to answer survey	Online shopping is huge, but you can't interview online shoppers, or can you? Surveymonkey is big. We can collect a lot of useful data from online surveying, and use that toward defining our target audience, our best means of distribution, and our selling prop
Most people shop online nowadays, or they go to a brick and mortar store	When shoppers are in need of something in a pinch, they'll go to a store. Else they shop online.
Find out what makes people shop online	Again, online surveys. Also think like an online shopper: Doesn't have to go to a store, can

	research products they find via the internet, from the comfort of their desks.
Good Product Branding: • A good brand stands throughout time • Broad enough appeal • Legit claims • Authentic story being told • Specific to skincare: backup those claims with evidence	What's a good brand for Fibrin-based skin cream? How can we craft our brand to be broadly appealing? Our claims will likely surround the use of fibrin; what are those claims? Again, back to that authentic story. What's the story for fibrin? Finally, scientific evidence is a must. Repeat of dermatologists, and surgeons.
Competing with other products • Don't look at it as a competition • What are you doing different/ better? • What are you addressing? Is it different from what is currently being addressed? • Would this change people's habits?	How do we separate from our competitor's products? Our address should be unique. Would the use of our product change the habits of our consumers in our benefit?
Word of mouth - slow • Marketing campaign to incentivize this • Word of mouth starts with a small group of people • Small, independent brands have done well (Tri dynamics (San Fran) - look at marketing campaign) • Why do consumers buy a product? - usually word of mouth, based on friends/ family opinions	While word of mouth is slow, it is also extremely effective. A major advertisement has far less effect than a personal friend or family member offering their own opinion. Our interviewer gave us examples of small independent brands to research. This may be great for getting started up.

How does marketing work?
• Not as hard to get clearance like a medical device company would
• Look at competitive market
    • How are they marketing their products?
    • Sales
    • What's our value proposition?
	Back to the regulations, where anything could be theoretically sold tomorrow. However, this theory did not originally take into marketing and means of selling. Once again, value proposition becomes very important.

<table>
<tr>
<td>

Go into stores - Sephora, Lord and Taylor, CVS/Walgreens → Ask what products sell the best, what kind of questions do they ask, what is your selling propositions

Research into salesmen/women - real world data on what customers are thinking
Department stores: Specific lines salesmen
CVS/Walgreens (also target) - array of products

</td>
<td>Interactions with salespeople and the everyday consumer is essential. The Accelerate Program itself pushed the “Get out of the building” mentality from the first class.

High-end stores will sell and attract a different group of consumers than a low end (compare Sephora to CVS). While the differences may appear non-correlating, the truth is our product could potentially sell in one place, the other, or both.</td>
</tr>
<tr>
<td>

How do you make money on a product? What should the structure of an SKU look like?

Distribution (based on where it’s being sold)

How much money do i have to spend on product and marketing?
What percentage flow to bottom line?

</td>
<td>There’s a complex mathematical equation that needs to be followed in order to answer the “how do we make money out of this?” question. However, one way of simplifying that question is to ignore the cost details of making the product and just envision the cost it would take to market and distribute first. Compare that to the general cost of making a product and figure out how much you would need to mark up to make money for yourself while paying everyone else along the way.</td>
</tr>
</table>

Table 34: Low-End Store Interview Insights and Takeaways

Interviews/Notes on Product Placement, Price, and/or Ingredients	Major Takeaways
Interview, Walgreens: Regular online shopper looking for all-natural face cream. All-natural component was most important to her. Price wasn’t a major factor. Normally shops online (bridgebox.com) but was in need of something immediately.	This shopper normally purchases her products online, but that day she had run out of supplies and had to go on an “emergency” run. Out of the entire aisle of skincare products, she had spent at ten minutes looking at only a few of the products. She took careful note of the ingredients each time. This was a highly valuable interaction to observe and ask about.
Product Placement, Walgreens: single aisle dedicated to skincare products. Depending on the product being sold, some product packaging	The eye-appealing packages appeared to be the ones that were selling most. In the sea of skincare products out there, many had eerily

was more eye-catching, if not on part of the aisle that catches a lot of consumer attention.	similar packaging color schemes. The product brands were taken note of to see if they could be found elsewhere on the shelves in other stores.
Discussion, Store Manager, Walgreens: The store manager offered to help me make a purchase on a product. I explained my intent for being there and she offered some consumer insight. She said that most consumers don't purchase skincare products at Walgreens. People more often buy these products a health food stores (e.g. Wegmans).	This was an interesting comment from the store manager, considering the amount of products I saw on the shelves right next to me. It may be worthwhile in the future to check out places like Wegmans and scope out buyer habits.
CVS interview: An older woman (60s) purchased a moisturizer from the small skincare section in the store. Upon asking her how she made her decision, she lifted up the product and read the "special ingredient" center labelled on the front. She laughed, admitting she has no idea what the ingredient really is. She wanted to moisturize the wrinkles on her face. Inexpensive, decent ingredients was what she claimed to be looking for.	While there wasn't much to choose from in the CVS, this woman made her purchase based on a label for an ingredient she knew nothing about. In truth, she may have just picked up the product and "this'll do just fine". Either way, this says a lot about consumer behavior. Hopeful to see this habit again.
Product Placement, Rite Aid: As there was no one in this section as I visited, I took careful note of all the products on the shelves. Many of the product brands seen in other stores were also found at Rite Aid. For many of them, their product placements were the same.	The fact that the products were found in the same or similar shelf spaces as in other stores tells us the amount of money some brands are paying to be at eye-level to the consumer. Hard to tell from the few visits made if this extra money for eye-level placement is working, but there is literature out there that backs this.
Product Placement, Price, Shaw's: Everything here was a little bit more expensive. There were also more options available in terms of product brands. Special note on some of the packaging: the more vibrant-color packaging, the more recognizable the brand (and also the more expensive)	Shaw's, like Rite Aid, had no foot traffic during my visit. Some of the new product brands that showed up, I had never seen before. The retail market appears to be packed with products, but at the end of the day it seems that marketing and packaging are playing a much larger role than the products themselves.
Double Interview, CVS: Two Spanish women had coupons for higher-end products. When	The appeal for higher-end products in what could be described as a low-end store was

asked if they regular buy the more normal products, they said no.	met by discounts to purchase the products. For these women, cost was important, but so was the perceived quality of the product. Why settle for what seems inferior when you can match the price with coupons? In the end, who really wins?

Table 35: Wholesale Manufacturer and Distributor Interview Insights and Takeaways

Major Questions/Insights/Comments	Team Takeaways
Pain points with drug products: • Regulator environment is severe in terms of drug products • Less severe with products that don't make a drug claim	If a product has drug-like properties, and the producer of said product makes claims based on those properties, then regulation is guaranteed to be there. However, if no drug claim is made, then regulations aren't as severe. Does this mean we can avoid making drug claims but still market a product with drug-like properties?
Don't market under therapeutic effect, pathologic indicator (cure disease) e.g. remove inflammation/redness Vs. Improve the way the skin looks and feels (marketing is important)	Again going back to the above takeaway. Note the difference: One claim could be to remove inflammation/redness (which would incline that physical properties of the skin are being altered by direct means). On the other side, improving the way the skin looks and feels can address the same issue of inflammation and redness, but without directly targeting it.
• Some Cosmetic ingredients are drugs, but marketed as a non-drug • Note: Claims are important in successfully marketing a cosmetic product as a nondrug	Arguably the largest surprise for the team. Drug products are being marketed as non-drug. What does this mean with respect to fibrin-based skin cream? How are we going to base our claims?
Marketing: • Fundamental questions: If there were no boundaries, what claims would i be able to make?	The team had no idea how to answer this at the time.
You can communicate information about the drug effects of the product as long as it is not communicated to the general public Provide data from established journals to professionals to back up our claims	A prominent clarification point: as producers, we are able to express the benefits of a drug product so long as we do not relay that information out to the open public. How do we tell people without telling people? The answer: doctors (dermatologists/surgeons)

Prescription drug products and cosmetic products marketed to professionals, while cosmetic products only marketed to consumers	To a professional, we are able to market a product as a drug product and as cosmetic. For consumers, only cosmetic. But, both the drug product, and the cosmetic, can be the exact same thing.

Decision making process behind choosing ingredients:
• Varies between professional and retail market
  ◦ Evidence is more important for professional market
  ◦ In order to create separation between 2 markets: credentialing the products for professional is more important than retail
	So it is possible to create a professional and cosmetic based product that are nearly identical. The professional product will likely be under a lot of scrutiny for its effectiveness and the science which backs that up. Keeping that in mind with the types of ingredients to choose in either case is vital for a lot of factors: cost, ease of production, scientific backing required, and so on.

◦ Consumer: All about novelty, price conscious
  ▪ Cost of materials/ drugs
Points of differentiation
Features/benefits
  ▪ Evidence of how the product works
	Our big approach with fibrin is novelty. Price is something the team has not heavily considered as of yet. Price is also associated with a number of costs. Said costs include manufacturing, materials cost, and of course time and resource to validate efficacy.

• Competitive Landscape
  ◦ Research required to know competition/ what they are selling
  ◦ Cost of entry into market and cost of competitors
	While it is great that our team is pursuing a novel product, we have to do our homework and see what the current competition is.

Cell Culture Evidence: • Based on what we consider the features and benefits of our product • Approaches based on our claim, lead to substantiation of the final product • Simple studies and Clinical studies ◦ Compare with competitor product: amount of people to enter clinical study to	The science has to specifically back up the claims being made. Our team's experiments need to follow along with our claims, which then have to compare against claims of a competitor. The experiments also have to compare against the science being used by the competitor. In any clinical study, population size is critical. As mentioned from one of the dermatologist interviews, some doctors won't even look at a study if its population size isn't above a certain number.

<table>
<tr><td>show effectiveness of the products
Go to individuals who do a lot of research with our type of products
Dermatologists
Can help define what are the best parameters to look at during our course of study</td><td>Reaching out to researchers and dermatologists who current with the experimental processes of testing products should be contacted.</td></tr>
<tr><td>What is the market place looking for right now?
Different answers depending on the consumers/ professionals</td><td>Based on our value proposition research, we believe the market is hungry for something new. That could mean anything, but it is a good start nonetheless.</td></tr>
<tr><td>What is professional market looking for when it comes to aesthetic products? What would you like to have? What is missing?
Cosmetic product business: Different biological pathways to producing beneficial effect on the skin, ways to present anti-aging effects on the skin</td><td>There is a lot of push to finding the most effective ways of delivering actives into the skin which cause desirable effects. The many difficulties have been discussed in the background section of this report. It is good to know that our approach with fibrin is one that is sought after in the industry.</td></tr>
</table>

Appendix III: Protocols for Fibrina Experiments

Appendix IIIa Protocol for Composition Experiment

Materials:

1 small whisk

16 oz. wide mouth jar, or recycled bottle/container (sterilized) [Glass Preferred]

½ cup Grape Seed Oil

¼ cup Emulsifying Wax

¾ cup Water

1 tbsp Glycerin

¼ tsp Vitamin E

12-15 drops Essential Oil

1/8 tsp Germall Plus

For a 50% Water-Based Cream:

Ingredient	Non-SI units	SI (in mls)	% to total
Grape Seed Oil	½ cup	118.294	31.5
Emulsifying Wax	¼ cup	59.147	15.75
Water	¾ cup	177.441	47.25
Glycerin	1 tbsp	17.7582	4.73
Vitamin E	¼ tsp	1.4798475	0.39
Essential Oil	12-15 drops	0.7	0.19
Germall Plus	1/8 tsp	0.73992375	0.2

Note: Adjust water content as needed to create more/less viscous cream.

Procedure:

1. In a small container such as a stainless steel measuring cup, combine emulsifying wax and grape seed oil.
2. Heat the emulsifying wax and grape seed oil over a pan of simmering water till the wax melts and stir till ingredients are thoroughly combined.
3. In the same pot of simmering water, but in a separate container warm the water so the oil and water reach approximately the same temperature.
4. Slowly drip the oil mix into the hot water while blending with the stick blender till the oil and water are thoroughly emulsified.
5. After it cools down to 120°F, blend in the optional additives.
6. Pour into container(s) before the moisturizer gets a chance to thicken.

Recommendations:

The team recommends a 60% water-based skin cream. A 60% water-based cream provides ideal properties for testing. A 50% water-based cream proves to be too viscous, and any % above 60% appears to have issues with maintaining non-separation between water and oil phases.

