# A Scaled and Semi-Automated Cell Encapsulation Process for a Shielded Cell-Based Platform for Chronic Diseases

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## Introduction

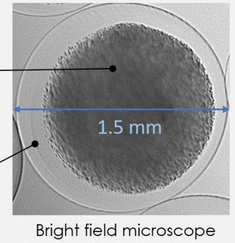
Historically, **allogeneic cell-based therapies** have faced two major challenges:

- Implanted allogeneic or xenogeneic derived cells are quickly rejected by the patient's immune system
- When these cells are protected from the immune system by encapsulation in biomaterials, the biomaterials themselves activate a foreign body response resulting in pericapsular fibrotic overgrowth (PFO) formation

We have previously described our **innovative modular platform** designed to a) support genetically engineered allogeneic cells (which produce therapeutic proteins) and, b) to shield them from the host's immune system (Barney ASGCT 2020):

**Inner Compartment:**
- **genetically modified human cells that express therapeutic protein**
- **modified alginate designed to optimize cell function**

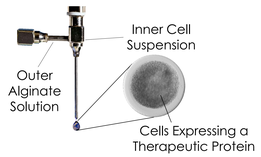
**Outer Layer:**
- **modified alginate chemically linked to small molecule to minimize PFO**




Bright field microscope image of a typical sphere

## Manual Encapsulation Method

- The manual encapsulation method was modified from published methods (Vegas et al., Nature Biotechnology 34 (3) 343-352) to generate two-compartment spheres using a coaxial needle.



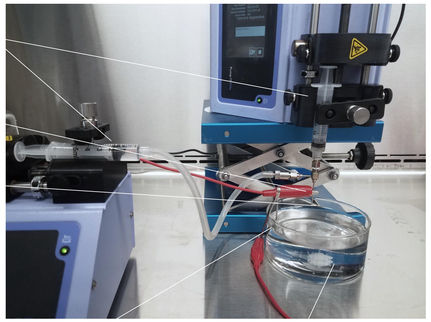

| Manual Encapsulation | |
|---|---|
| Batch Scale | 5 mL |
| Rate | 10 mL/hr |
| Droplet Rate Control | Manual |

Pumps extrude inner and outer alginate solutions at a pre-determined rate

High voltage is applied to the needle tip to accelerate droplet formation rate at tip

Droplets are extruded through coaxial needle

*Voltage can drift during a run which affects sphere formation rate unless manual adjustments are consistently made throughout the process to product a batch with consistent size.*



Bath is grounded

Crosslinked spheres are collected in crosslinking bath throughout the run

The manual system is limited to low batch volumes and requires manual input to maintain control over the droplet rate and resulting sphere size. **An automated and scaled method** was required to supply the first-in-human clinical trial for our lead candidate SIG-001 for hemophilia A (SIG-001-121, Shapiro ASH 2020, NCT04541628).

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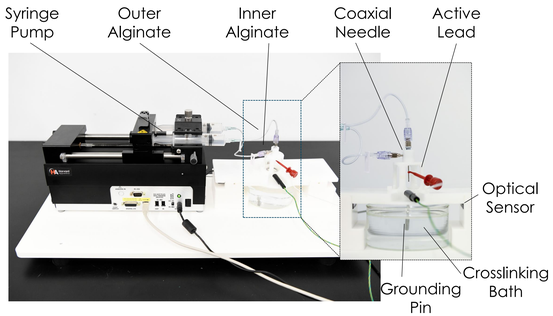
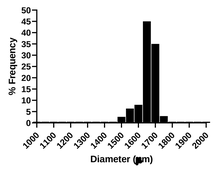
## Semi-Automated Encapsulation Method Development

### A Semi-Automated Method Was Developed to Automate Manufacturing and Increase Batch Scale

- The manual encapsulation method was modified to create a semi-automated batch encapsulation platform, which consists of:
  - A dual syringe pump to extrude the inner and outer polymer solutions through tubing into the coaxial needle
  - A crosslinking bath with custom sterilizable bath cover to hold the needle, sensors, and grounding pin for aseptic assurance and position control
  - Optical sensors to measure droplet rate and automate voltage adjustment using a custom controller (*not shown*)
- With this system, **the batch scale and droplet rate were increased four-fold** compared to the manual process, while maintaining the same total process time.
- The control of the droplet rate and resulting sphere size was automated, resulting in a **narrow distribution of sphere diameter and morphology within a batch**.




