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# Correcting FVII Deficiency Using Coagulation Factors Produced *in Vivo* by Encapsulated Engineered Allogeneic Cells

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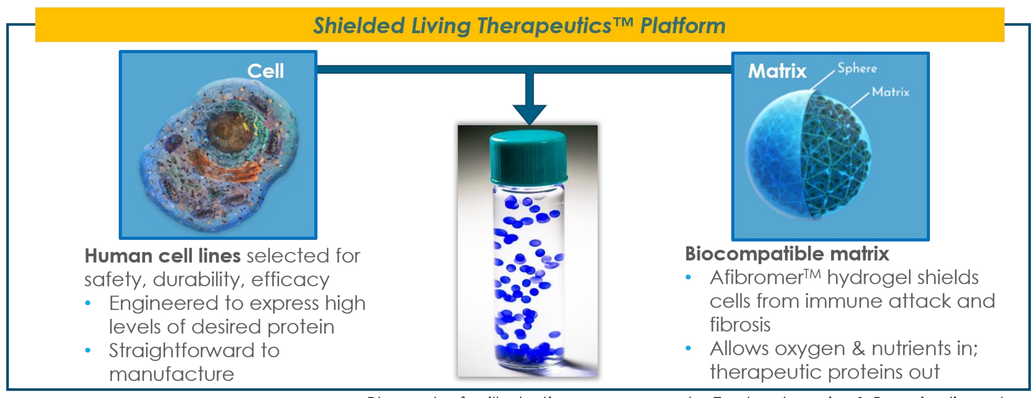
## Introduction

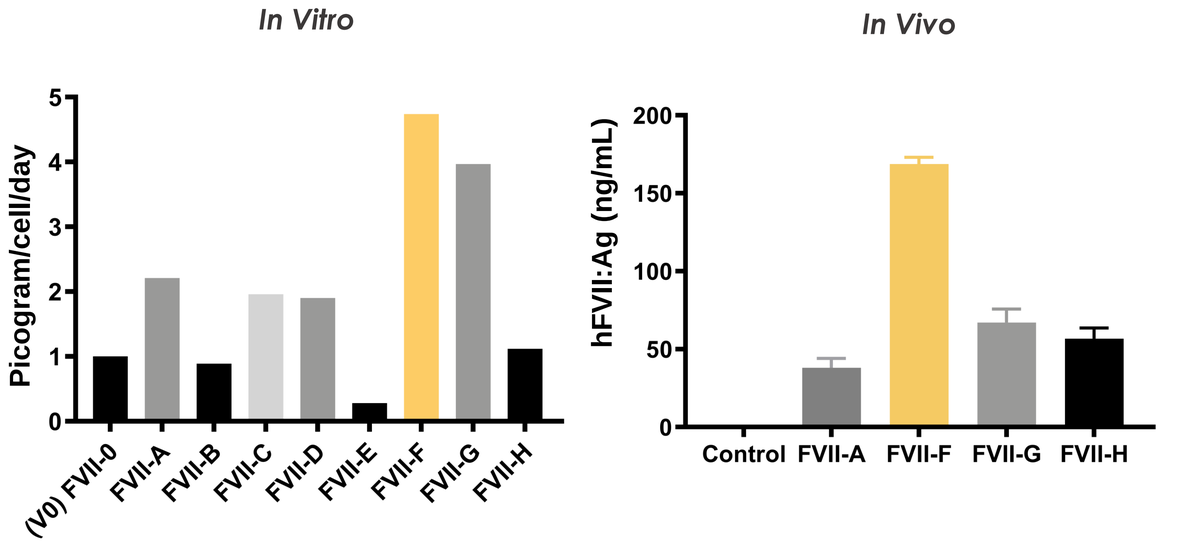
- Factor VII Deficiency is an autosomal, recessive rare bleeding disorder
- Current therapy is primarily on demand due in part to **short half-life of the activated factor**
- There are **no ongoing clinical trials** for novel therapies for this disease (as of 1/2020)
- We are proposing an **innovative approach** using **cell therapy** with **shielded**, genetically modified human allogeneic cells that can deliver **long-term, sustained human FVII zymogen** (hFVII) production *in vivo*
- Delivery of the precursor (zymogen) form of FVII vs the activated form will also allow for **longer availability** in circulation




*Blue color for illustration purposes only; Each sphere is ~1.5mm in diameter*

## Methods and Results

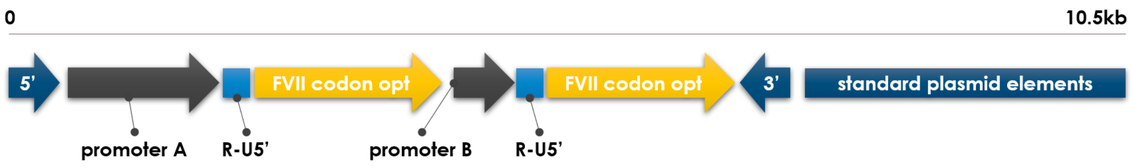
### Figure 1. Construct Optimization for hFVII Zymogen Production




- We generated a range of constructs that express hFVII
- Each was used to transfect the chosen human cell line in order to assess the level of hFVII protein expression *in vitro*
- The protein levels were assessed using ELISA