Appendix IIIb Protocol for Exploratory Degradation Experiment:

Materials:

- Fibrin powder (Grind fibrin threads into particles the size of grains using scissors other grinding method)
- PBS Solution (Or any other neutral buffer)
- Petri dishes
- Protease (Bromelain, Serrapeptase, Nattokinase)
- Microscope

Procedure:

1. Take 5 mls of PBS solution into each of the petri dishes to be used for the experiment
2. Place 1, 2.5, 5, and 10 mgs of protease into separate petri dishes.
3. Place the petri dishes onto an orbital shaker; else use a magnetic stirrer to continuously mix the solution.

4. Take images of the solutions every other hour beginning at the first hour (1,3,5,7, etc.). Images should be taken with a microscope using imaging software.
5. Record any observations throughout the time periods. Take special note of the visible fibrin threads both through the microscope images and with the naked eye.

Special Notes: Using too little protease will prevent the degradation reaction from continuing over time. Observations showing little to no change in degradation between time periods is normal/expected with the lower quantities of protease (1, 2.5 mg).

Appendix IIIc Protocol for UV Spectroscopy Degradation Analysis Experiment

Experiment Objective:

To quantify the rate of retinol-additive being release through the degradation of fibrin-based particles via the use of protease.

Materials:

100mg – 100ul Retinol-infused fibrin-based particles
20 - 60x15mm tissue culture dishes
100ml – PBS solution
200mg – protease in lyophilized powder form (bromelain, nattokinase, serrapeptase, plasmin, lumbrokinase)
SpectraMax 250 UV Spectroscopy Plate Reader
24-Well Plate
96-Well Plate

Procedure:

1. Prepare 100mg of retinol-infused fibrin threads using the protocol for producing additive-infused fibrin threads.
2. Grind, pulverize, or chop threads into particles (grains). The particles can be anywhere from 500um to up to 2mm in length. Smaller particles preferred. Experimental set-up is shown in Figure 51 below.

Figure 51: Cutting of Threads into particles using small scissors. Threads cut onto weigh plate for measuring

3. Add 5mg of the retinol-infused fibrin-based particles into each of the 20 tissue culture plates. Ensure as much of the fibrin particles are sitting in solution and not statically clung to the walls of the plate.
4. Add 5ml PBS solution to each of the plates. Use a 1000ul Micropipetter to spray statically clung particles down to the bottom of the plate and into solution. Experimental set-up is shown in Figure 52 below.

Figure 52: Tissue Culture Dishes all prepped with 5mg fibrin particles and 5ml PBS. On the Right: Eppendorf Tubes prepped with varying amounts of protease

5. Introduce 6,7,8,9, and 10 mg of the chosen protease to 4 of the tissue culture plates.
6. At 1,3,5, and 7 hours, take a 500ml sample of one of each of the 6,7,8,9 and 10 mg protease plates. Take images of the plates from which each of the samples just before prior to taking the sample.

7. Place the 500ml samples in the 24 well plate. Rake a clear sample from each plate; be careful not to take up any of the protease powder grains, nor any degrading fibrin particles.
8. Take 3 100ul samples of each of the 500ml samples onto the 96 well plate.
9. Create a standards group consisting of 1,3,5,10,20, and 30 ul of 25ug/ml retinol in ethanol solution within the 96-well plate.
10. Create a control group consisting of PBS solution.
11. Run the SpectraMax 250 UV Spectroscopy plate reader at a wavelength of 325nm.
12. Record results on an excel sheet and observe the recorded quantities read by the plate reader for patterns. Experimental results are shown in Figure 53 below.

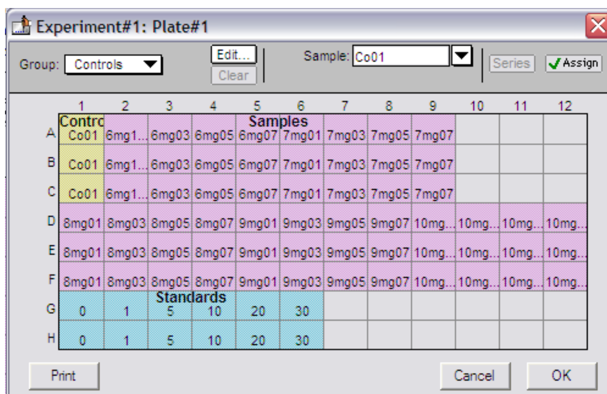


Figure 53: Plate Reader Setup. 6,7,8,9 and mg protease degradation measured over 1,3,5, and 7 hours. Actual Standards used: 1,3,5,10,20 and 30 ul retinol in ethanol. Control group was PBS only

Note: Fibrin-based Particles experience static cling and thus are difficult to transport from one container to another. When cutting/grinding threads into particles, be sure that the particles are being cut/ground into their final container of use if possible.

Below in Tables 36 and 37 is experimental data for the standard curve and data for different concentrations of Bromelain at different concentrations over hours 1, 3, 5, and 7.

Table 36: SpectraMax 250 Data Values for UV Spec. Analysis Experiment

Bromelain	6 mg	7 mg	8 mg	9 mg	10 mg	Control
1 hour	0.243	0.244	0.242	0.238	0.251	0.216
	0.244	0.238	0.243	0.245	0.265	0.222
	0.238	0.235	0.249	0.233	0.255	0.224
Average	0.2417	0.239	0.2447	0.2387	0.257	0.2207
3 hour	0.257	0.245	0.258	0.238	0.253	0.221
	0.262	0.241	0.257	0.25	0.263	0.225
	0.246	0.241	0.245	0.253	0.251	0.23
Average	0.255	0.2423	0.2533	0.247	0.2557	0.2253
5 hour	0.278	0.297	0.287	0.306	0.31	
	0.295	0.295	0.288	0.306	0.307	
	0.299	0.283	0.283	0.756	0.289	

Average	0.2907	0.2917	0.286	0.456	0.302	
7 hour	0.3	0.295	0.312	0.308	0.328	
	0.298	0.292	0.301	0.309	0.324	
	0.288	0.268	0.292	0.302	0.327	
Average	0.2953	0.285	0.3017	0.3063	0.3263	

Table 37: Standard Curve Values for Retinol in Ethanol Solutions within PBS Solution

Standard Curve:				Avg.
1	0.295	0.289	0.284	0.2893
3	0.394	0.403	0.402	0.3997
5	0.515	0.512	0.508	0.5117
10	0.898	0.923	0.908	0.9097
20	1.575	1.635	1.634	1.6147
30	2.067	2.134	2.113	2.1047

Appendix IV: Data and Results from Fibrina Experiments

Appendix IVa: Fibrin Exploratory Experiment Images

No PBS hour 0

Results displayed in Figures 54 to 58.

General Notes: Without PBS as a medium, bromelain powder is inactive to the fibrin particles.

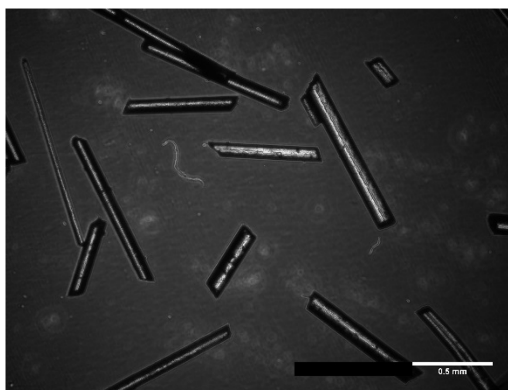


Figure 54: Fibrin Control Hour 0, Pre-PBS

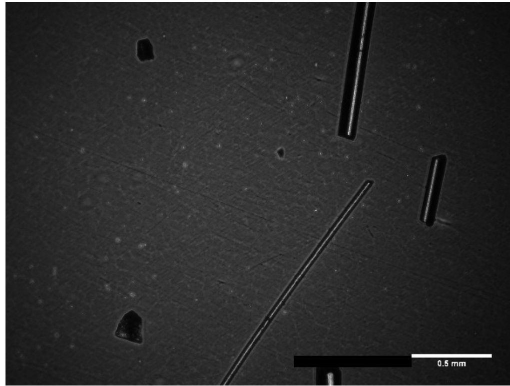


Figure 55: Fibrin 1mg Hour 0, Pre-PBS

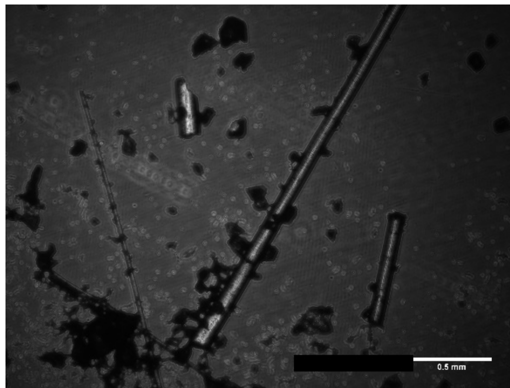


Figure 56: Fibrin 2.5mg Hour 0, Pre-PBS

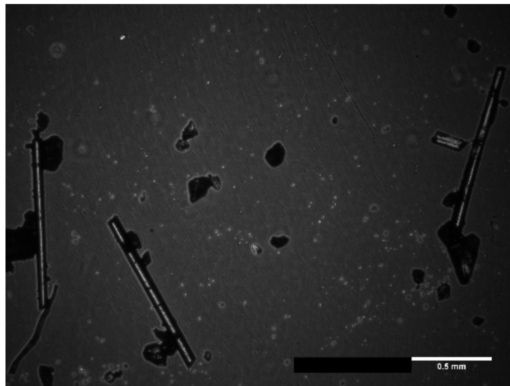


Figure 57: Fibrin 5mg Hour 0, No PBS

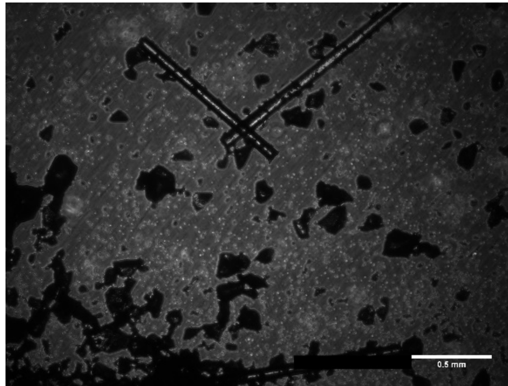


Figure 58: Fibrin 10mg Hour 0, No PBS

PBS Hour 0

Observations: With the introduction of PBS, the fibrin particles immediately begin to swell. No immediate notice of degradation among fibrin threads except from the 10mg dish, which works very fast towards complete fibrin degradation. Results are shown in Figures 59-63.