| Semi-Automated Batch | |
|---|---|
| Batch Scale | 20 mL |
| Rate | 40 mL/hr |
| Droplet Rate Control | Automated |

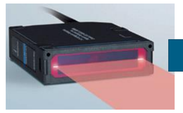


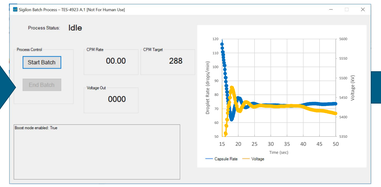

### Software Was Developed to Automatically Adjust Voltage to Maintain a Droplet Rate at Set Point

- Software with a graphical user interface was developed to run the system and automatically control the droplet rate.
- The droplet rate is measured by a droplet sensor, and a PID loop controls the voltage to maintain a consistent droplet rate, resulting in a narrow diameter distribution within the batch.

PID Controller with Graphical User Interface

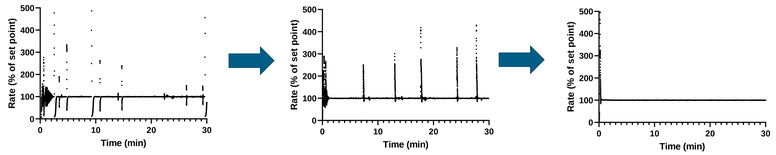
Optical Sensor



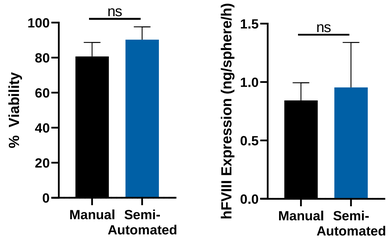



Voltage Supply

Control software was optimized to maintain a **consistent droplet rate.**




### Manual and Semi-Automated Batch Methods Produce Comparable Spheres




- Spheres were produced using the manual and semi-automated methods, and critical quality attributes were measured.

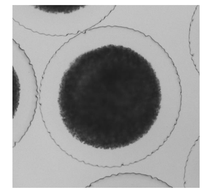
Spheres produced in the manual and semi-automated procedures have **equivalent viability and potency**.

### Droplet Rate Can Be Further Increased By 2-Fold Without Impact to Sphere Morphology

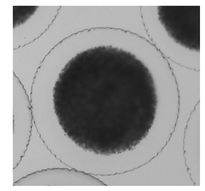
- Further increasing the droplet rate will allow for even greater volume of product to be produced within one manufacturing run.

Droplet rate was increased 1.5-fold and 2-fold from the baseline and **concentric spheres with acceptable morphology were produced.**

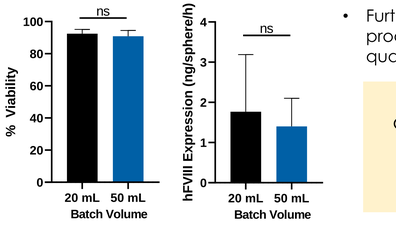
**1.5x Increase in Droplet Rate**



**2x Increase in Droplet Rate**



### The Scale of Semi-Automated Batch Encapsulation Can Be Further Increased 2.5-Fold




- Further scaling the batch volume will enable production of both larger doses and greater quantities of doses.

Batch volume was scaled an additional 2.5-fold to 50 mL with a consistent droplet rate, and **the resulting spheres had equivalent viability and potency.**

## Conclusions

- The increased sphere formation rate achievable in this system **allowed an order of magnitude increase in the volume of spheres** that can be produced while maintaining a short residence time in the crosslinking bath, **without impact to sphere quality.**
- The semi-automated manufacturing method described here is being **used for the ongoing first-in-human clinical trial of SIG-001 in hemophilia A.**
- There is ongoing work to further scale and automate the manufacturing process for future manufacturing for the hemophilia A program, and for our pipeline programs in other rare blood disorders, lysosomal disorders, type 1 diabetes, and other serious chronic illnesses.

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