

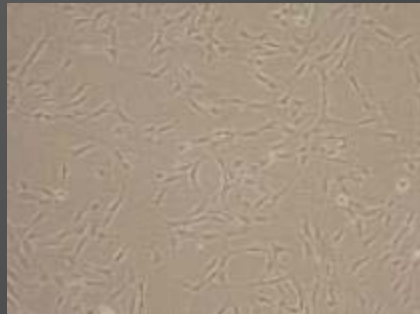
# CLOSED CULTURE SYSTEMS

## CLINICAL PRODUCTION POINTS TO CONSIDER WHEN IMPLEMENTING THE WAVE BIOREACTOR

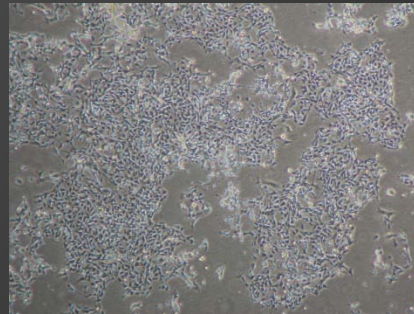
Scaling Up Cell Culture: Application of Closed Cell Culture  
Systems in Clinical Research

PACT Webinar - May 8, 2014

Darin Sumstad, CLS-Technical Lead



MSC's



Glioblastoma



UCB T-Regulatory Cells

# Disclaimer

- ⦿ This discussion will be based on the implementation of the GE WAVE bioreactor system in our production processes. The speaker does NOT endorse the specific purchase of this or any other equipment discussed.

# Objectives

- Discuss WAVE platform
- Outline techniques utilized for WAVE implementation into existing academic cGMP production lab
- Discuss supporting equipment used to make this process easier.
- Discuss “lessons learned” and solutions to production bottlenecks.
- Questions?



# History

- ◎ Why evaluate a WAVE BioReactor?
  - Current processing workflow will allow for expansions in excess of 20 billion cells - the 'old fashioned' way
    - High sterility risk
    - Extremely labor intense
    - PI's always want more.....
  - Incoming external projects already established on platform

# History



VS



Space saving, processing time, and a decrease in contamination risk make bioreactors an attractive alternative to traditional culture flasks.

Provided by



# History



## ○ Evaluate Current Production Processes

### ● Traditional T-Flasks, Cell Factories, Culture Bags

#### ○ Open System

- Internal processes developed to 'close' as much as possible (Rigging sets, etc.)

#### ○ Equipment

- Standard – CO2 incubator, PVC tube sealer, Sterile connecting device (SCD)