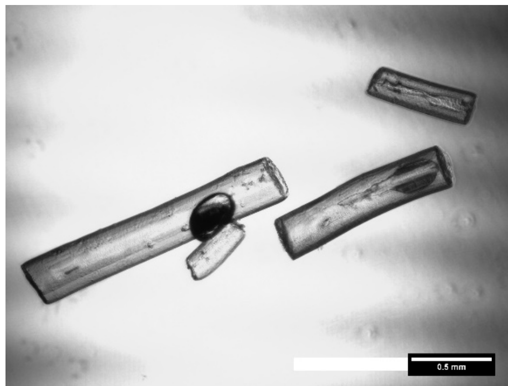


Figure 59: Fibrin Control Hour 0

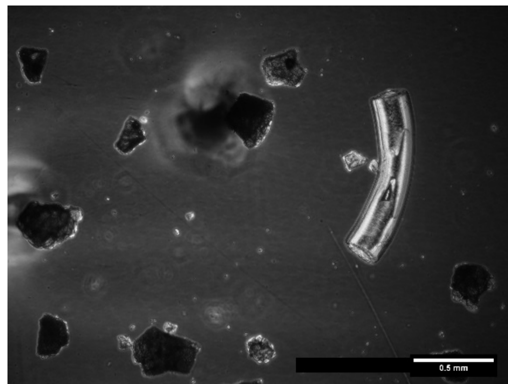


Figure 60: Fibrin 1mg Hour 0

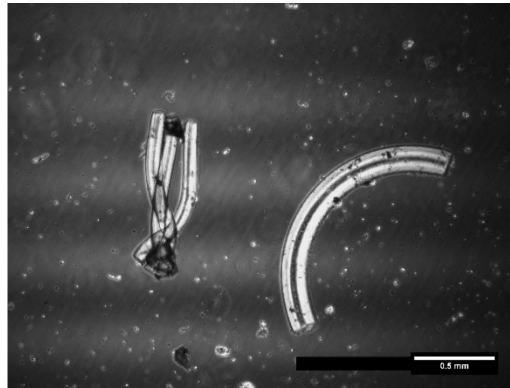


Figure 61: Fibrin 2.5mg Hour 0

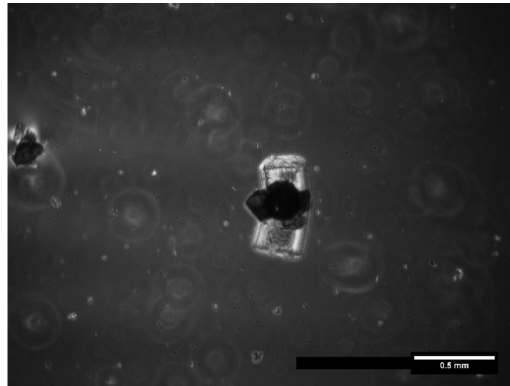


Figure 62: Fibrin 5mg Hour 0, PBS Added

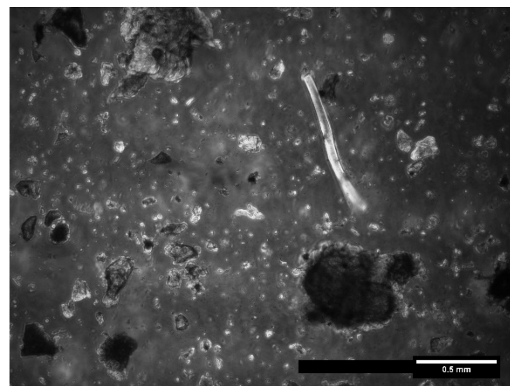


Figure 63: Fibrin 10mg Hour 0, PBS Added

PBS Hour 1

Observations: Fibrin particles are beginning to degrade across all plates, with the exception of the control. The 10mg dish showcases particles which are showing prominent signs of degradation. From the naked eye, it was noted that some of the smaller particles in each of the

plates has degraded. This occurrence was noticed much more prevalently when going up in concentration. Results are shown in Figure 64-68.

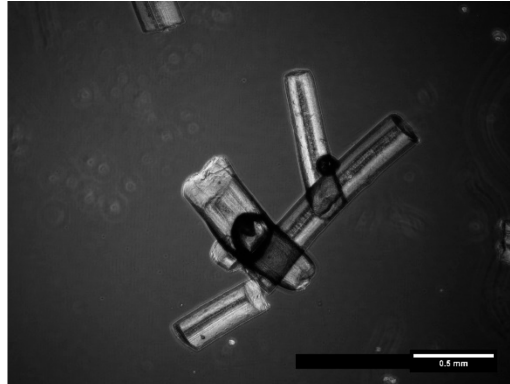


Figure 64: Fibrin Control Hour 1

Figure 65: Fibrin 1mg Hour 1

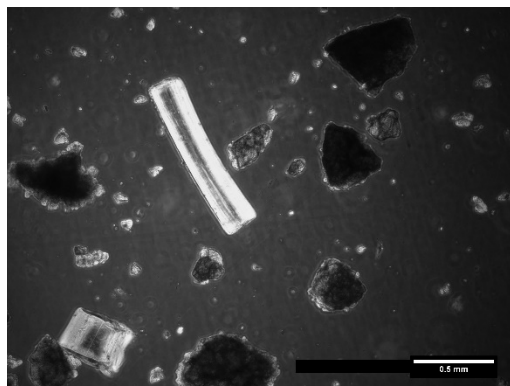


Figure 66: Fibrin 2.5mg Hour 1

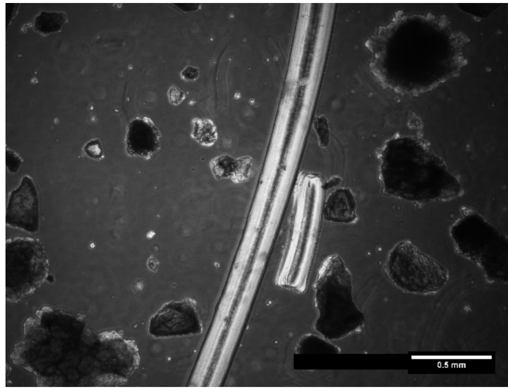


Figure 67: Fibrin 5 mg Hour 1

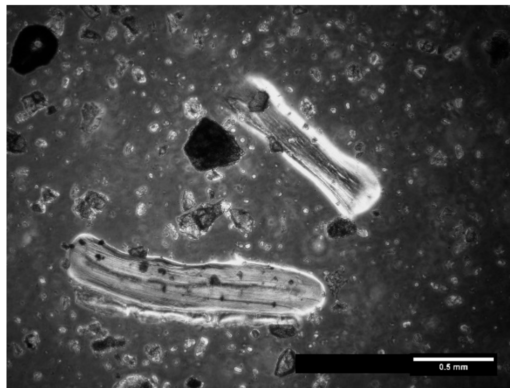


Figure 68: Fibrin 10mg Hour 1

PBS Hour 3

Observations: The 10 mg dish has completely broken down all fibrin particles. The 5mg dish is almost complete as well. The 1mg dish appears to have shown little progress in particle degradation. Hard to determine where the 2.5mg dish is in terms of whether it is continuing to degrade or not. Within all of the non-control dishes, it is difficult to determine whether floating particles are smaller fibrin particulate or if they are particulate from the bromelain powder that is slowly solubilizing. Results are shown in Figures 69-73.

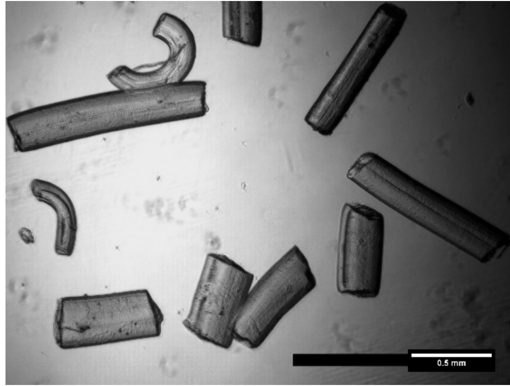


Figure 69: Fibrin Control Hour 3

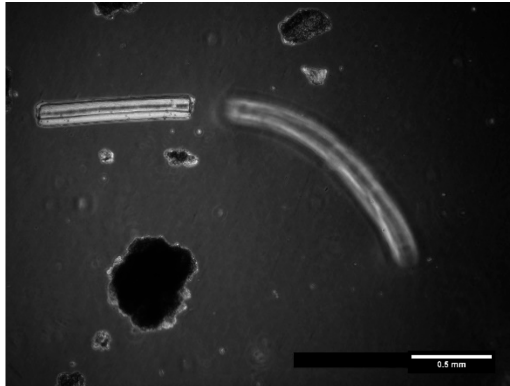


Figure 70: Fibrin 1mg Hour 3

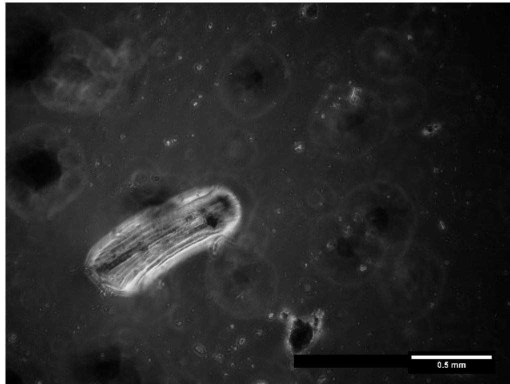


Figure 71: Fibrin 2.5mg Hour 3

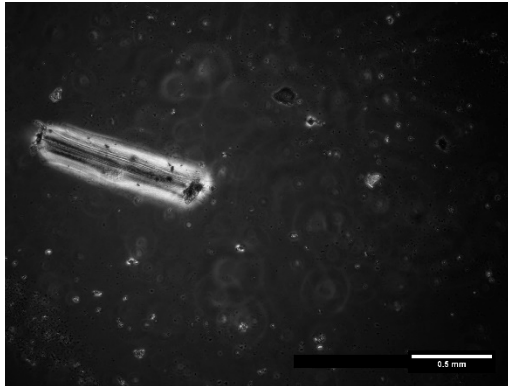


Figure 72: Fibrin 5mg Hour 3

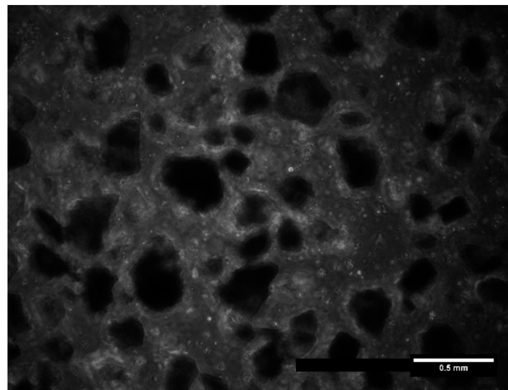


Figure 73: Fibrin 10mg Hour 3

PBS Hour 5

Observations: Both the 5mg and 10mg dishes are now fully degraded. The 2.5 mg dish has made more progress but appears to be slowing down. The 1mg dish has made very little progress since hour 3. Results are shown in Figures 74-78.

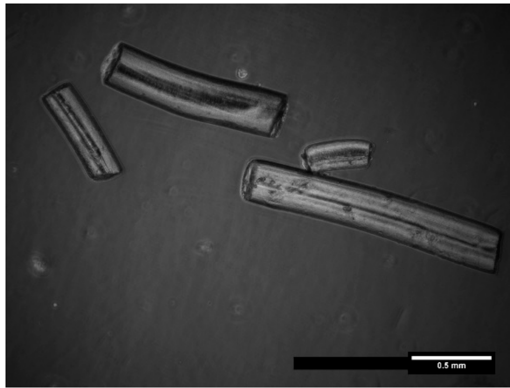


Figure 74: Fibrin Control Hour 5

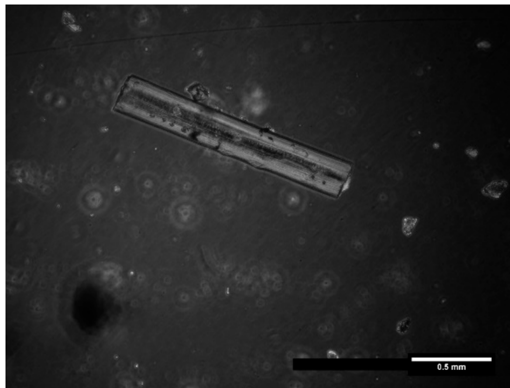


Figure 75: Fibrin 1mg Hour 5

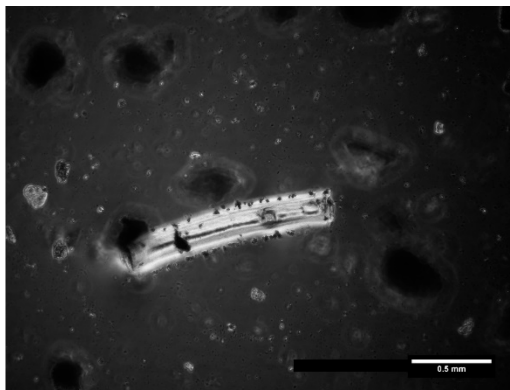


Figure 76: Fibrin 2.5mg Hour 5

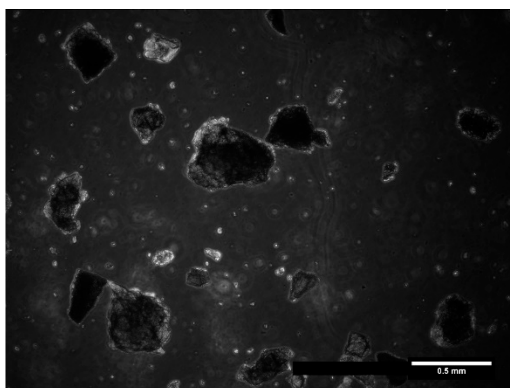


Figure 77: Fibrin 5mg Hour 5

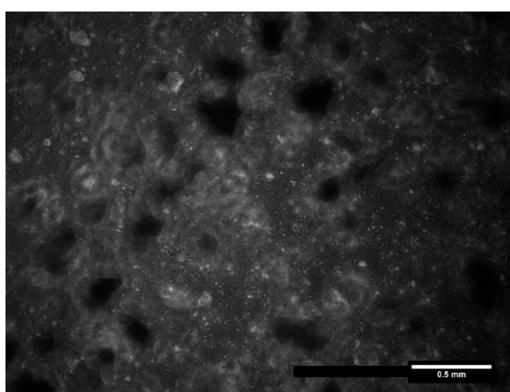


Figure 78: Fibrin 10mg Hour 5

PBS Overnight Observations

Observations: At this point, all of the dishes have sat for over 12 hours. The resulting images appear drastically different due to unforeseen evaporation of PBS. The control dish showcases fibrin particles that appear to be entrapped in a light film that is presumably PBS? Each of the other dishes lack this film-like appearance, but are significantly evaporated. Both the 1mg and 2.5mg plates still show some fibrin particles. There is still nearly the same amount of particles in the 1mg plate as there was the night prior. The 2.5mg plate has also shown little change. Two images were taken to showcase different areas of the dish. In one area, a fibrin particle can still be seen, whereas in another there appears to be nothing more than mixed particulate that could be fibrin or bromelain. The 5mg and 10mg plates have shown no change since the previous night. Results are shown in Figures 79-84.

