**The Creation of Active Human Factor X**

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ABSTRACT

 Factor X is important in the process of coagulation in living creatures and the absence of such often constitutes a life or death situation. High risk factors can be assumed if coagulation is not completed in an allotted amount of time. During the coagulation cascade, prothrombin is converted to thrombin by the active Factor X (Xa). If Xa is not present or created in blood, it is unable to clot and stop bleeding. Our attempt to clone Xa by using both mRNA and DNA and to create bacteria that produced Xa, was unsuccessful, but the creation and transformation of one exon, Exon 6, was successful. Great strides were taken to create the other two exons to later be ligated together to complete the Xa gene sequence, but our attempt was not completed.

INTRODUCTION

Coagulation is a process that most people fail to understand or appreciate. It is necessary for the vitality of animals and humans. In order to coagulate, Factor X (also known as the Stuart factor) is a necessity. The active Factor X protein (Xa) reacts with prothrombin to make thrombin. Thrombin, in the coagulation cascade (Figure A) (Raber), reacts with fibrinogen to make fibrin, the cross-linked building blocks that form a blood clot.

Figure : Coagulation Cascade

 Factor X is located on Chromosome 13 and consists of over 26,000 bp and eight Exons. Xa consists of a portion of Exon 6, all of Exon 7 and all of Exon 8 for a total of 721 bp. The active gene sequence is as follows (Accession Numbers L00395, L00396, and N00045):

**atcgtgggaggccaggaatgcaaggacggggagtgtccctggcaggccctgctcatcaatgaggaaaacgagg gtttctgtggtggaaccattctgagcgagttctacatcctaacggcagcccactgtctctaccaagccaagagattcaaggtgagggtaggggaccggaacacggagcaggaggagggcggtgaggcggtgcacgaggtggaggtggtcatcaagcacaaccggttcacaaaggagacctatgacttcgacatcgccgtgctccggctcaagacccccatcaccttccgcatgaacgtggcgcctgcctgcctccccgagcgtgactgggccgagtccacgctgatgacgcagaagacggggattgtgagcggcttcgggcgcacccacgagaagggccggcagtccaccaggctcaagatgctggaggtgccctacgtggaccgcaacagctgcaagctgtccagcagcttcatcatcacccagaacatgttctgtgccggctacgacaccaagcaggaggatgcctgccagggggacagcgggggcccgcacgtcacccgcttcaaggacacctacttcgtgacaggcatcgtcagctggggagagggctgtgcccgtaaggggaagtacgggatctacaccaaggtcaccgccttcctcaagtggatcgacaggtccatgaaaaccaggggcttgcccaaggccaagagccatgccccggaggtcataacgtcctctccattaaagtga**

Discrepancies were found between the Xa sequence from mRNA (Accession Number K01886) and from the actual exon sequence found above. We chose to continue our project with that in mind, but for further analysis and PCR with exons, our sequence to order primers from was the sequence determined by the exons.

The primary goal of our project was to create a bacterial system that will produce the Xa protein in order to facilitate the reaction of prothrombin to thrombin in the coagulation cascade.  We may then be able to test this protein and enter this part in the Biobricks library.

METHODS

*RNA Methods:*

Venous blood from one healthy volunteer was collected on several occasions into heparinized blood collection tubes as well as Yellow-Top (Type A) BD Vaccutainer tubes by trained phlebotomy personnel.

We began by following the RNA Isolation from Human Peripheral Blood protocol created by GMB (Genomic Medicine Biorepository). We then ran a Formaldehyde Agarose (FA) gel (Pitra) to verify that our RNA isolation worked. Gel ran at 70 volts for one hour.

The same RNA Isolation protocol was completed again, making small corrections to the initial procedure. Changes included maintenance of an RNAse free working environment, careful follow-through of protocol precisely as stated (e.g. allowing RBC lysis buffer to fully lyse the cells), and thorough performance of all steps (did not omit ethanol washing steps as did in first attempt).

After obtaining a small, visible white pellet of RNA in three of four tubes via isolation, we continued by adding 50 µl of RNAse-free water to each of the four tubes in order to elute the pellets. Our RNA samples were then run through an FA gel prepared as before for verification.

After confirmation of RNA by FA gel, we ran four reactions through a RT-PCR using the Qiagen One Step RT-PCR Protocol to create many copies of the double stranded DNA from mRNA to later use in transformation. The reaction consisted of two samples with 1 µl of RNA and another two samples with 5 µl of RNA. The following two primers were added to the master mix for our RT-PCR.

