

Analytical Development Considerations for the Shielded Living Therapeutics™ Platform: A Novel Encapsulated Cell-Based, Non-Viral Platform for Delivery of Therapeutic Proteins

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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

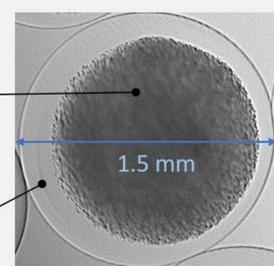
We have previously described our **innovative modular platform** designed to a) support genetically engineered allogeneic cells (which produce therapeutic proteins) and, b) to shield them from the host's immune system (Barney ASGCT 2020):

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

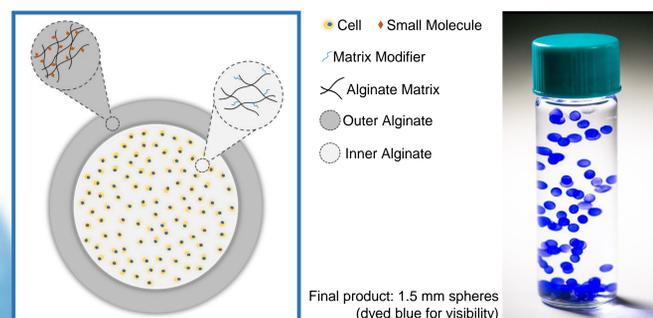


Bright field microscope image of a typical sphere

Analytical Support

- The **novel structure and material components used for cell encapsulation** minimize PFO while maximizing health and protein production of the cells and allowing for potential long-term applications of the platform
- To ensure highest quality of our product candidates, **we developed a novel set of critical quality attributes (CQAs)** for each sphere component as well as the drug product.

Raw Materials	Intermediates	Encapsulation	Drug product
Alginate	Modified alginate (inner compartment and outer layer)	Empty sphere	Sphere with encapsulated cells
SM modifier (outer layer)			
Matrix modifier (inner compartment)			
Cell bank/plasmid	DS: Genetically modified cells		



Final product: 1.5 mm spheres (dyed blue for visibility)

SM: small molecule; DS: drug substance; DP: drug product; CQA: critical quality attribute; MW: molecular weight

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Results

CQAs of Modified Alginates

Modified alginate solution:

- Alginate is a mix of linear polysaccharides
- Important to identify and understand properties of alginate materials
- Our DP contains modified inner compartment and outer layer
- Physicochemical properties of the alginate conjugates are critical to controlling quality of the DP

Category	Attribute	Rationale
Modification level	• Ratio of SM/alginate conjugates	Outer layer directly accounts for immune response reduction and PFO minimization.
		Inner compartment affects cell function, such as viability, and expression of the therapeutic protein.
Polymerization; MW distribution	• Viscosity	Alginate size influences sphere formation and <i>in vivo</i> performance as the physical shield.
Solution property	• Concentration	Physical properties of the solutions affect sphere performance and thus the function of encapsulated cells.
	• pH	
Safety	• Osmolality	DP safety.
	• Appearance	
Safety	• Process-related impurities	DP safety.
	• Product-related impurities	
	• Biosafety	

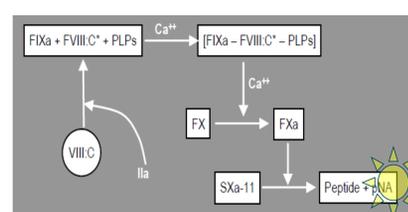
CQA analytical assays:

- Ratio of outer layer SM/alginate conjugate
- Ratio of inner compartment matrix modifier/alginate conjugate
- Conjugate concentration (inner and outer)
- Viscosity
- Residual SM
- Conjugation reaction intermediates and other process-related impurities

CQAs of Drug Products

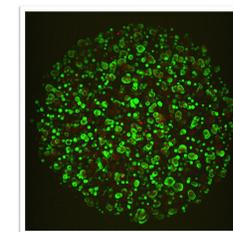
Characterization of DP includes (1) expression and release of drug substance from encapsulated cells and (2) physicochemical property of the sphere

Protein Activity



hFVIII Chromogenic Assay (Hyphen BioMed: Biophen product insert)

Cell Viability



Cell viability in the explanted sphere after 6 months

Category	Attribute	Rationale
Potency	• Protein activity	Cell viability, expression of the functional protein in the genetically modified cells, and its secretion from the sphere are critical attribute of the drug product.
	• Protein expression level	
	• Cell viability	
	• Viable cell number	
Morphology	• Defective spheres	Sphere morphology, size, integrity are important attribute of sphere quality.
	• Sphere size	
General Characteristics	• Mechanical strength	These attributes are critical to maintain the sustainable functions of the encapsulated cells.
	• pH	
	• Osmolality	
	• Sphere concentration	
Safety	• Purity	DP safety
	• Process- and product-related impurities	
	• Biosafety	

CQAs of Spheres

Mechanical Properties of Spheres

Initial Fracture

Drop in force past a threshold to represent the first fracture point during stress

Brittleness

Distance at which the initial fracture is measured

Absolute Positive Force

Maximum force up to catastrophic failure of the sphere

Recovery

Morphology recovery over time after compression at a specified level (without breaking the sample)

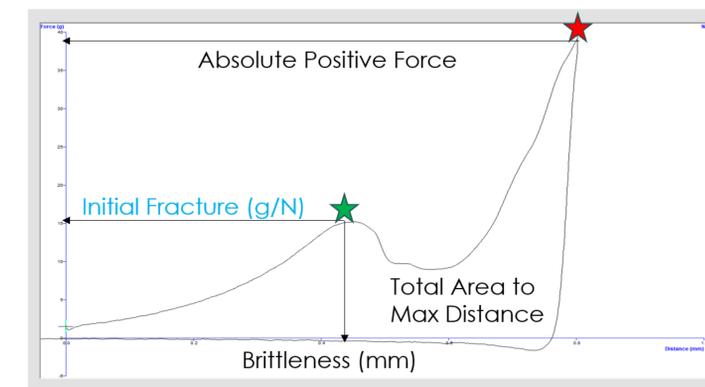
Elasticity

% force relative to initial (100% = perfectly elastic)

Relaxation

Force change during compression at a specified level for a specified time period (without breaking the sample)

Force (g) vs distance (mm)



Conclusions

- Emerging new drug modalities need novel analytical strategy and techniques to support evaluation of product safety, quality, and efficacy
- We have **developed a variety of analytical techniques to characterize modified alginates, spheres, and drug product to support the analytical development of the SLTx platform**
 - Ratio and concentration of alginate conjugate
 - Mechanical properties of sphere
 - Process and product-related impurities
 - Drug substance and drug product release assays

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