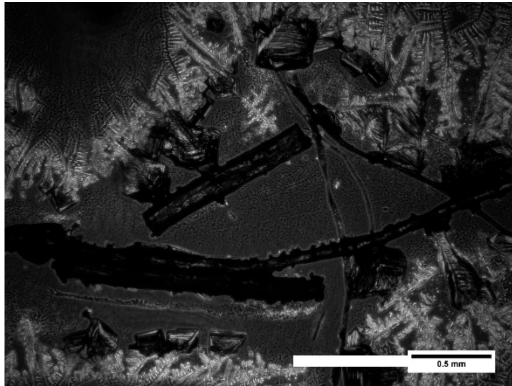


Figure 79: Fibrin Control Overnight

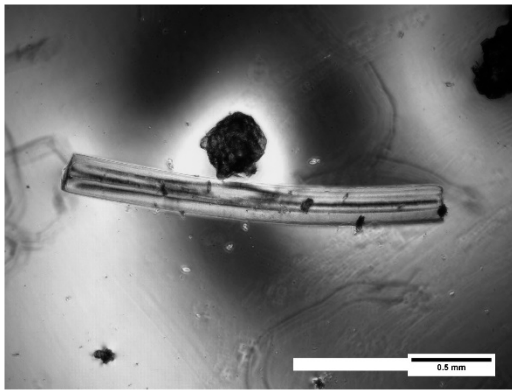


Figure 80: Fibrin 1mg Overnight

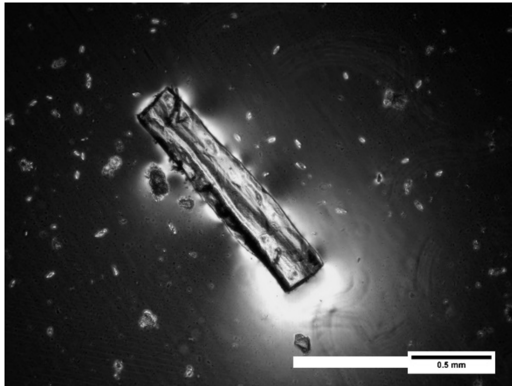


Figure 81: Fibrin 2.5mg Overnight (Image 1)

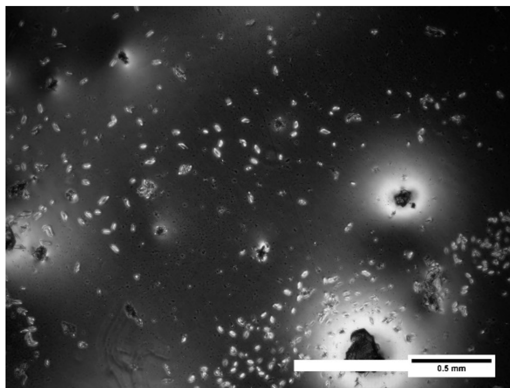


Figure 82: Fibrin 2.5mg Overnight (Image 2)

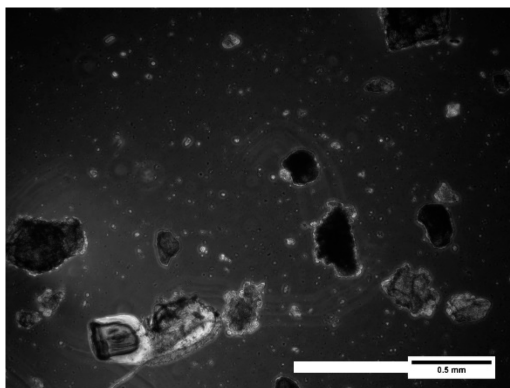


Figure 83: Fibrin 5mg Overnight

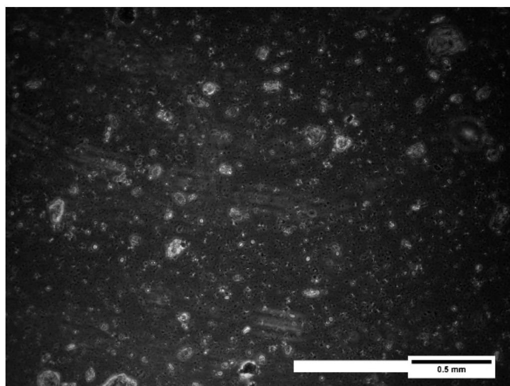


Figure 84: Fibrin 10mg Overnight

Appendix IVb: UV Spectroscopy Data

Table 38: Raw UV Spectroscopy data from UV spectroscopy experiment

Bromelain	6 mg	7 mg	8 mg	9 mg	10 mg	Control
0 Hour	0.222	0.230	0.231	0.236	0.224	0.220
	0.232	0.232	0.233	0.240	0.225	0.223
	0.225	0.230	0.235	0.237	0.220	0.217
Average	0.226	0.231	0.233	0.238	0.223	0.220
1 hour	0.243	0.244	0.242	0.238	0.251	0.216
	0.244	0.238	0.243	0.245	0.265	0.222
	0.238	0.235	0.249	0.233	0.255	0.224
Average	0.242	0.239	0.245	0.239	0.257	0.221
3 hour	0.257	0.245	0.258	0.238	0.253	0.221
	0.262	0.241	0.257	0.250	0.263	0.225
	0.246	0.241	0.245	0.253	0.251	0.230
Average	0.255	0.242	0.253	0.247	0.256	0.225
5 hour	0.278	0.297	0.287	0.306	0.310	0.220
	0.295	0.295	0.288	0.306	0.307	0.223
	0.299	0.283	0.283	0.756	0.289	0.218
Average	0.291	0.292	0.286	0.456	0.302	0.220
7 hour	0.300	0.295	0.312	0.308	0.328	0.221
	0.298	0.292	0.301	0.309	0.324	0.220
	0.288	0.268	0.292	0.302	0.327	0.222
Average	0.295	0.285	0.302	0.306	0.326	0.221

Table 39: Corresponding data to Figure 32 from UV Spectroscopy Results

Hour	Control	6mg	7mg	8mg	9mg	10mg
0	0.220	0.226	0.233	0.238	0.223	0.220
1	0.222	0.242	0.239	0.245	0.239	0.257
3	0.224	0.255	0.242	0.253	0.247	0.256
5	0.221	0.291	0.292	0.286	0.456	0.302
7	0.221	0.295	0.285	0.302	0.306	0.326

Table 40: Corresponding data to Figure 34 from UV Spectroscopy Standard Curve

Standard Curve:				Avg.
0	0.221	0.220	0.225	0.222
1	0.295	0.289	0.284	0.289
3	0.394	0.403	0.402	0.400
5	0.515	0.512	0.508	0.512

10	0.898	0.923	0.908	0.910
20	1.575	1.635	1.634	1.615
30	2.067	2.134	2.113	2.105

Code for UV Spectroscopy Statistical Analysis

```
%% MATLAB Data Analysis MQP
%% Two Way ANOVA Analysis - UV Spec

UV_Spec = [0.243    0.244   0.242   0.238   0.251   0.216;
0.244   0.238   0.243   0.245   0.265   0.222;
0.238   0.235   0.249   0.233   0.255   0.224;
0.257   0.245   0.258   0.238   0.253   0.221;
0.262   0.241   0.257   0.25    0.263   0.225;
0.246   0.241   0.245   0.253   0.251   0.23;
0.278   0.297   0.287   0.306   0.31    0.234;
0.295   0.295   0.288   0.306   0.307   0.237;
0.299   0.283   0.283   0.756   0.289   0.239;
0.3 0.295   0.312   0.308   0.328   0.241;
0.298   0.292   0.301   0.309   0.324   0.246;
0.288   0.268   0.292   0.302   0.327   0.249;];


[p, tbl] = anova2(UV_Spec,3);
```

Appendix IVc: AFM Surface Roughness Images

Results of the AFM surface roughness are shown in Figures 85-98.

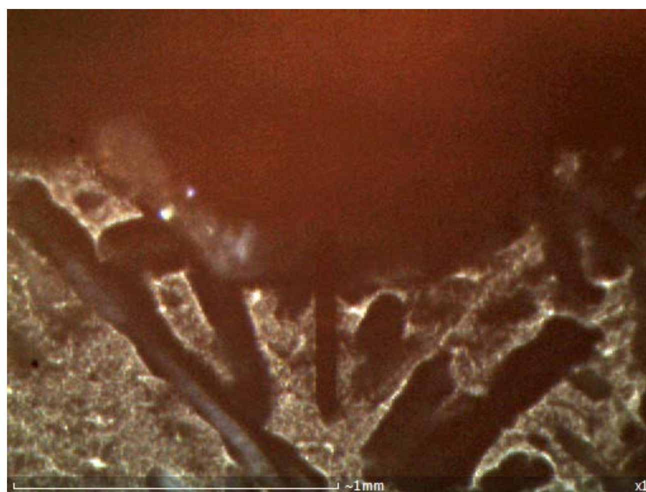


Figure 85: AFM Top-down image of Fibrin Particle Sample Plate

Figure 86: Side Image of Fibrin Particle Sample Plate under AFM

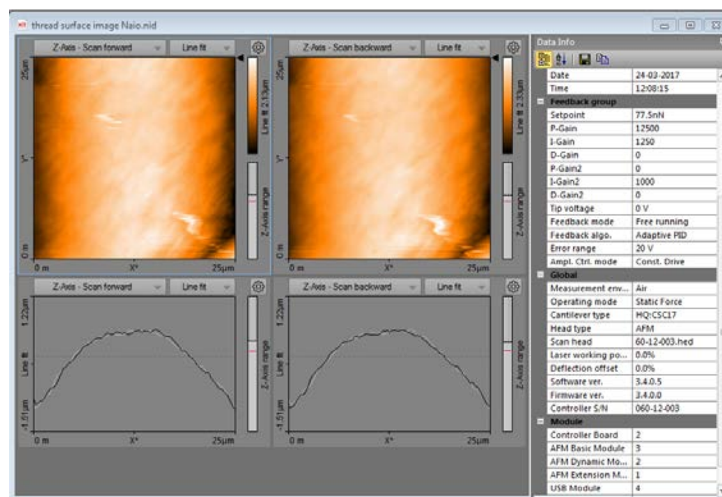


Figure 87: AFM image of Fibrin Particle with Data Info

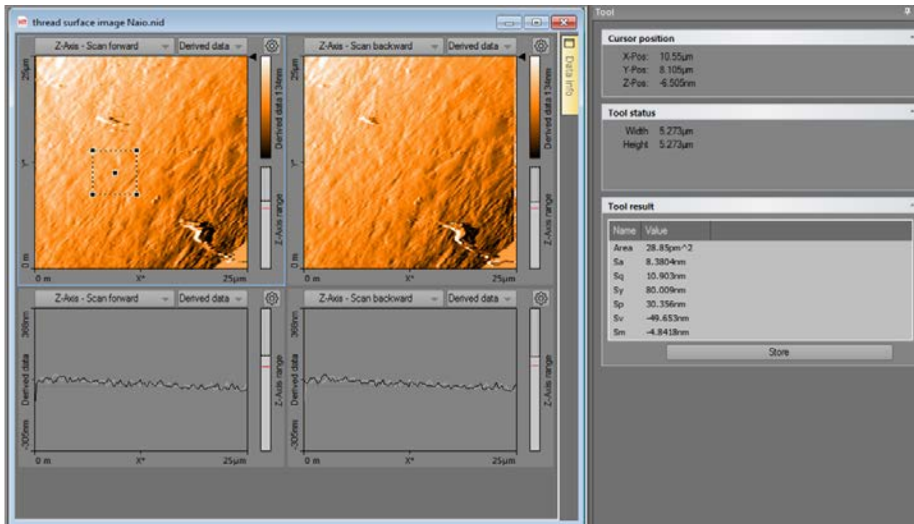


Figure 88: Derived Data of the AFM image in Figure 79, with Area Roughness Calculation for near-center cross section.