Forward Primer #1:

 5’ **TCTAG**ATGATCGTGGGAGGCCAGG 3’

Reverse Primer #1:

5’ **TGATCA**CTTTAATGGAGAGGACGTT 3’

After RT-PCR, all samples, along with the pBluescript vector, were placed through a restriction digest, using the SpeI and XbaI restriction enzymes, to cut both ends for later ligation. The vector was purified using the Qiagen PCR Product Purification Kit and stored at -20ºC. To confirm success of the RT-PCR and restriction digest, all samples were run on a 1.0% agarose gel. Bands were cut and purified with the Qiagen Gel Extraction Purification Kit.

The gel electrophoresis that was run in order to determine if there was any DNA present in our sample was unsuccessful. In order to continue with this aspect of the project we needed to find DNA in our sample so that it could be purified and put through another PCR with additional primers. As a result of this set-back, we proceeded with our experiment by using DNA instead of RNA as our source for obtaining the Xa gene sequence.

*DNA Methods*:

We first isolated and purified the DNA by using the DNeasy Blood and Tissue Kit from Qiagen. We used human blood from the same volunteer that was used in our RNA isolation. We ran two samples with 100 µl of blood each. During elution we added 200 µl of Buffer AE twice to make four different samples in 1.5 ml microcentrifuge tubes. Samples 1 and 2 were from the first elution and samples 3 and 4 were from the second elution. We then ran a 1.0% agarose gel to verify that our DNA isolation worked.

We ordered primers to amplify out our Exons 6 and 8, with overhangs that included parts of Exon 7 to complete a jump template PCR (Figure B):



Figure : Jump Template PCR Diagram

With these overhangs, we planned to do a second PCR by using our Exons 6 and 8 with the overhangs as primers to amplify out Exon 7, which in total would be our whole Xa sequence.

We ordered the following primers (the primer number corresponds to the diagram above):

Primer 1 (this includes three random base pairs which allow XbaI restriction enzyme to attach, the XbaI restriction site, a start codon since the start codon is included in the full Factor X gene, but not our Xa sequence, and the first 20 bp of Exon 6):

ttatctagatgatcgtgggaggccaggaat

 Primer 2 (last 20 bp of Exon6 plus the first 20 bp of Exon 7, all in reverse complement):

tcctcattgatgagcagggcctgccagggacactccccgt

Primer 3 (last 20 bp of Exon 7, plus first 20 bp of Exon 8):

gagattcaaggtgagggtaggggaccggaccacggagcag

Primer 4 (last 20 bp of Exon 8, plus SpeI restriction site, all in reverse complement):

actagtcactttaatggagaggacg

Primer #3 had ability to fold over and bind to itself (Integrated DNA Technologies), so for our PCR, we tested four different annealing temperatures (65°C, 59°C, 55°C and 50°C) to see which was the most ideal. We also tested two different amounts of DNA in our PCRS (10 µl vs. 1 µl) for a total of sixteen PCR products. Verification of all the PCR products was completed on a 1.0% agarose gel. Exon 6 and 8 were purified out of the gel using the Qiagen QIAquick Gel Extraction Kit.

Exon 6 and 8 were then used as our primers in a jump template PCR to amplify Exon 7 to achieve our full length Xa sequence. This would be possible due to the Exon 7 overhangs created in our first set of primers listed above. Seven PCR reactions were run:

1. 0.8µl of each Exon 6 and 8 with 5.0µl of DNA;

2. Negative control with Exon 6 and 8;

3. 0.8µl of Exon 6 and 8, as well as 0.8 µl of Primers 1 and 4 from above (used to try to isolate out the whole sequence including introns) with 5.0µl of DNA;

4. Negative Control with all of the same primers;

5. 2.0µl of each Exon 6 and 8 with 5.0µl of DNA;

6. 2.0µl of Exon 6 and 8 and Primers 1 and 4 with 2.0 µl of DNA

7. Positive Control

Annealing stage was set at 59°C. All PCR products were verified on a 1.0% agarose gel.

A second attempt at our template jump PCR used only Exons 6 and 8 as primers at 50°C, 53°C, and 55°C, as well as separate reactions using only primers 1 and 4 at the same temperatures. The amount of DNA template used in each was 5.0µl. Products were again verified on a 1.0% agarose gel.

