# Optimization of Shielded Encapsulated Cell Therapy for Hemophilia and Beyond

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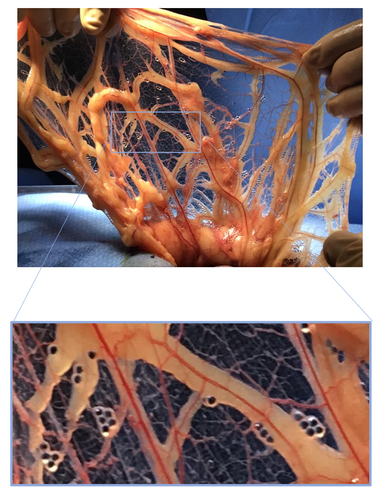
## Introduction

- **Cell therapy** is an attractive option for treatment of various chronic diseases due to the ability to modify the cell genome to express therapeutic molecules.
- Many biomaterials (e.g. alginate hydrogels) can be used to physically shield the allogeneic cells from the recipient's immune system. **These barriers are effective at protecting the cells, but they themselves can elicit a foreign body response resulting in pericapsular fibrotic overgrowth (PFO).**
  - PFO blocks the nutrients from coming in and the therapeutic protein from coming out resulting in a significant challenge to durability, and thus, utility, of this type of therapy.
- Recently, a group of molecules were identified that **significantly minimize the PFO** in rodents and NHPs when conjugated to alginate biomaterials, **allowing the encapsulated cells to remain functional long-term.**[1]



- Sigilon Therapeutics has licensed this technology from MIT and further developed **a category-defining platform to engineer biocompatible, encapsulated cell therapeutics** with the goal of applying it to a **wide range of chronic conditions**.

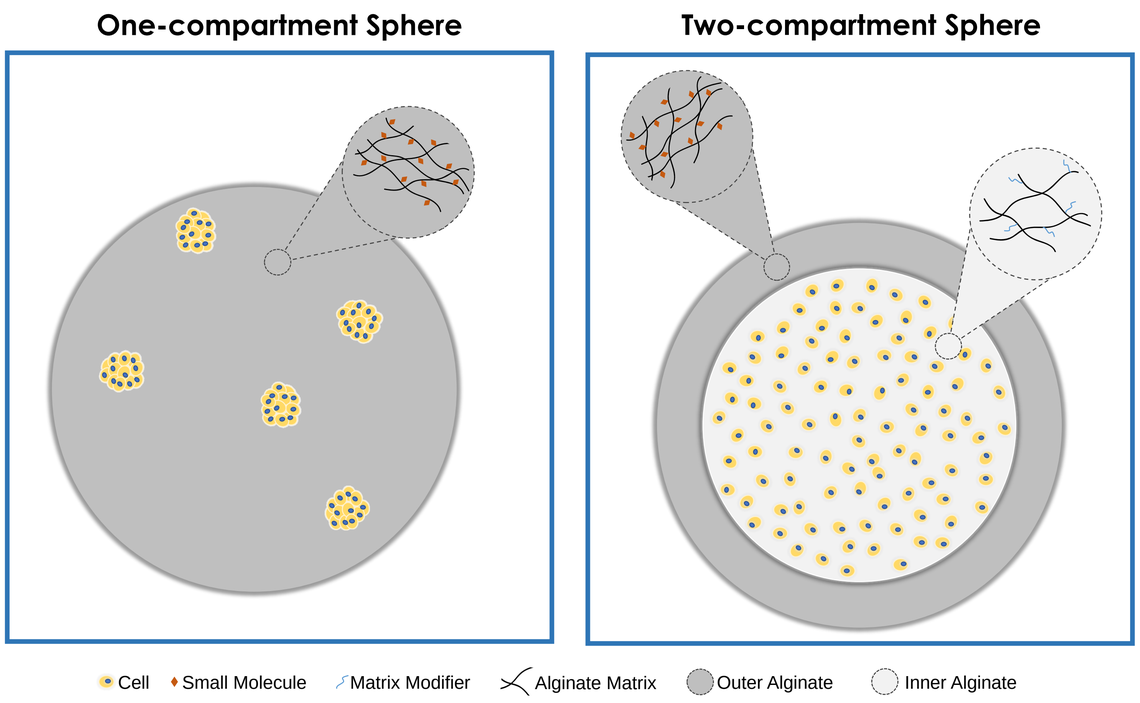
**Objective:**

- **To expand the scope of this technology to a broad range of therapeutic areas.**