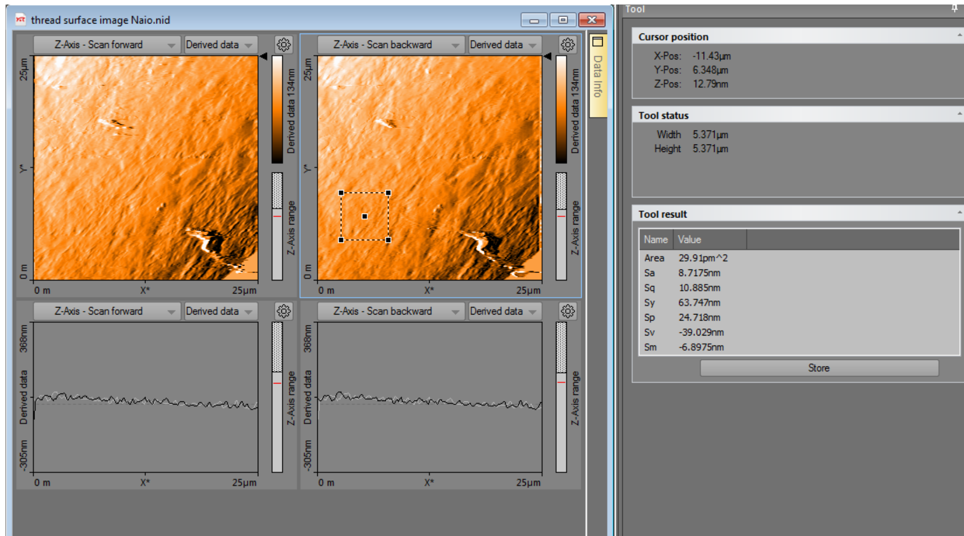


Figure 89: Derived Data of the AFM image in Figure 79, with Area Roughness Calculation for lower-left cross section.

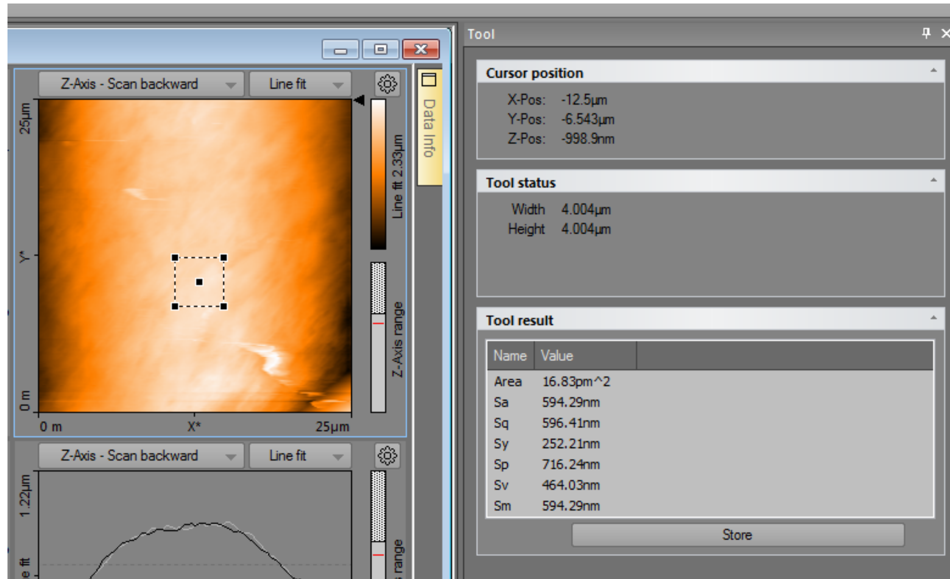


Figure 90: Near-center area roughness calculations from backward scan of Figure 79

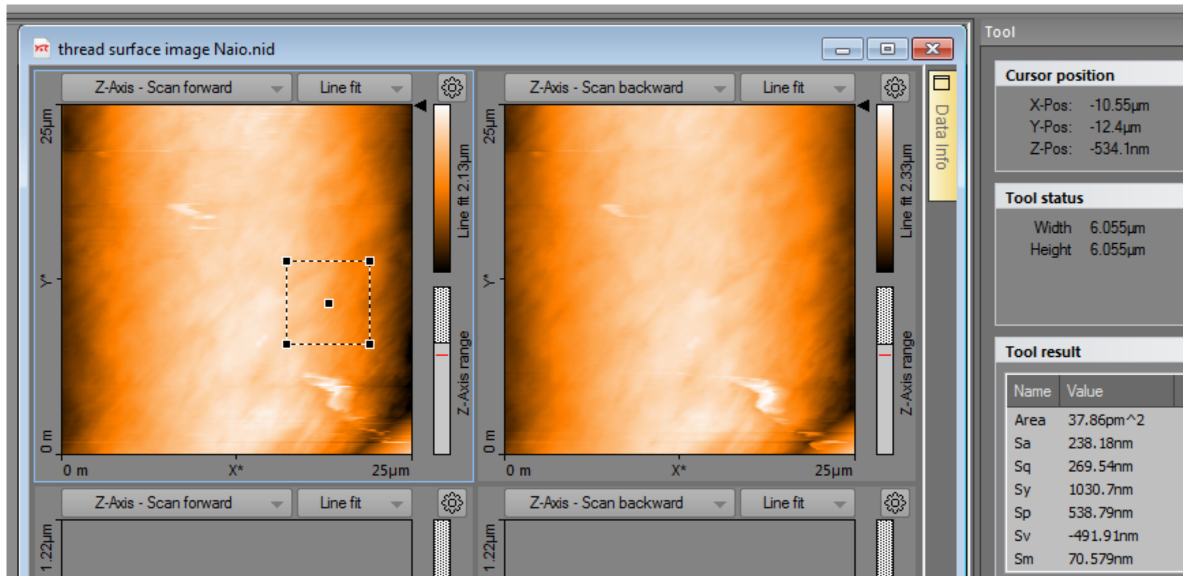


Figure 91: Near-Edge area roughness calculations from forward scan of Figure 79

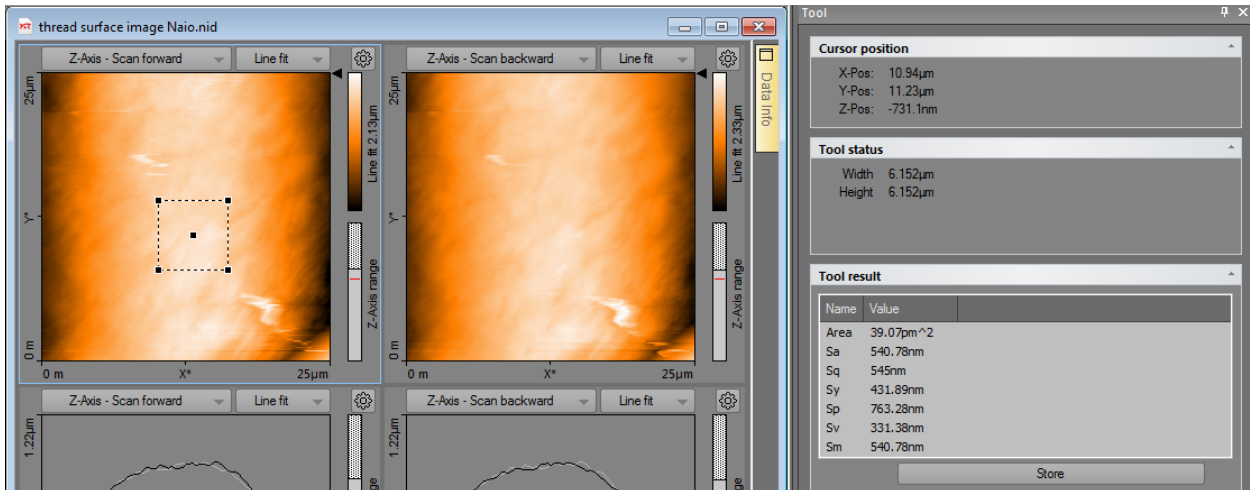


Figure 92: Center cross section area roughness analysis on forward scan of Figure 79

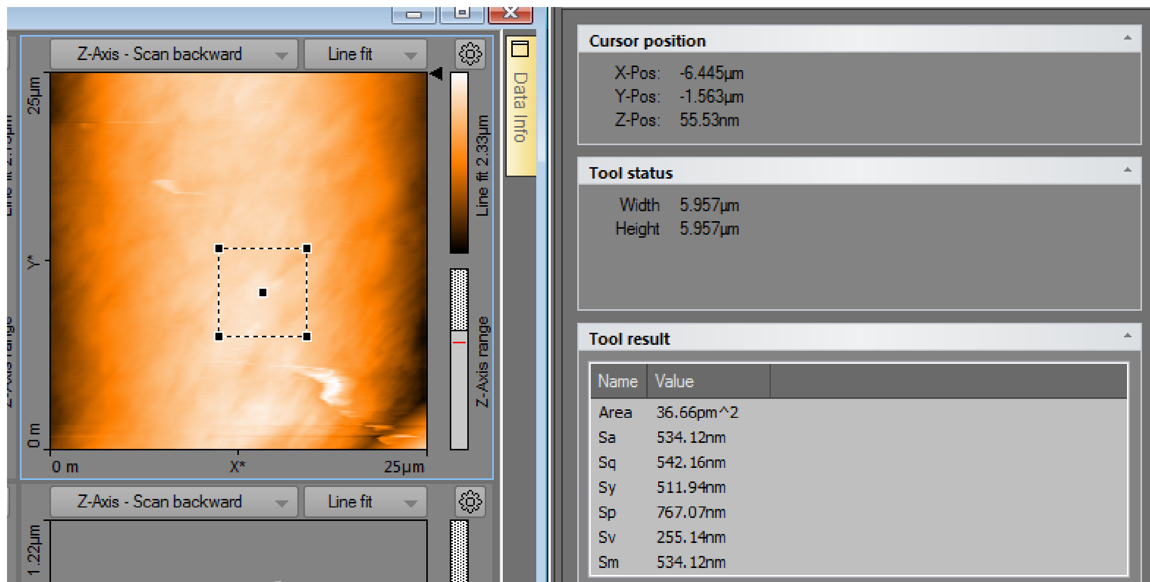


Figure 93: Near Center cross-section area roughness calculations from backward scan

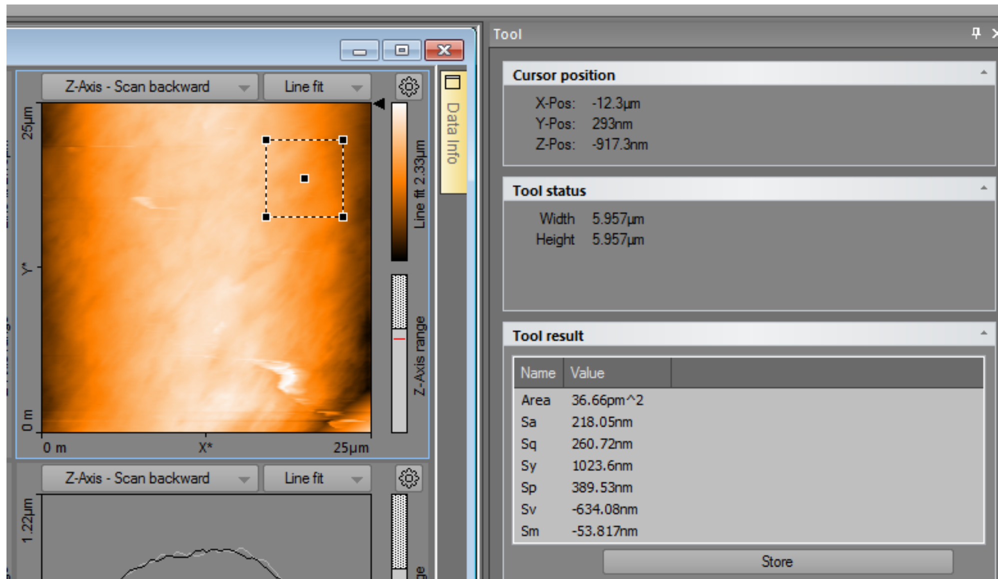


Figure 94: Top-Right Cross section area roughness calculations from backward scan