Exon 7 is approximately 125 bp, so creation using seven oligo sets was also attempted (Figure C).



Figure : Exon 7 Creation with Oligos

The following primers were ordered:

Primer 1: ctagagccctgctcatcaatgaggaaaacgagggtttctg

Primer 2: tggtggaaccattctgagcgagttctacatcctaacggcag

Primer 3: cccactgtctctaccaagccaagagattcaaggtgagggtag

Primer 4: gatcactaccctcaccttgaatct

Primer 5: cttggcttggtagagacagtgggctgccgttaggatgtag

Primer 6: aactcgctcagaatggttccaccacagaaaccctcgtttt

Primer 7: cctcattgatgagcagggc

To ligate the primers together, 2µl of each of the seven primers were placed in a PCR tube. PCR setup included a “Touch Down” protocol that started the ligation at 70°C and dropped 1°C every minute for 60 minutes. Ligation with the pBluescript vector was completed.

*Transformation Methods:*

We also wanted to gain experience with ligating a gene into a plasmid vector and inserting that plasmid vector into an *E. coli* cell to transform the resulting colony genetically. To ligate the cloned gene from Exon 6 and Exon 8 into a vector we used Promega pGem T-Easy Vector System Protocol and JM109 High Efficiency Competent Cells *(E.coli* DH5α). This system provides us with a color indicator. If the cells contain the plasmid we synthesized, it will turn white, if there isn’t a plasmid containing our gene, the colonies will remain blue.

The first transformations ran utilized the gene fragments for Exon 6 and Exon 8. For the vector ligation protocol, we used 0.5 µl of Easy Vector and 3.5 µl of PCR product. For transformation protocol, we added 10 µl of ligation product to 40 µl of cells.

The second transformation used Exon 7 and the same competent cells, but the pBluescript vector that we purified was used in place of the pGem T-Easy Vector System. Six samples were run including 5 µl of PCR product, 1 µl of PCR product, 7 µl of a 1:10 dilution of PCR product, 1 µl of a 1:10 dilution of PCR product, 7 µl of 1:10 dilution of vector and 1 µl of 1:10 dilution of PCR product. Dilutions were run to determine what concentration of PCR product worked more efficiently.

 Two hundred micro liters of each transformation were plated on LB plus Ampicilin plates and were incubated at 37ºC for 24 hours. The numbers of blue and white colonies were recorded.

One white colony was inoculated into 5 ml of liquid LB media and 8 µl of Ampicilin. A total of six colonies from each of Exon 6 and Exon 8 plates were transferred into media. These were left in the incubator overnight shaking at 250 rpms at 37ºC. Growth was recorded from each tube.

 Tubes that grew were used to make glycerol stocks by adding together 680 μL of cells along with 320 μL of glycerol. These are stored in -80ºC freezer.

The innoculum tubes that grew were also used to create minipreps using the Fermentas Gene Jet Plasmid Miniprep Kit to create purified plasmids.

To lyse the DNA fragments exon 6 and exon 8 out of the plasmid vector, we set up a restriction digest using the restriction enzyme, EcoRI, which can be found on both sides of our gene fragment in the plasmid. Seven microliters of DNA were combined with 1.0 μl of EcoRI.

To lyse the DNA fragments for exon 7 out of our PBluescript vector we used the restriction enzymes XbaI and SpeI We used the same amount of DNA as before, but we chose to use 0.5 μl of each enzyme.

All digests were verified on a 1.0% agarose gel. Ran for 30 mins at 150 volts.

Two samples from Exon 6 and two samples from Exon 7 were sent for sequencing (Iowa State University).

RESULTS

*RNA Results:*

 We initially were under the impression that it was not going to be possible to use DNA, our first choice, to isolate the Xa gene that we were aiming to clone. At the time it was thought that using DNA sequences of the Xa gene was not ideal because the DNA of the entire Factor X gene has over 26,000 bp –far too much for PCR. We also knew that if we decided to work with DNA we would have to deal with a number of exons and attempt to ligate them together. It has become apparent that at that moment in time we did not do a substantial job researching because it turns out that DNA could in fact be used as our Xa consisted of only three exons - a substantial number for isolation. As a result of our initial lack of understanding of the factor X gene in DNA, we decided to obtain the gene from RNA, which provides 721 base pairs for PCR, a number that can be successfully run through the PCR machine.