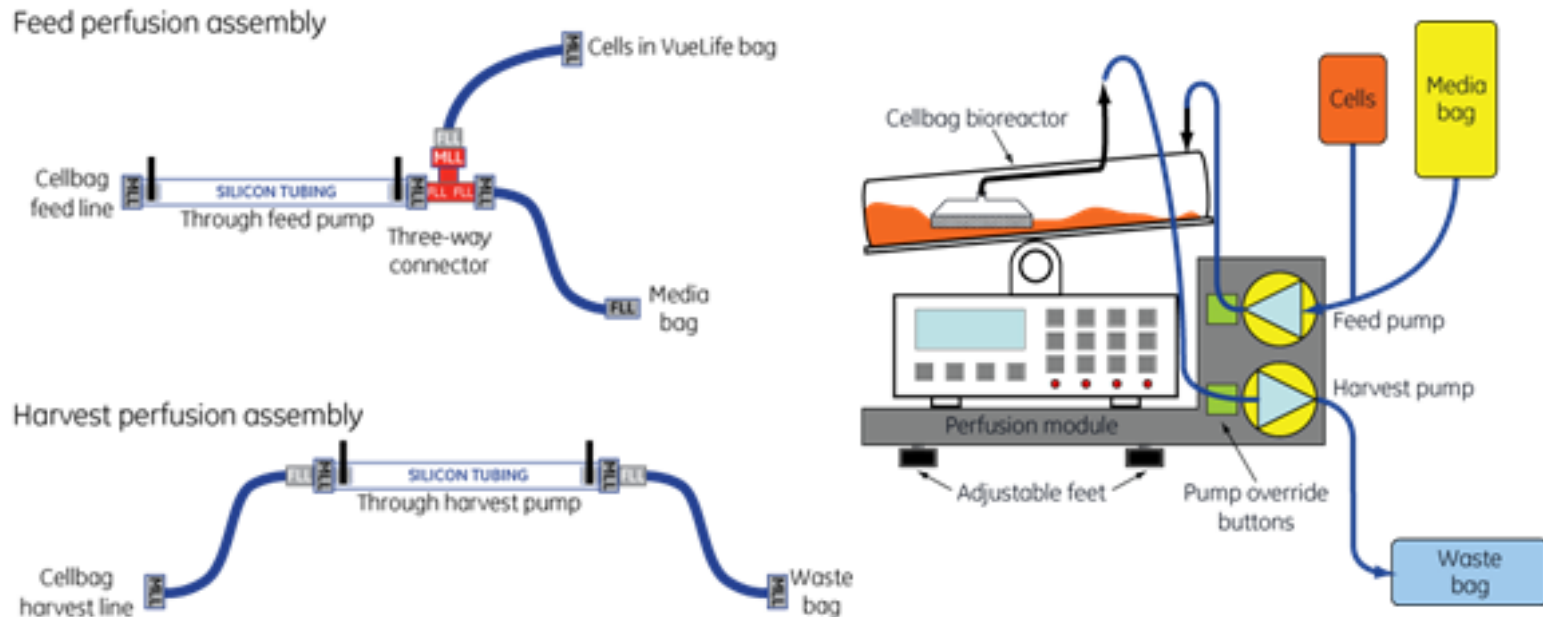
#### ○ Media Requirements

- Low/Medium – Max required ~ 50L



# Getting Started

## The WAVE Bioreactor™ 2/10 system and perfusion setup



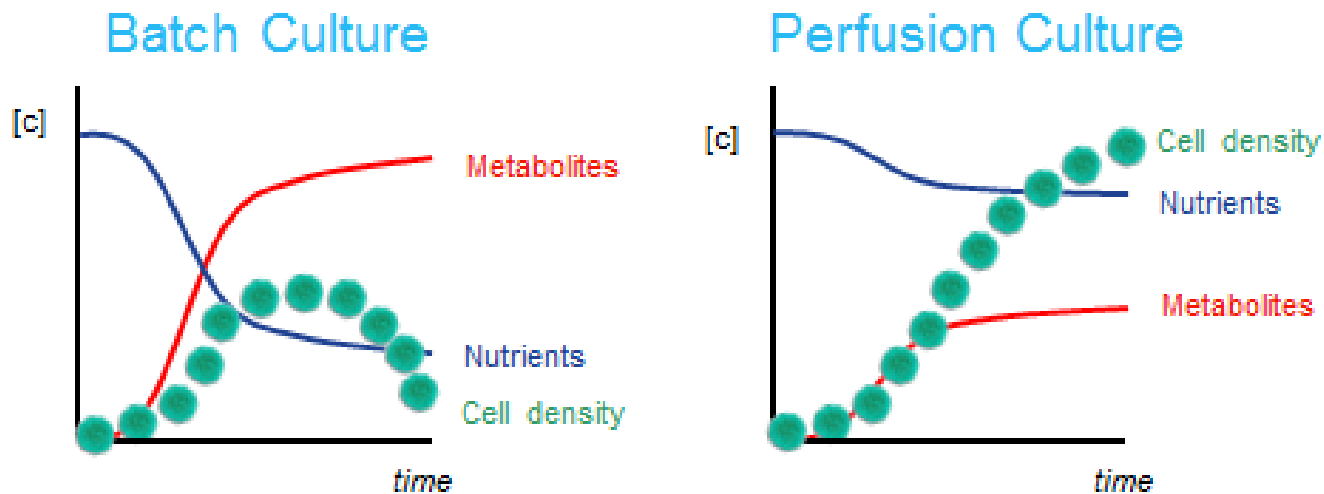
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# Getting Started

## The WAVE™ Bioreactor

Replacement of essential growth nutrients



Provided by





# Getting Started

- ◎ New processing workflows would be required to accommodate our evolution into large scale bioreactor processing
  - Equipment management
  - Media preparation management
  - Fluid transfer management
  - Training

# Getting Started

## ⦿ Materials Compatibility

- Can already stocked items be used for the culture?
  - Review supplied production procedure and/or provide researcher with a spreadsheet template to complete.
  - Compare with available materials and re-distribute for acceptability approval
- Not all materials will be available in-house