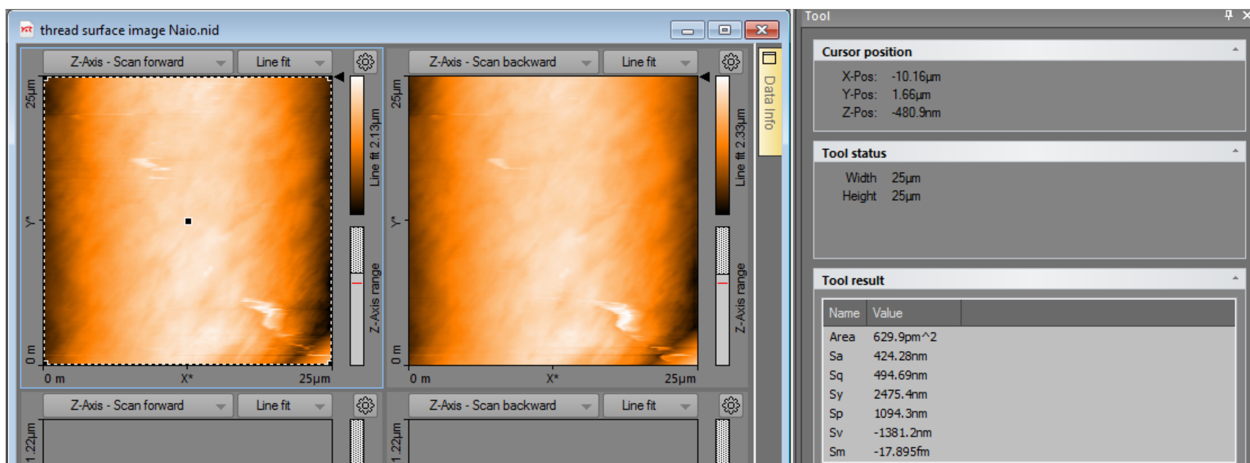


Figure 95: Whole image area roughness calculations for forward scan

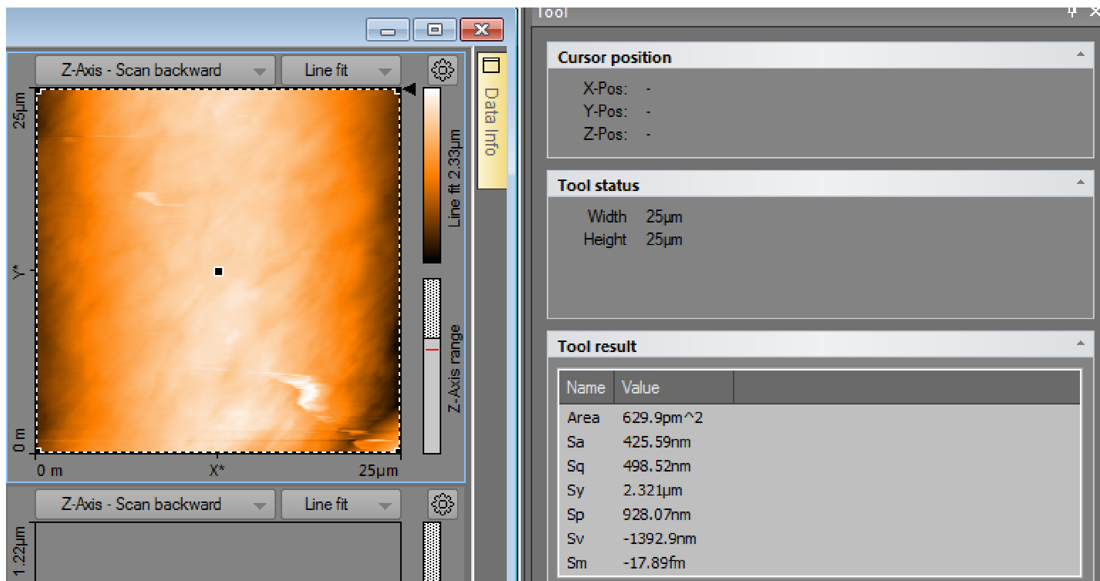


Figure 96: Whole Image area roughness calculations from backwards scan

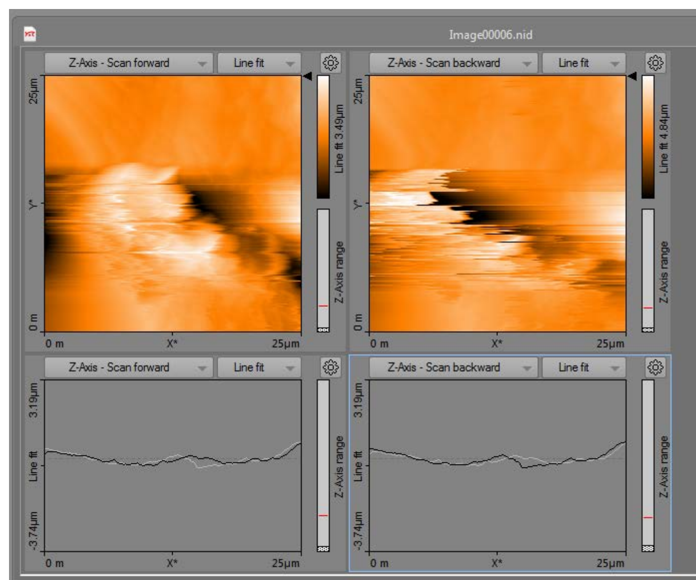


Figure 97: Unreadable imaging of cut face of Fibrin Particle

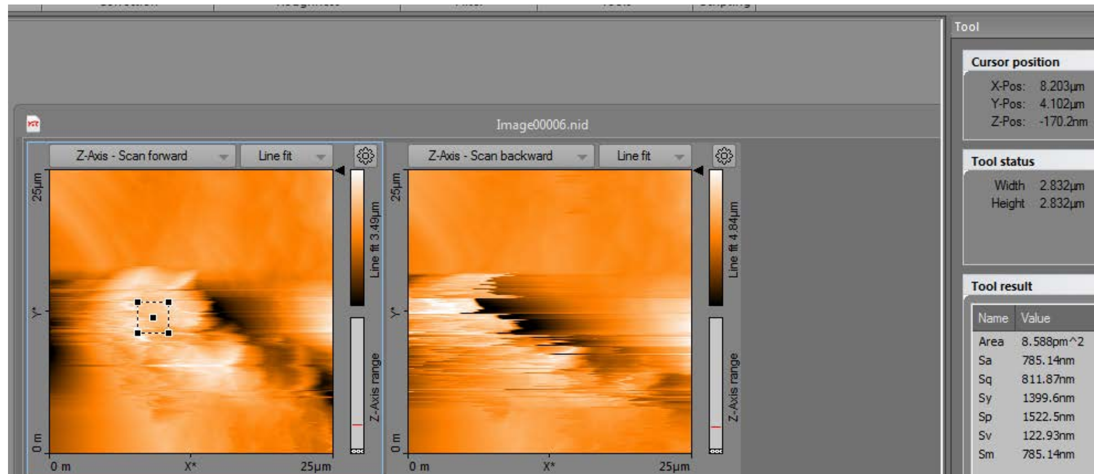


Figure 98: Attempted area roughness calculation for cut face particle

Appendix IVd: Channels UV Sub-Experiment Results

Objective:
- Identify whether retinol is seeping out of threads during extrusion or not.

Hypothesis: Given the ratio of retinol to HEPES during the drawing of a singular thread, the overall concentration gradient is forcing the retinol to disperse out into the HEPES and not stay into thread.

Materials:
- Retinol-Infused Fibrin Microthreads (25mg/ml retinol in ethanol solution, 100ul ROL in EtOH for one batch)
- HEPES
- 5ml multi-channel tub
    -Specs: 19 wells, well radius: 7mm. Well length: 180mm. Max Volume: 14 mls (Half-Cylinder)
- SpectraMax 250
- Bromelain Powder

Procedures:

Part 1: Drawing and Collecting Samples

1. Produce a single batch of retinol-infused fibrin microthreads using the protocol for producing additive infused fibrin threads. Please note that instead of using the regular 500ml tub, instead use a series of small channels as individual tubs, much like the one seen in Figure 99 below:

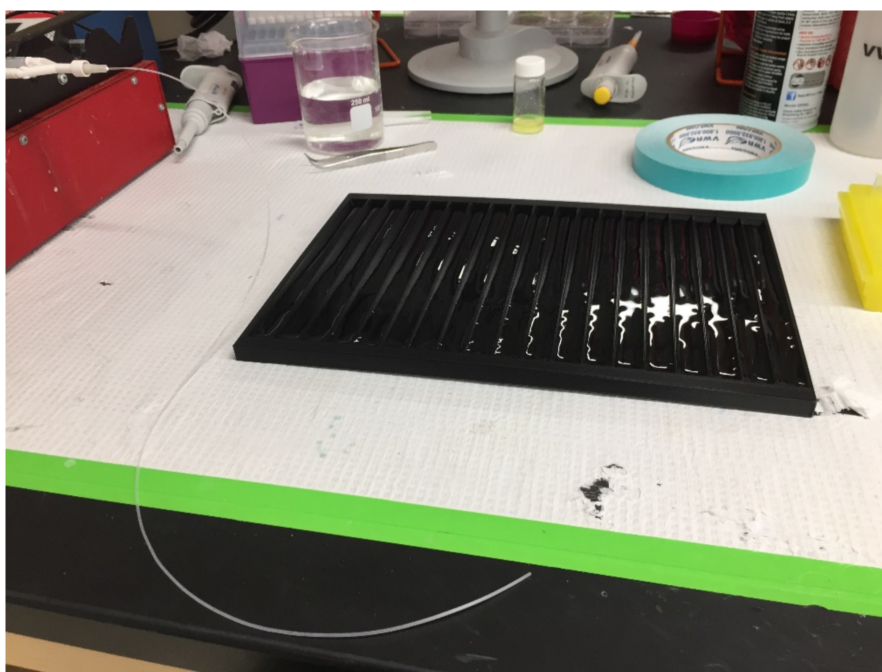


Figure 99: A custom 3D Printed multi-channel design (has 19 channels)

Use 5ml of HEPES for each channel.

2. Draw a single thread into as many of the wells as possible. As a rule of thumb, take about 15 seconds drawing within each well.
3. Remove the threads from the channels once they are ready to be removed. DO NOT empty the channels for cleaning.
4. Allow the threads to dry. **Bonus:** collect droplets from the threads as they are drying or during any part of their removal from the channels (i.e. when you are lifting them from the channels using tweezers and they are suspended in mid-air.

Part 2: Prepping Samples and Controls

5. Collect the 5ml HEPES from each of the channels separately and place them in a 12-well plate (use as many as needed). See Set-up in Figure 100.
6. Create controls using retinol in ethanol solution (0,10,25,50,100ul respectively) and HEPES (fill to 5mls). See Set-up in Figure 101.
7. Once the threads are dried, bundle them, and weigh them in milligrams. Write down the weight before placing the bundled threads into a single 5ml well of HEPES. **Hint:** the bundled threads can be kneaded into a ball with your fingers to make placement into the well easier.
8. Based on the weight (in mg) of the batch of retinol-infused fibrin threads, add 10x bromelain powder to the fibrin batch well*.

9. Create a separate control of just HEPES and the same quantity of bromelain powder used in step 8.
10. Create a final well using the collected droplets from the drying threads (we used 100ul) and fill the rest up to 5mls with HEPES.
11. Place the wells on a horizontal orbital shaker for 3 hours.

*Based on the results of the exploratory fibrin degradation experiment, where it takes around 3 hours to fully degrade 1mg of fibrin with 10mg of bromelain.

Part 3: UV Spectroscopy Data Collection

12. After waiting for 3 hours, examine each of the wells and write down observations.
13. Take 100ul samples of each sample and control (in triplicate) and organize them onto a 96-well plate for reading.
14. Take readings for each sample on the 96-well plate using the Spectra Max 250 plate reader. Results are shown in Figures 102-103.
15. Transfer the results from the plate reader over to an excel file for further statistical analysis.

