Synthetic production of Factors VIII, IX, and X to speed up coagulation in hemophiliacs

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Hemophilia is a genetic disorder in which blood is unable to clot normally, leading to excessive and severe bleeding. Currently, some existing solutions are clot-preserving medications, fibrin sealants, and physical therapy. Many of these treatments involve replacing the function of an essential blood-clotting protein called Factor VIII, a protein that many hemophiliacs lack, rather than directly restoring Factor VIII. Other treatments involve replacement therapy, which includes providing missing recombinant factors, but complications such as developing antibodies to clotting factors may occur. Therefore, the purpose of this study is to research the deficiency in the blood coagulation pathway and speed up the clotting process in hemophiliacs by creating an applicant that will be applied onto the skin lesion. Those with hemophilia A, one of the most common forms of this condition, have a Factor VIII deficiency, which makes them unable to activate Factor X. This ultimately hinders them from utilizing the enzyme prothrombinase to convert prothrombin to thrombin and fibrinogen to fibrin, which is the main protein involved in blood clotting. Thus, we propose a system that produces Factors VIII, IX, and X in order to speed up the convergent pathway in coagulation. Furthermore, we plan to implement a kill switch as a safety mechanism to control the system's effects. Eventually, this system would be brought inside of the skin microbiome through a topical applicant.

Keywords: Hemophilia, coagulation, Factor VIII, Factor IX, Factor X

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Background

One of the basic functions to maintain homeostasis in the human body is blood coagulation, a series of steps which ultimately leads to reduction of bleeding at the site of an injury. However, in some individuals, the ability for blood to clot is reduced or inhibited completely; an example of such a disorder is known as hemophilia. Hemophilia is a genetic disorder that causes the affected person's blood to not clot properly, leading to severe bleeding from both internal and external wounds. It is estimated that about 400,000 people worldwide suffer from this disease, with hemophilia A occurring in one of every 5,000 live male births. Complications such as deep internal bleeding, damage to joints, infection, and adverse reaction to hemophilia treatments can occur. This usually occurs because blood vessels in hemophiliacs narrow and platelets fail to form a hemostatic plug, causing bleeding to last longer than normal. Furthermore, about seventy-five percent of individuals that suffer from hemophilia do not receive adequate treatment for it (National Hemophilia Foundation 2020).

In order for blood clotting to properly occur, several coagulation factors are required in a cascade of reactions consisting of two pathways: the intrinsic and extrinsic pathways. The intrinsic pathway is activated by vascular system trauma, platelets, exposed endothelium,

collagen, or other chemicals, while the extrinsic pathway is just primarily activated by vascular system trauma. The blood coagulation cascade begins when collagen, a structural protein in the blood vessel, forms complexes with high-molecular-weight kininogen (HK), kallikrein, and Factor XII. These are further converted into Factors IX and VIII, which begins the start of the extrinsic pathway. The intrinsic pathway has a minor role in initiating blood coagulation as patients with HK, kallikrein, and Factor XII usually do not have bleeding disorders. Factors VIII and IX, proteins made by the liver, are essential, as they activate Factor X and begin the coagulation process (U.S. National Library of Medicine 2020a,b). Factor X converts the protein prothrombin into thrombin which then separates fibrinogen into fibrin and activates the platelets in the bloodstream (U.S. National Library of Medicine 2020c, Narayanan 1999, King 2019). The platelets then bind onto exposed collagen from the wound and form a temporary hemostatic plug. Once this loosens and an unstable plug forms, Factor XIII will activate to cross-link the fibrin and strengthen the clot (Smith, Travers and Morrisey 2015, Kaur 2020). Hemophilia A, the most common form of hemophilia, is the deficiency of Factor VIII, and hemophilia B is the deficiency of Factor IX. A rarer version, known as hemophilia C, can also occur due to a factor XI deficiency (Centers for Disease Control and Prevention 2020). See Figure 1 for a visual model of the intrinsic and extrinsic pathways.

Current solutions to hemophilia include gene therapy, injection of deficient clotting factors into the patient's bloodstream, and several types of medication (Doshi and Arruda 2018). The clotting factor concentrates are either derived from human plasma or from a genetically engineered Factor VIII concentrate. A major drawback of gene therapy is the high cost and a lack of worldwide treatment access (Beck 2018). Furthermore, the effects of gene therapy weaken over time. In the past, cryoprecipitate, which is made by thawing recently frozen plasma, has been used as a mainstream blood clotting substance. It is rich in Factor VIII, but there is no method of sterilizing it to eliminate viruses such as HIV in the plasma. Other medications such as Emicizumab and Desmopressin Acetate, also known as DDAVP or Stimate, may replicate or release more Factor VIII into the bloodstream, though they do not replace Factor VIII directly. Because Factor VIII is not actually being replaced, these treatments may not prevent the development of inhibitors, which can hinder the effectiveness of the treatment (Centers for Disease Control and Prevention 2019). Additionally, DDAVP and Stimate will only be effective for hemophiliacs with mild to moderate cases of hemophilia A (National Cancer Institute 2020, Centers for Disease Control and Prevention 2020). Because our system produces Factors VIII, IX, and X, it is a direct supplement for the factors the patient may be deficient in. Please refer to Table 1 for a comparison of different treatments and solutions to hemophilia.