One vital aspect to working with RNA is the degradation by ever-present RNAses. The keeping of an RNAse-free working environment is crucial to the successful extraction of RNA. RNAses are microorganisms that are found virtually everywhere—from the surfaces in which we work, to the air we breathe and the water we drink. They are nearly inescapable as they are not harmed even by autoclaving. Extra special care was taken throughout this aspect of our experiment in the hope that RNAses would not ruin our wanted sample of RNA. We had to quickly learn how to keep sterility in our work environment, using only RNAse free pipette tips, centrifuge tubes, and solutions. Diethyl pyrocarbonate (DEPC), a solution that binds to RNAses and disables their function was added to both our RBC lysis buffer and our PBS solution at a 1/100 ratio. When running a gel, we had to follow a specific protocol (Pitra), making sure that the entire gel assembly was RNAse free, including the solutions themselves.

Overall, the first attempt to extract RNA from human blood was unsuccessful. After working through the extraction process by following the protocol, we put our samples through a gel in order to confirm the presence of RNA. Unfortunately, our sample showed no evidence of RNA at all. If we did in fact isolate a small portion of RNA, it was less than 1 ng/µl because otherwise there would have been a visible band on our gel at roughly 750 base pairs.

A number of things can help explain why we did not get the results desired. One issue could be that the protocol advised us to re-suspend our pellet using 1 ml of RBC lysis buffer and transfer the contents into a 1.5 ml microcentrifuge tube. This task seemed a bit absurd given the fact that our pellet of red blood cells totaled roughly 4 to 5 ml by itself. We proceeded as depicted in the protocol by adding the 1 ml of RBC lysis buffer and let it stand for 5 minutes to allow the buffer to do its job. Without putting a lot of thought into it, we assumed that we just had way more red blood cells than normal and transferred the small amount of supernatant found on the top of the pellet into two 1.5 ml tubes, leaving the majority of pellet behind. We later realized when attempting our second RNA extraction that we did not give the buffer enough time to lyse the cells. On our second attempt, we took our time, shaking the tube gently and allowing most of the pellet to re-suspend.

 Another minor problem was the appearance of our pellet after lysing. According to the protocol, we were to repeat the lysis steps over again as needed until the pellet was no longer red in color. After adding RBC lysis buffer 10 times during our first attempt, the pellet was still red in color, whereas on our second attempt, we continued the process after four lysing steps, even though our pellet was red in color.

One reason why this extraction did not work the first time could be because of our failure to take extra care in preventing our sample from coming in contact with RNAses. In the initial attempt at extraction, the pipette tips, RBC lysis and PBS buffers, and micro-centrifuge tubes used were not RNAse free. The chance of RNAses coming in contact with our solution at some point was fairly high, rendering it very likely that much of our desired RNA became degraded during the process. Another reason as to why RNA isolation was unsuccessful could be because of the amount of initial pellet used. During our first attempt, only the surface of the pellet obtained in the isolation was re-suspended and used. This cut down on the amount of RNA available for extraction dramatically.

Yet another reason for failure can be found in the washing step of the protocol where we were to rinse the pellet with 0.5 ml of ice-cold 75% ethanol. At the time of our first extraction, we were under the impression that this step was not needed so we proceeded with the experiment by skipping over the washing. It was later discovered that this step is important, as it is needed in removing any salts present in our solution. (Epicentre Biotechnologies).

In the second attempt at RNA extraction from human blood, extra precautions were taken to ensure that we obtained as much product as possible. In addition to using RNAse-free supplies, every step to the protocol was followed as closely as possible. Perhaps most importantly, the entire 4 ml of pellet obtained at the beginning of the experiment was used. This RNA isolation produced approximately 10-50ng/µl of RNA.

 

Figure : RNA Isolation Gel Verification. Lane 1: RiboRuler Slow RNA Ruler, Lane 2: Sample 1, Lane 3: Sample 2, Lane 4: Sample 3, Lane 5: Sample 4

After isolation, we ran our RT-PCR and restriction digest, but found that we did not see any appropriate bands. After over a month of work, we decided to continue our project with DNA in hopes of better results.

*DNA Results:*

Our DNA Isolation yielded enough DNA for the rest of our project. We found that in all of our samples, we obtained approximately 20ng/µl when compared to our ladder in lane 1 (Picture B). We had precise bands at well over 1500 bp, which means that we isolated and purified a very large fragment in each sample, which we used in our PCRs. Since sample one contained the largest concentration of DNA, we chose to use this sample and freeze samples 2, 3, and 4 in the -80°C freezer.