Results:

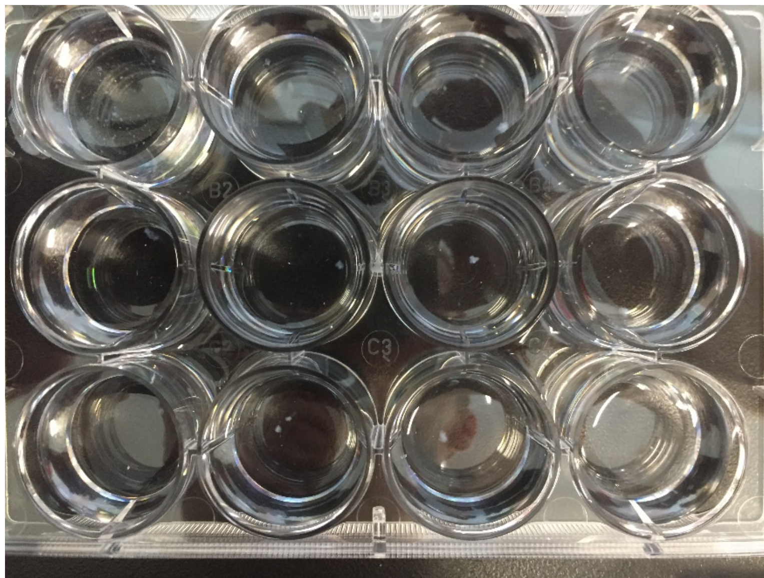


Figure 100: Channels 1-12 transferred over to a 12-well plate

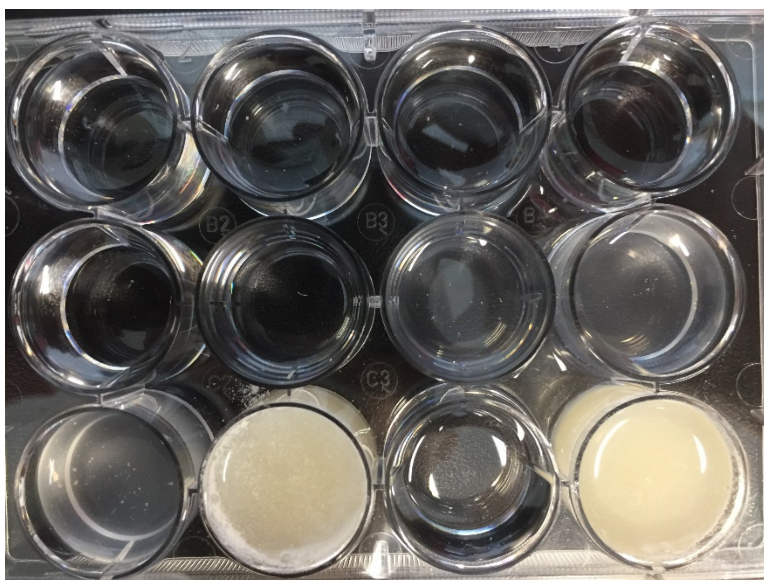


Figure 101: Top-row:Channels 13-16;
Mid-row starting from Left: 0,10,25,50ul ROL in EtOH standards;
Bottom-row from Left: 100ul ROL in EtOH, 37mg fibrin batch + 370mg bromelain, 100ul collected droplets from hung threads, 370mg bromelain

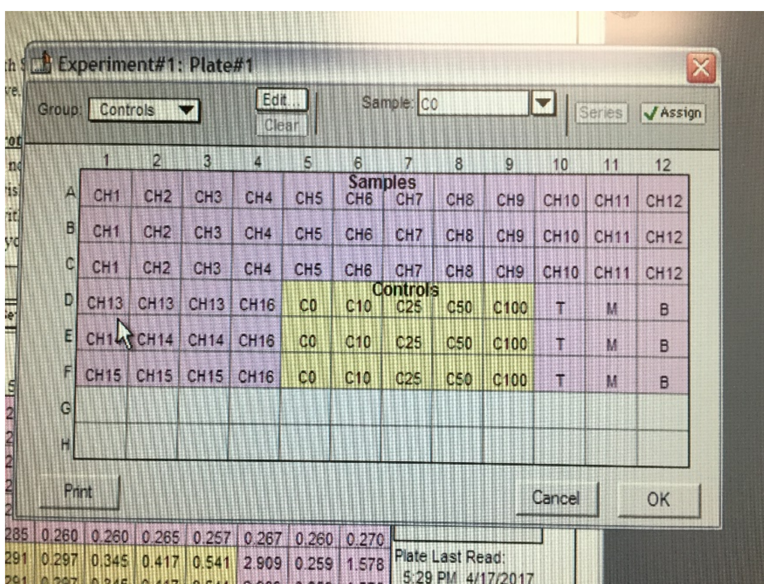
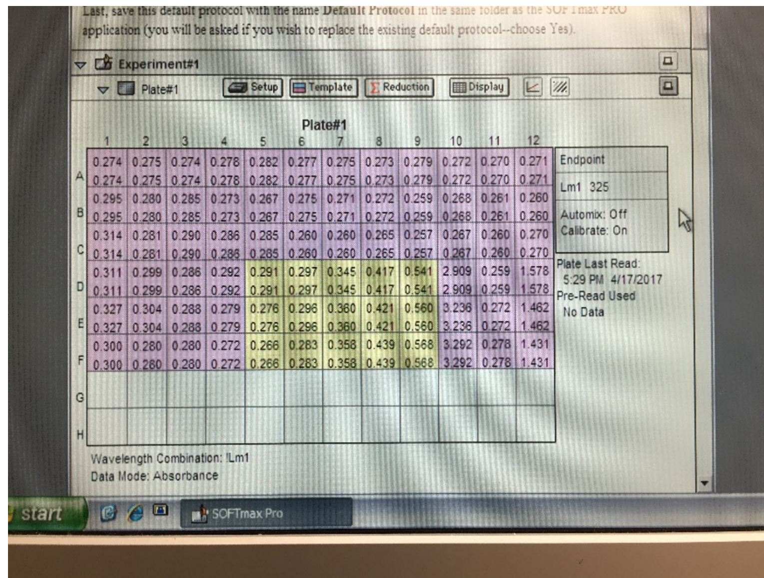


Figure 102: SpectraMax 250 template for UV Spec testing; CH - Channel; C# - Control/Standard, amount of ROL in EtOH (ul); T - Threads+Bromelain; M - Droplets from threads; B - 370mg Bromelain



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.274	0.275	0.274	0.278	0.282	0.277	0.275	0.273	0.279	0.272	0.270	0.271
	0.274	0.275	0.274	0.278	0.282	0.277	0.275	0.273	0.279	0.272	0.270	0.271
B	0.295	0.280	0.285	0.273	0.267	0.275	0.271	0.272	0.259	0.268	0.261	0.260
	0.295	0.280	0.285	0.273	0.267	0.275	0.271	0.272	0.259	0.268	0.261	0.260
C	0.314	0.281	0.290	0.286	0.285	0.260	0.260	0.265	0.257	0.267	0.260	0.270
	0.314	0.281	0.290	0.286	0.285	0.260	0.260	0.265	0.257	0.267	0.260	0.270
D	0.311	0.299	0.286	0.292	0.291	0.297	0.345	0.417	0.541	2.909	0.259	1.578
	0.311	0.299	0.286	0.292	0.291	0.297	0.345	0.417	0.541	2.909	0.259	1.578
E	0.327	0.304	0.288	0.279	0.276	0.296	0.360	0.421	0.560	3.236	0.272	1.462
	0.327	0.304	0.288	0.279	0.276	0.296	0.360	0.421	0.560	3.236	0.272	1.462
F	0.300	0.280	0.280	0.272	0.266	0.283	0.358	0.439	0.568	3.292	0.278	1.431
	0.300	0.280	0.280	0.272	0.266	0.283	0.358	0.439	0.568	3.292	0.278	1.431
G												
H												

Figure 103: Results from UV Spectroscopy reading

Appendix V: CAD drawings for Automated Fibrin Microthread Production Mechanism

Figure 104: CAD image for Automated Fibrin Microthread Production Mechanism Final Design