1: Bochenek Nat Biomed Eng 2018; *see image for a representative result in NHPs after two weeks*

## Methods and Results

### Figure 1. Two-Compartment Sphere Development



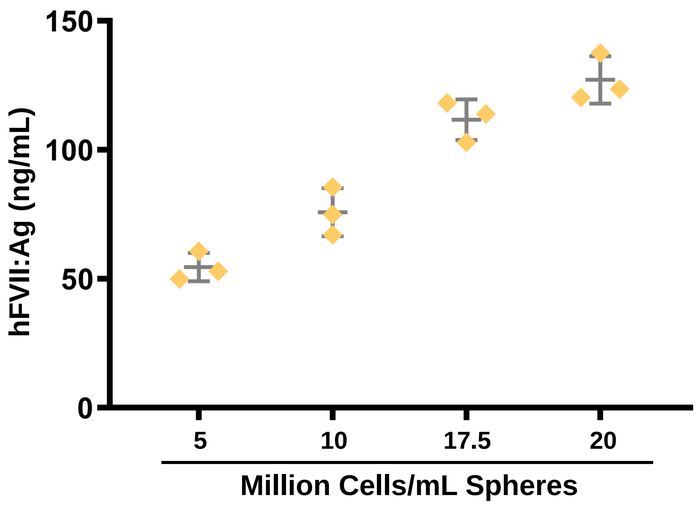

- For the encapsulation technology to be broadly applicable, the spheres needed to be modified for encapsulation of cells capable of expressing a variety of therapeutic proteins.
- The optimal cell line is an adherent, epithelial cell line which has different matrix and cell-contact requirements compared to islets.

**Two-compartment spheres have optimized:**

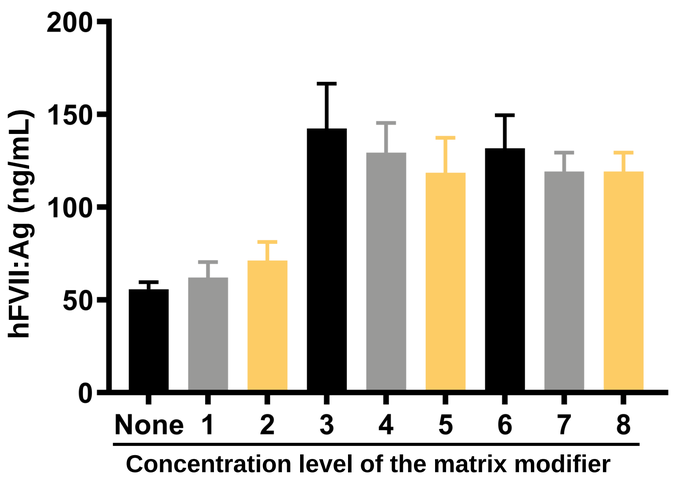
- ✓ Cell architecture and density
- ✓ Inner compartment material
- ✓ Outer layer thickness
- ✓ Outer layer material

### Figure 2. Inner Compartment Cell Density Optimization

- A constant total volume of spheres containing different numbers of genetically modified cells were administered IP in nude 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