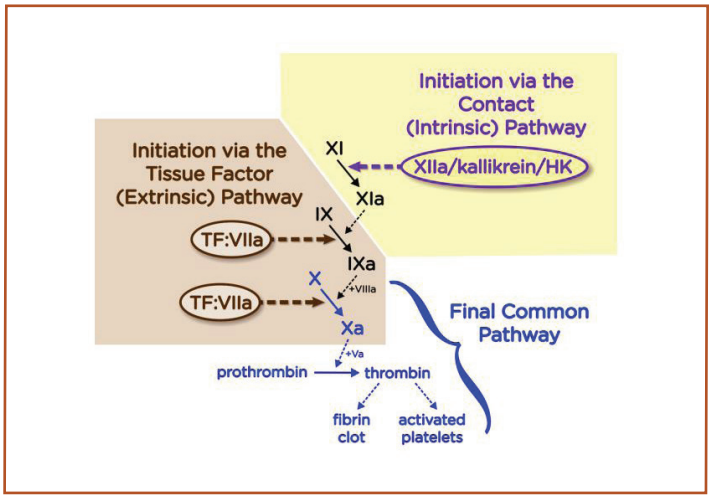

Figure 1. *A simplified visual model of the intrinsic and extrinsic pathways in blood coagulation. As shown in the figure, the two pathways converge to become the final common pathway.*

Table 1. Treatments and Solutions to Hemophilia

Gene Therapy	• Very high cost Gene Therapy • Not accessible worldwide • Effects weaken over time • Administered by weekly transfusions
Cryoprecipitate	• No way to sterilize plasma • Administered by transfusion
Emicizumab	• Does not directly replace Factor VIII, which hinders its effectiveness • Administered by injection
DDAVP/Stimate	• Does not directly replace Factor VIII • Is only effective for patients with mild to moderate cases • Administered by injection (DDAVP) or nasal spray (Stimate)
Our proposed solution	• Will directly replace Factors VIII, IV, and X • Administered by topical spray

Systems level

Our proposed system is designed to activate the coagulation pathway for blood clotting for hemophiliacs. A topical applicant, such as a spray, will be developed for the hemophiliac to put on the open wound in order to directly target the coagulation pathway. The spray-on method of administering the bacteria will be an easier and more cost effective solution compared to other current solutions, as mentioned in the "Background" section. The system will express the F8, F9, and F10 genes, which will ultimately produce Factors VIII, IX, and X. This will ensure that patients with hemophilia A can be treated. The effectiveness of this system will be determined in the future; see the "Future Experimentation" section for further details.

Device level

After much research, we are unable to determine a chassis that will not only be suitable for our system but will also not have any health risks or complications when applied onto an open wound. Therefore, we do not have a chassis for our proposed system. We plan to find a chassis that will be able to effectively transform and express Factors VIII, IX, and X. We may also use multiple strains of E. coli, each producing one of the proteins, so that the order that the proteins are produced in will not unbalance their production as much as only using one strain of E. coli for our chassis. Additionally, the chassis should not introduce any harmful risks or complications to the patient.

Parts level

Our proposed system begins with the constitutive promoter BBa_I14034. By having a constitutive promoter, this will enable continuous transcription of its associated gene. This is essential because it would be difficult to detect an environment where there is an over clotting of blood. This type of promoter is needed because its intent is to take the form of a bacterial spray, which performs the function of coagulation, rather than to detect a site of bleeding. However, once the coagulation process is complete, we would need to utilize a kill switch to destroy the chassis itself. This ensures that the system will not continuously transcribe, even when the production of the clotting factors are not necessary. Please refer to the "Safety" section for more information. The constitutive promoter (BBa_I14034) will be followed by the standard ribosome binding site BBa_B0034. Next, our system will contain the following genes to create Factors VIII, IX, and X: the F8 gene (NM_000132), F9 gene (NM_000133), and F10 gene (NM_000504), respectively. The system will finish with a standard terminator, BBa_B0015. This is a double terminator and the most commonly used terminator for prokaryotes. Even though a kill switch would be present in our proposed system, a terminator is still required so the transcription process continues and terminates. This overall transcription process, from promoter to terminator, will be completely destroyed once the kill switch is activated. Please reference Figure 2 for a visual model of our proposed system.