#5 #4 #3 #2 #1

50

1500

Figure : Verification of DNA Isolation. (L to R) Lane 1: Fermentas RiboRuler Low Range DNA Ruler (50, 100, 200, 400, 850, 1500). Ladder, Lane 2: DNA Isolation Sample 1, Lane 3: DNA Isolation Sample 2, Lane 4: DNA Isolation Sample 3, and Lane 5: DNA Isolation Sample 4.)

PCR using our isolated DNA with Primers 1 through 4 to create our Exons 6 and 8 was successful. What seemed to be our Exon 6 at approximately 75 bp was seen at all temperatures and DNA amounts. Exon 8, at approximately 625 bps, could be found at all temperatures and amounts, except 1.0µl at 65°C. This could be due to the high temperature or the folding of Primer 3 back on itself.

 

Figure F: Verification of Exon 6 PCR Products. See Table 1 for Gel Contents.

 

Figure G: Verification of Exon 8 PCR Products. See Table 2 for Gel Contents.

Table : Contents of Exon 6 Gel Verification

|  |  |
| --- | --- |
| LANE: | CONTENTS: |
| 1 | 10.0µl of Fermenta DNA Fast Ruler Ladder (50, 200, 400, 850, 1500) |
| 2 | 8.4µl of DNA at 65°C |
| 3 | 8.4µl of DNA at 59°C |
| 4 | 8.4µl of DNA at 55°C |
| 5 | 8.4µl of DNA at 50°C |
| 6 | 1.0µl of DNA at 65°C |
| 7 | 1.0µl of DNA at 59°C |
| 8 | 1.0µl of DNA at 55°C |
| 9 | 1.0µl of DNA at 50°C |
| 10 | Negative Control |
| 11 | Positive Control |

Table : Contents of Exon 8 Gel Verification

|  |  |
| --- | --- |
| LANE: | CONTENTS: |
| 1 | 10.0µl of Fermenta DNA Fast Ruler Ladder (50, 200, 400, 850, 1500) |
| 2 | 8.4µl of DNA at 65°C |
| 3 | 8.4µl of DNA at 59°C |
| 4 | 8.4µl of DNA at 55°C |
| 5 | 8.4µl of DNA at 50°C |
| 6 | 1.0µl of DNA at 65°C |
| 7 | 1.0µl of DNA at 59°C |
| 8 | 1.0µl of DNA at 55°C |
| 9 | 1.0µl of DNA at 50°C |
| 10 | Negative Control |

The first and second attempts with the jump template PCR reactions to amplify Exon 7 with Exons 6 and 8 as primers were unsuccessful as were the reactions with Primers 1 and 4 to isolate the entire Xa sequence including introns. We found that even our positive control did not work in the second attempt, which in turn shows us that something was wrong in our second PCR as a whole.

*Transformation Results:*

The plate containing Exon 6 had 77% growth of assumed transgene containing plasmids and the plate containing the exon 8 transformation had 75% growth of assumed transgene containing plasmids (Table 3). The fact that there was growth on our negative control leads us to believe that there were mutant cells present in our starting cell stock or there was some contamination in our transformation. This changes the efficiency of our exon plate growth as some of the cells must have developed resistance to Ampicillin and in turn grew on our plate giving us mixed results. The reason our positive control turned all blue instead of white is because we used pBluescript as our vector instead of pGem T-Easy vector. Exon 7 had no blue colonies and we believe that this may have to do with there not being any X-gal added to the plates as we did not make them ourselves. Since time was of the essence, we did not have time to make new plates and regrow them.

Table 3: Colony Count of Transformation LB+Amp Plates

|  |  |  |
| --- | --- | --- |
| Exon/ control | # White colonies | # Blue colonies |
| Positive Control | -- | 1,000+ |
| Negative Control | 30 | -- |
| Background Control | 150 | 5 |
| Exon 6 | 500+ | 150 |
| Exon 8 | 150 | 50 |
| Exon 7 | 500+ | 0 |

We selected our innoculation tubes according to whether they showed turbidity or not. Observations of opaqueness of the tube constitutes turbidity. Turbidity means growth and will tell us if our cells we innoculated the tubes with grew. The tubes that showed growth included all of the exon 6 samples, except 6A, but only 8D and 8F from exon 8. All of Exon 7’s inoculums grew. We created minipreps and glycerol stocks of all the samples that grew.