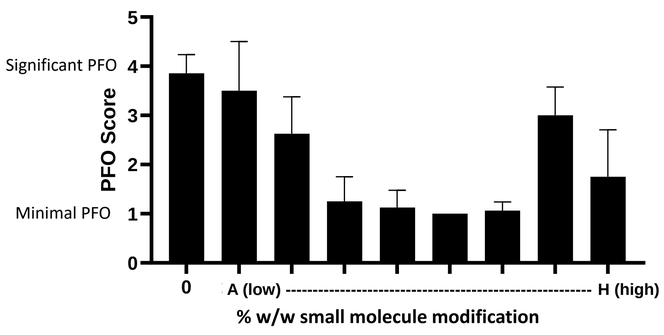



### Figure 3. Inner Compartment Material Optimization

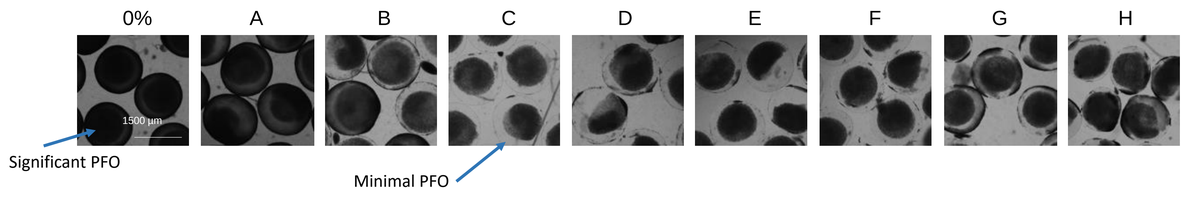



- Spheres containing different levels of the matrix modifier were administered IP in nude mice
- Blood samples were collected 14 days after administration
- hFVII antigen levels in mouse plasma were measured by ELISA
- N=3 per group; bars show mean + SEM

### Figure 4. Outer Layer Optimization




- Alginate polymers were prepared with varying levels of small molecule conjugation (0, A-H).
- Spheres were prepared with 20 million cells/ml of matrix forming solution, and implanted into C57/BL6 mice (n=4 per group).
- Spheres were explanted at day 7 and assessed for PFO using brightfield microscopy.
- Spheres were scored from 1 (minimal PFO) to 4 (significant PFO)



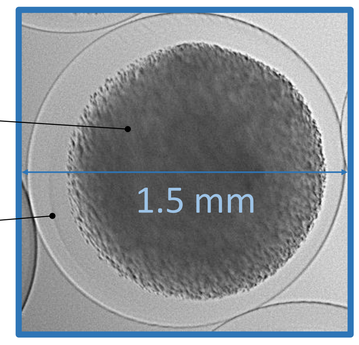

### Figure 5. Final Product: Human Cell Line Modified with a Non-Viral Vector to Express Therapeutic Protein, Encapsulated within Alginate Spheres

**Inner compartment:**

- **genetically modified cells**
- **alginate matrix**

**Outer layer:**

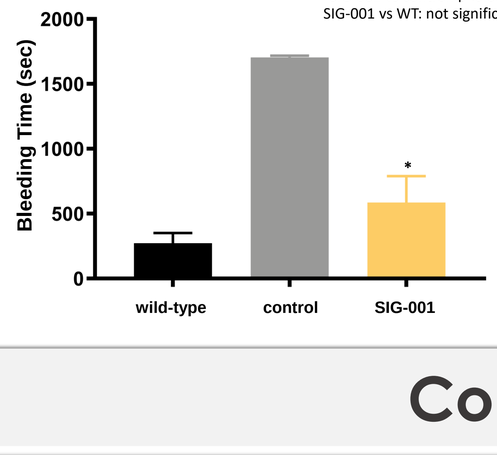
- **small molecule modified alginate matrix**




Brightfield 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 6. Platform in Action: SIG-001 Corrects the Tail Bleeding Phenotype *in vivo* in Hemophilia A Mice[2]




- SIG-001 was placed in the IP space of male FVIII Hemophilia A (HA) mice via laparotomy (SIG-001, n=8).
- Control groups included male wild-type mice (wild-type, n=7), and FVIII HA mice with spheres containing unmodified cells (control, n=8).
- Mice were observed daily and the bleeding time assay was conducted on day 7.

2: Carmona ASH 2019

## Conclusions

- **We have developed an innovative platform that can be used across a broad range of chronic diseases**
- **The novel structure and material components minimize the PFO while maximizing health and protein production of the cells and allow for potential long-term applications of the platform**
- **Preclinical proof of concept shown for hemophilia A, FVII deficiency, MPS I and Fabry**
- **First-in-human clinical trial in hemophilia A to open in 2020**

**ACKNOWLEDGEMENTS:** The authors would like to acknowledge the team at Sigilon Therapeutics for guidance, laboratory work and helpful discussions. The work presented in this poster was fully funded by Sigilon Therapeutics, Inc.

Presented at the 23rd Annual Meeting of the American Society for Gene and Cell Therapy (ASGCT), held from May 12th-15th, 2020 in virtual format.