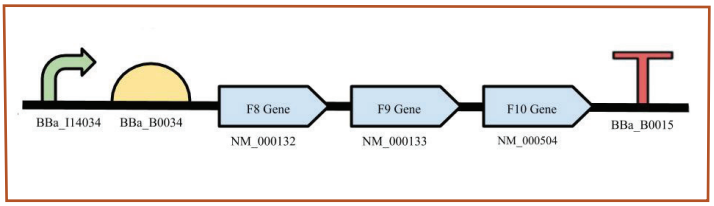

Figure 2. *Our proposed system to produce coagulation Factors VIII, IX, and X. BBa_I14034 is a constitutive promoter that induces the transcription of the F8, F9, and F10 genes to produce Factors VIII, IX, and X. BBa_B0034 is the RBS used in this system, and BBa_B0015 is the terminator in our proposed system.*

Safety

Before we implement our proposed system, we must consider a number of possible health risks and tests in order to determine the construction and safety of our system. Our system plans to increase the levels of Factor VIII, Factor IX, and Factor X in hemophiliacs. However, recent studies have shown that long-term elevation of Factor VIII above a baseline of 150% clotting activity can cause an increased risk of venous thrombosis (Chandler, Rodgers, Sprouse, et al. 2002). Venous thrombosis is the formation of blood clots in blood vessels in deep or superficial veins that impede the blood flow, and can lead to other serious health complications and symptoms (University of California San Francisco Health 2020). Thus, when we begin testing, we must carefully test and regulate the amount of Factor VIII produced by our proposed system to ensure safe levels of Factor VIII in hemophiliacs.

Additionally, we will need to be careful with the actual implementation of our system. Therefore, we will add a kill switch to our system. A possible kill switch to include in our system is the CcdA/CcdB Type II Toxin-antitoxin system. In this system, the ccdA gene produces the antitoxin CcdA in E. coli to block the effects of the toxin CcdB by binding to the toxin (UniProt 2004a, Erental, Kalderon, Saada, et al. 2014). When activated, the ccdb gene can overproduce the CcdB toxin, leading to an SOS response that induces apoptosis-like death due to the destruction of chromosomal DNA (UniProtKB 2004b). Since we do not have a definite chassis, the CcdA/

CcdB Type II Toxin-antitoxin system is only a possible kill switch we could use in our system. We expect that introducing a kill switch will prevent many harmful side effects should our proposed chassis enter the bloodstream. There are also other unknown potential side effects and concerns regarding the implementation of our system which will require careful and lengthy testing (Ansari, Ahmed, Matsangos, et al. 2016).

Discussions

The purpose of this system is to speed up the blood coagulation process in hemophiliacs in order to reduce excessive bleeding. Since the lack of Factor VIII is the most common type of hemophilia, hemophilia A, this system aims to replace the missing factor through the incorporation of the F8 gene and hasten coagulation through the F9 and F10 gene. This system is more advantageous in comparison to current treatments, as this system utilizes the gene encoding the factors necessary for normal blood coagulation. However, treatments such as Plasma-derived Plasma Concentrates directly utilize clotting factors, posing potential harm to the recipient, as the factors are collected from a number of human samples.

The system will be incorporated into a bacterial spray. The reason we chose a spray as the bacterial applicant is because a spray does not require one to have direct contact with the wound; it is also easy to apply the spray onto the wound. However, a downside to using a bacterial spray is that during usage, one might spray an excess amount which might impact the feedback cycle of blood coagulation. Thus, we will need to account for the correct dosage of the spray. In addition, when a spray is applied, the area covered may not be clearly visible, which may cause a part of the wound to be left untreated. There may also be potential issues and complications with aerosolizing our proposed system, so more research must be done in order to determine the efficacy of using a bacterial spray as our applicant.

Future Experimentation

Having completed most of the background research, the next step is to build the system. However, various parts in our system need to be tested individually as all of our research has been theoretical. Once we test that our system works as expected we plan to administer a kill switch in our system to act as a repressor to the BBa_I14034 promoter, possibly implementing the Toxin-antitoxin system. The efficiency and system compatibility of the kill switch with the promoter needs to be studied as the promoter is a constitutive promoter and will require a form of repression once the end of the blood coagulation pathway is reached. Therefore, the kill switch's ability to detect the level of unclot blood in the wound and accordingly attach to the promoter needs to be researched for the system to be successful. This will be a challenging task to accomplish due to the variety of sizes and depths of wounds which makes the task of detecting the level of unclot blood difficult. Mechanisms used inside the human body may be replicated in the system, but making it also accomplish the task of repressing the promoter might pose a challenge.

Furthermore, the issue of internal bleeding within hemophilias will require further research, since a system applied to the surface of a wound cannot address internal complications.

The other parts in our system are ones that are commonly used in synthetic biology, so they should not pose a functional challenge individually. However, testing the parts together is also crucial to the success of the system. If successful, we could potentially test our proposed system on patients with hemophilia A. However, much research still needs to be done in order to begin testing our proposed system and to ultimately test our system on hemophiliacs.

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