After our restriction digest to determine if our sequence was there, we analyzed our results on a gel (Figure F). We found that what we believe is our Exon 6 in samples 6D and 6E.



200

3000

#8 #7 #6 #5 #4 #3 #2 #1

Figure : Gel Verification of Restriction Digest of Exon 6 and 8. (R to L) Ran 5μL of each digest product plus 3 μL of 6x orange loading dye for 30 minutes at 150 V. Lane 1: Sample 6B, Lane 2: Sample 6C, Lane 3: Sample 6D, Lane 4: Sample 6E, Lane 5: Sample 6F, Lane 6: 10 μl of Fermentas O’Range 200 bp DNA Ladder (200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 2600, 2800, 3000bp), Lane 7: Sample 8D and Lane 8: Sample 8F.

There seems to have been leakage from lane 6 into lane 7. We wonder if other lanes were mixed together as well. This leakage is a result of not taking the gel comb out carefully before pouring buffer solution into gel electrophoresis machine. In lane 3 and 4 we see a band under 200 bps. Also present around 3,000 bps, our vector can be seen in the same lanes. Lanes 7 and 8 show a loss of production due to over-dilution during the mini-prep synthesis as 350 µL were added to the elution instead of 35 µL. Samples 6D and 6E were sent for sequencing.

 We again attempted to innoculate Exon 8 from both our glycerol stocks and plates, but nothing grew either time. We came to the conclusion that there must have been something wrong with our initial transformation. We found that none of our plasmids contained any of the Exon 8 fragment. Due to time constraints, we were unable to go any further with Exon 8.

 Our restriction digest for Exon 7 showed that there was no gene insert present in any of our plasmids.

 As a result from our sequencing, we found that we did in fact clone and transform our Exon 6 into the vector. This was verified by performing a Blast search on NCBI website.

DISCUSSION

 We have learned much through this process in our hopes of creating a bacterial system that can produce Xa.

 Working with blood can be tricky, but working with RNA can be even worse. Sterile, RNase free work environments must be kept at all times.

Background work must be done thoroughly before planning a project. In our case, we should have looked more into the DNA portion before planning our project using only RNA. We found that DNA is more stable and can be used just as easily if not easier than RNA.

Although most of our project was unsuccessful, we were able to clone and transform Exon 6 into a plasmid, which has never been done before. Although not useful by itself, it does show that the individual exons can be cloned from DNA. If more time would have been available to us, we would have been able to continue our quest in successfully cloning and transforming Exons 7 and 8, which could then be ligated together with Exon 6 to form the complete Xa sequence. If and when this should occur, you could transform this Xa sequence into a bacterial vector, which would in turn hopefully produce the functional Xa protein to turn protrombin into thrombin. Although we do not believe that this will be an immediately available application sometime in the near future, we have proved that it could be a possibility for future scientists.

It has been shown that one can mutate Factor X to create mice that can not coagulate their own blood, it should be available likewise to in turn to the same to mutate Factor X to “turn on” and be able to coagulate (Dewerchin). Once this can be done, others can also looking into evolving Xa to also become an anti-coagulation factor for humans with an over abundance of Xa.

CONCLUSION

The great importance of Factor X cannot be under minded.  Its significance can be depicted in people who have a Factor X, or Stuart-Prower, deficiency (National Hemophila Foundation).  In the rare event that someone inherits this autosomal recessive deficiency, hemorrhagic symptoms are prominent.  The individual may have frequent nosebleeds, easy bruising, soft tissue hemorrhages, and bleeding of the joints.  It is even thought by researchers that the entire absence of Factor X is incompatible to life.

Today, virtually the only way to help patients with this condition is to give them a transfusion of blood plasma.  The major dilemma is this is only a quick fix as the body quickly uses up the Factor X.  If successful, this experiment may be the beginnings of a solution to this problem.  By directly giving a patient Factor X, most likely intravenously or possibly via the capillaries, there would no longer be a need for blood transfusions and cauterizations.  The patient would no longer have to live their entire life in defense.  In order to complete such a task, much study and research would have to go on after this project is finished.  Researchers would have to perfect the procedure and ultimately mass-produce a product that can help Factor X deficient people all over the world.

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