- Spheres containing cells genetically modified with different hFVII-expressing constructs were administered IP in NSG mice
- Blood samples were collected 14 days after administration
- hFVII antigen levels in mouse plasma were measured by ELISA
- N=3 per group; data are represented as mean+SEM

### Figure 2. Final hFVII Construct in SIG-009




- The final construct used for development of SIG-009 product contains two copies of codon optimized human FVII cDNA, each under control of an independent promoter

### Figure 3. Inner Matrix Optimization for Optimal hFVII Zymogen Protein Levels



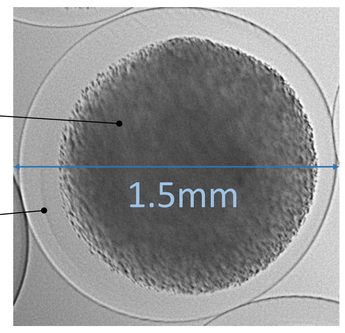

- Spheres containing different levels of the matrix modifier were administered IP in NSG mice
- Blood samples were collected 14 days after administration
- hFVII antigen levels in mouse plasma were measured by ELISA
- N=3 per group; bars show mean+SEM

- A constant total volume of spheres containing different amounts of genetically modified cells were administered IP in NSG mice
- Blood samples were collected 14 days after administration
- hFVII antigen levels in mouse plasma were measured by ELISA
- N=3 per group; bars show mean+SEM

### Figure 4. SIG-009: Cell Line Modified with a Non-Viral Vector to Express hFVII Zymogen, Encapsulated within Alginate Spheres

**inner compartment:**
- **genetically modified cells**
- **alginate matrix**

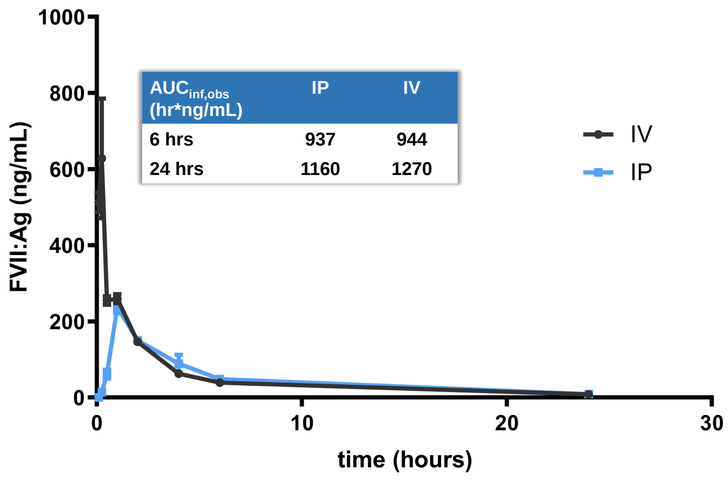
**outer layer:**
- **small molecule modified and unmodified alginates**




Bright field microscope image of a sphere with genetically modified cells

The spheres are sufficiently porous to allow gasses, nutrients, and secreted proteins to freely diffuse

### Figure 5. hFVII Injected IP in NSG Mice Has Similar Bioavailability as hFVII injected IV

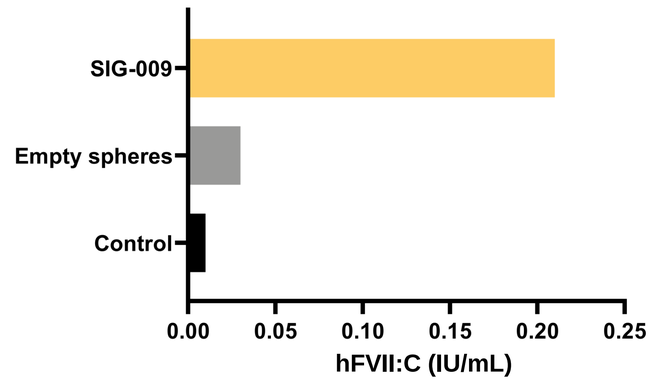



| $AUC_{inf,obs}$ (hr*ng/mL) | IP | IV |
|---|---|---|
| 6 hrs | 937 | 944 |
| 24 hrs | 1160 | 1270 |

- hFVII recombinant protein was injected IV or IP in NSG mice
- Blood samples were collected over time
- hFVII antigen level in mouse plasma were determined by ELISA
- Bioavailability was determined using AUC (inset table)

### Figure 6. hFVII Zymogen Produced by SIG-009 Rescues Clotting Activity of FVII-Deficient Human Plasma

- SIG-009 or empty spheres were incubated in the conditioned medium for 16 hours
- A fraction of the medium was collected and added to the human plasma obtained from FVII-deficient patients
- Clotting activity was measured using FVII chromogenic assay




## Conclusions

- **SIG-009 can deliver dose-dependent plasma levels of hFVII zymogen *in vivo***
- **SIG-009 could reduce the risk of acute bleeds, particularly highly dangerous intracranial bleeds, by delivery of sustained levels of hFVII zymogen**
- **Our technology platform allows for re-dosing if needed, and has the potential for use in pediatric patients without concerns for decreased efficacy due to changes in the size of the liver**

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