Figure 105: CAD of 80/20 Frame

Figure 106: CAD of Chain Guiding Platform

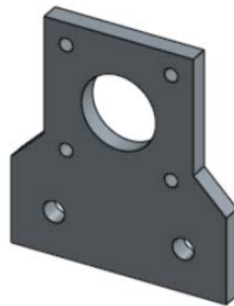


Figure 107: CAD of Stepper Motor 1 Holster

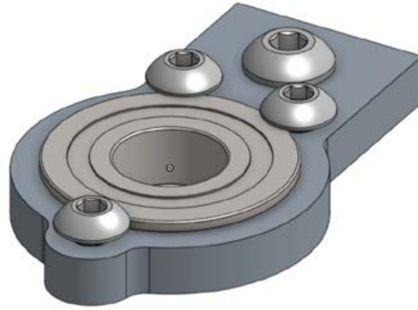


Figure 108: CAD Assembly of Drive Shaft Bracket and Ball Roller Bearing

Figure 109: CAD Assembly of Drive Shaft Bearing Assembly, Sprockets, and Hex Bar

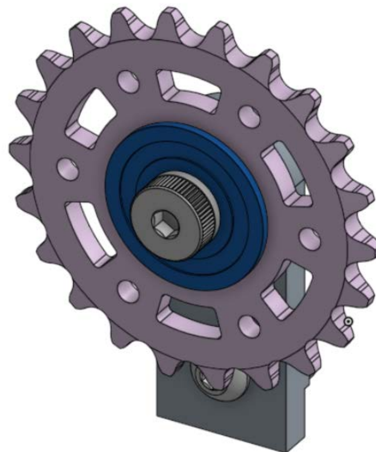


Figure 110: CAD Assembly of Bearing, Bracket, and Sprocket

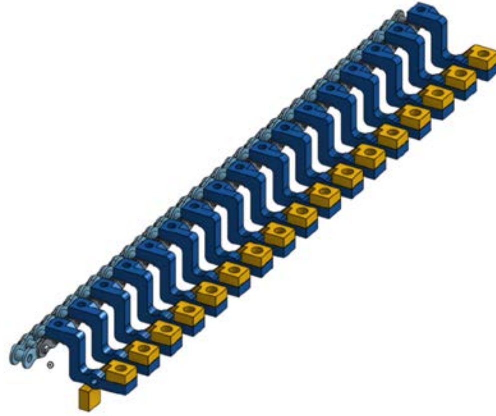


Figure 111: CAD Assembly of Chain and Magnetic Clamps

Figure 112: CAD of 80/20 Gantry Frame

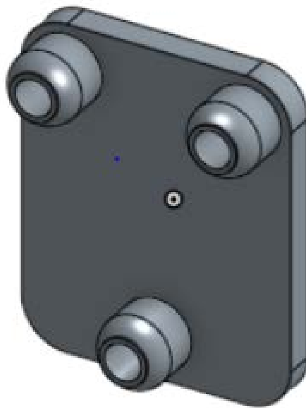


Figure 113: CAD of Slider for Extruder

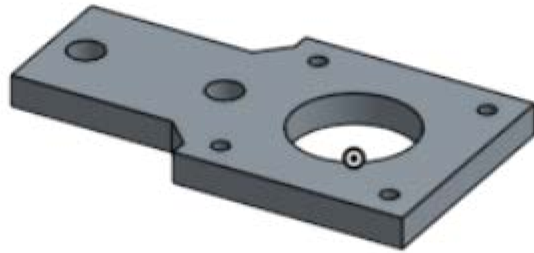


Figure 114: CAD of Holster for Stepper Motor 2

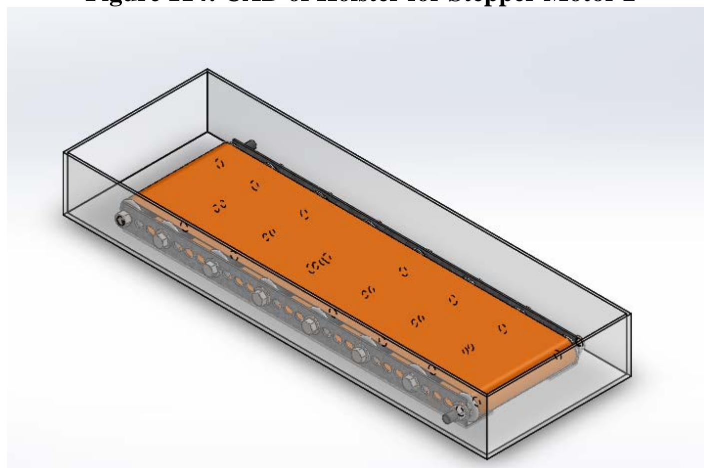


Figure 115: CAD Assembly of Tub and Conveyor

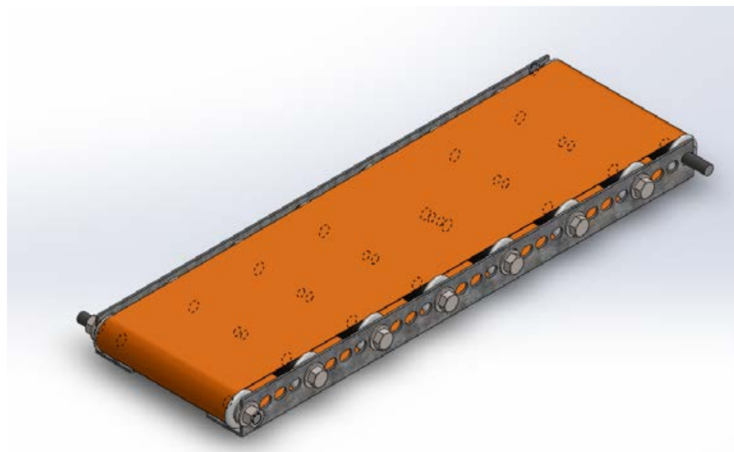


Figure 116: CAD Assembly of Conveyor

Figure 117: CAD of L-frame 1

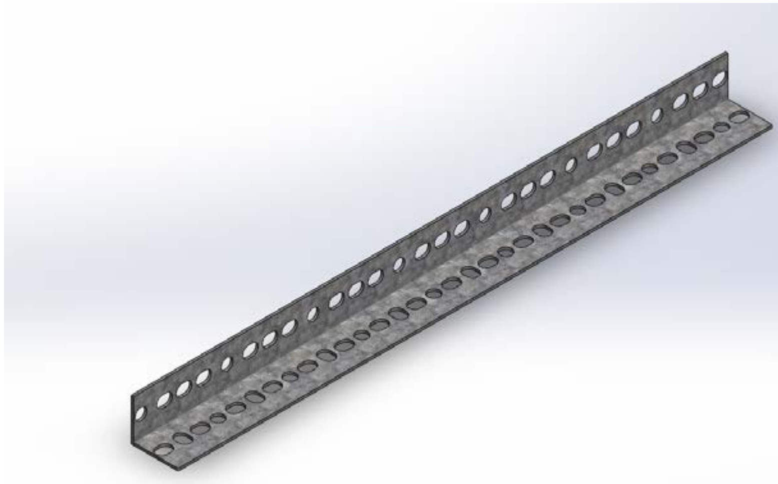


Figure 118: CAD of L-frame 2

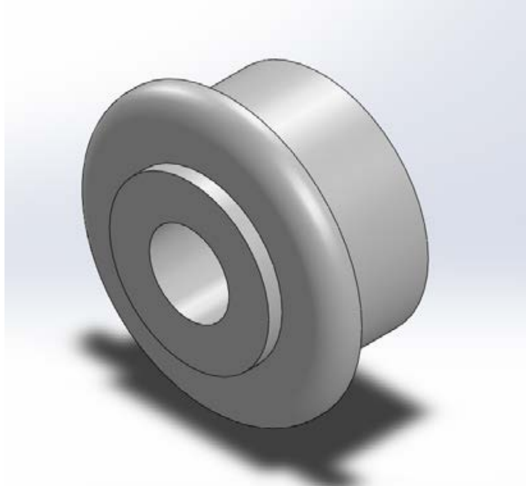


Figure 119: CAD of Bearing for Conveyor Roller

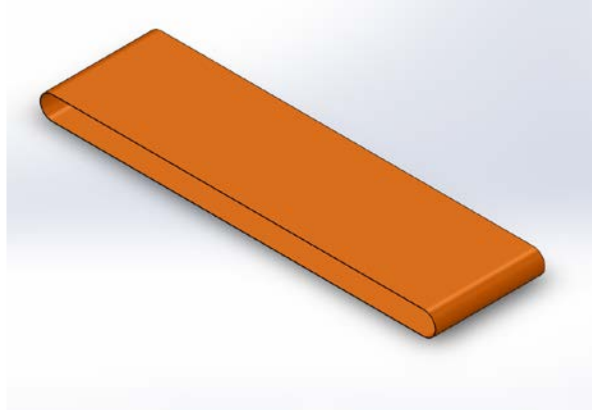


Figure 120: CAD of Urethane Conveyor Belt

Figure 121: CAD of PVC for Conveyor Roller Assembly

Figure 122: CAD of Threaded Rod for Conveyor Drive Roller Assembly

Figure 123: CAD of 3/8 in Hex Bolt

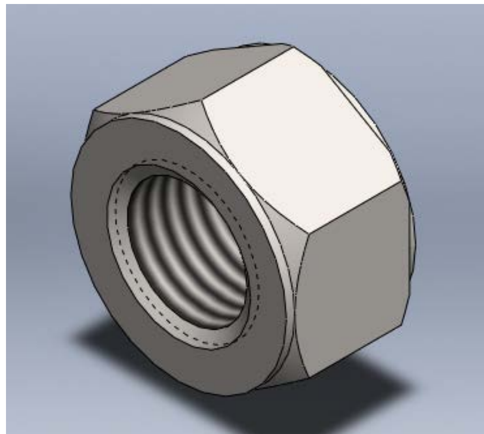


Figure 124: CAD of 3/8 in Hex Nut

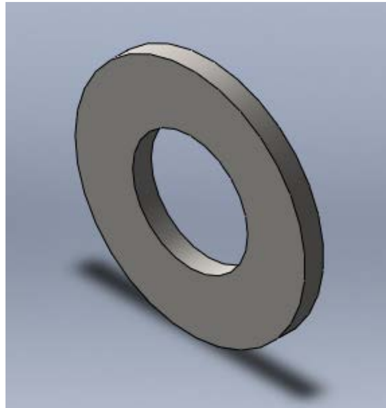


Figure 125: CAD of 3/8 in Washer

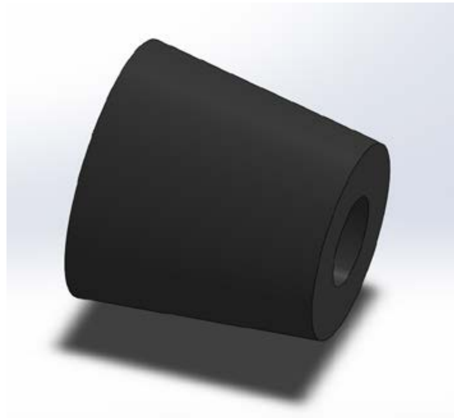


Figure 126: CAD for Rubber Bung to Provide Friction Between Threaded Rod and PVC of Conveyor Drive Roller

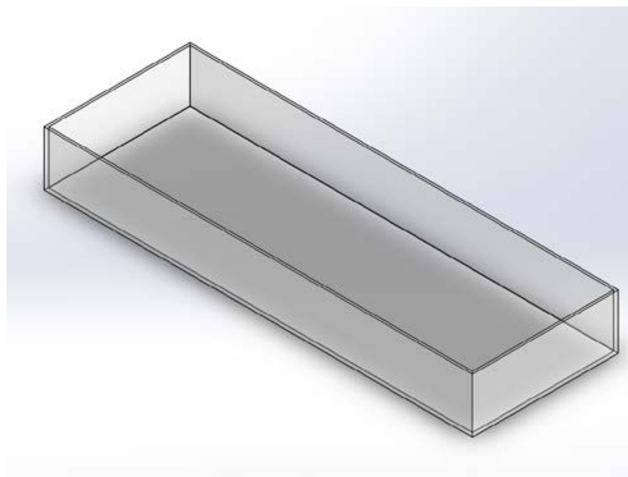


Figure 127: CAD of Acrylic Tub

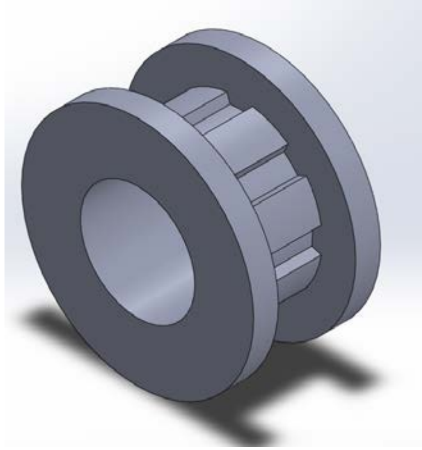


Figure 128: CAD of Pulley for Conveyor Drive Roller for Coupling with Hex Bar

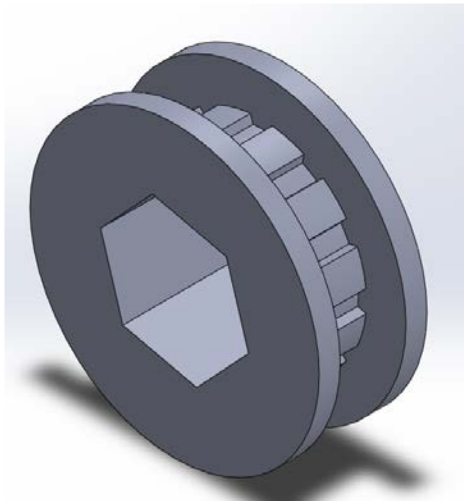


Figure 129: CAD of Pulley for Hex Bar Drive Shaft

Appendix VI: Conversion Preliminary Testing Results and Design Analysis

Table 41 shows the initial imaging analysis of the different conversion methods. The teammates' Design Analysis of the conversion mechanism are shown in Figures 42-43 respectively.

Table 41: Initial Testing Conversion Method Image Analysis - Fibrin Particle Length Measurements

Preliminary Testing of the Conversion Methods		
Method	**Measurement (mm)**	
Grinding or Kneading	0.7	
	0.4	
	0.5	
	0.3	
	0.2	
	0.42	Average
Dual-Action Rotational Disks	1	
**The Full Fibrin Microthreads are not pictured for this method*	0.8	
	3.2	
	2.3	
	1.5	
	1.76	Average
Manual Trimming	0.1	
	0.3	
	0.7	
	0.4	
	0.1	
	0.32	Average
Dual Automated Trimmer Blades	0.3	
	0.4	
	0.1	
	0.2	
	0.2	
	0.24	Average

Table 42: Design Matrices for the other teammates

	Filter System	Stabilize Threads	Bundle Threads	Collect Particles	Safe	Simple	Adaptable	Automated	Total
Weight	*12*	*12*	*11.5*	*16*	*21*	*5*	*6*	*0*	
Design 1	1	3	4	4	5	4	2	2	295
Design 2	1	3	5	4	5	3	2	4	301.5
Design 3	4	3	3	5	5	4	3	2	341.5

	Filter System	Stabilize Threads	Bundle Threads	Collect Particles	Safe	Simple	Adaptable	Automated	Total
Weight	*12*	*12*	*11.5*	*16*	*21*	*5*	*6*	*0*	
Design 1	1	2	3	3	5	4	2	2	255.5
Design 2	1	3	4	4	5	2	2	2	285
Design 3	4	4	5	5	5	4	4	2	382.5

Table 43: Pairwise Comparison Charts for the other Teammates

Pairwise Comparison	Filter System	Stabilize Threads	Bundle Threads	Collect Particles	Safe	Simple	Adaptable	Automated	**Total**
Filter System		0	0	0	0	1	0	1	2
Stabilize Threads	1		0	0	0	1	1	1	4
Bundle Threads	1	1		0.5	0	1	1	1	5.5
Collect Particles	1	1	0.5		0	1	1	1	5.5
Safe	1	1	1	1		1	1	1	7
Simple	0	0	0	0	0		1	1	2
Adaptable	1	0	0	0	0	0		1	2
Automated	0	0	0	0	0	0	0		0

Pairwise Comparison	Filter System	Stabilize Threads	Bundle Threads	Collect Particles	Safe	Simple	Adaptable	Automated	**Total**
Filter System		0	1	0.5	0	1	1	1	4.5
Stabilize Threads	1		1	0	0	1	1	1	4
Bundle Threads	0	0		0	0	1	1	1	3
Collect Particles	1	1	1		0	1	1	1	6
Safe	1	1	1	1		1	1	1	7
Simple	0	0	0	0	0		1	1	2
Adaptable	0	0	0	0	0	0		1	1
Automated	0	0	0	0	0	0	0		0