# Materials Compatibility

## PACT Cells - Required Materials

**\*Please complete as thoroughly as possible\***

### Media Supplies:

<u>Description</u>	<u>Vendor</u>	<u>Catalog #</u>	<u>Notes</u>
DMEM	Gibco/Invitrogen	12440-053	
FBS	Hydrex	SH30070.03	Using lot ARD26314
L-glutamine 100x	Gibco/Invitrogen	25030-081	
2-Mercaptoethanol	Gibco/Invitrogen	21985-023	
Gentamicin	Quality Biological	120-098-031	
Collagenase IV	Sigma	C1889	
D-MEM/F- 12	Gibco/Invitrogen	11330-057	
Fibronectin	Becton Dickinson	356008	Human natural
Trypsin 0.05%	Gibco/Invitrogen	25300-054	
Versene	Gibco/Invitrogen	15040-66	
PBS	Gibco/Invitrogen	10010-023	

# Materials Compatibility

## PACT Cells - Materials Compatibility

Media Supplies:	Cell Therapy				Research Lab		Acceptable?
	Specification	Description	Manufacturer	Man Catalog #	Manufacturer	Catalog #	
	CH138879	IMDM	Gibco/Invitrogen	12440-053	Gibco	12440-053	☑
	CH100089	FBS	Hydrex	SH30070.03IR	Hydrex	SH30070.03	☑
	CH100014	L-glutamine 100x	Gibco/Invitrogen	25030-081	Gibco/Invitrogen	25030-081	☑
	CH100134	2-Mercaptoethanol	Gibco/Invitrogen	21985-023	Gibco/Invitrogen	21985-023	☑
	CH100138	Gentamicin	Quality Biological	120-098-031	Quality Biological	120-098-031	☑
	CH100139	Collagenase IV	Sigma	C1889-50MG	Sigma	C1889	☑
	CH100133	D-MEM/F-12	Gibco/Invitrogen	11330-057	Gibco/Invitrogen	11330-057	☑
	CH100140	Fibronectin	Becton/Dickenson	358008	Becton Dickson	358008	☑
	CH100141	Poly-D-Lysine	Sigma	P1024-10MG			☑
	CH100135	Trypsin 0.05%	Gibco/Invitrogen	25300-112	Gibco/Invitrogen	25300-054	☑
	CH100136	Versene	Gibco/Invitrogen	15040-066	Gibco/Invitrogen	15040-66	☑
	CH100137	PBS	Gibco/Invitrogen	10010-049	Gibco/Invitrogen	10010-023	☑
	CH100807	dH2O	Gibco/Invitrogen	15230			☑

# Media Management

## ◎ Base Media Configurations

- Traditionally offered in bottles – not ideal for closed system processing
- Aseptically transferring from hard to soft wall vessel is challenging



# Media Management

## ◎ Base Media Configurations

- Most media manufacturer's will 'custom fill' into bags, but this requires a substantial initial investment of media (300 - 400L) to be economically viable
  - If custom fill is an option for institution, media preparation / storage of large volumes of media may be difficult
  - High risk –
    - Premature trial closure – large monetary value tied up in media – that will eventually expire!
    - Storage – Manufacturer may not be willing to store
      - Current capacity to hold on-site
      - Additional capital equipment investment
      - Institutional liability – power loss, equipment failure, etc.

# Equipment

## ⦿ Equipment Requirements

- Bioreactor
  - Multiple required – coverage required for downtime
- Tube Sealer / SCD – larger tubing not compatible with current models
- Accessory equipment
  - Uninterrupted power supply(UPS), real-time equipment monitoring, CO2 supply access



# Equipment

## Large volume cell harvest

- Additional equipment is required for the efficient processing of final cultured product.
  - Traditional centrifuge not optimal
  - CellSaver 5+, Cobe 2991, LOVO, etc.
- Validation required





# Equipment

	Cobe 2991	Cell Saver	LOVO
Max Process Volume	Unlimited*	Unlimited*	5L*
Observed NC Recoveries	> 70%	> 80%	> 95%
Process Rate	125 ml/min**	250 ml/min	150 ml/min
Min Final Product Volume (mL)	50	70	50
*Theoretical / > 10L processed on LOVO with similar results			
**3 min centrifugation time			

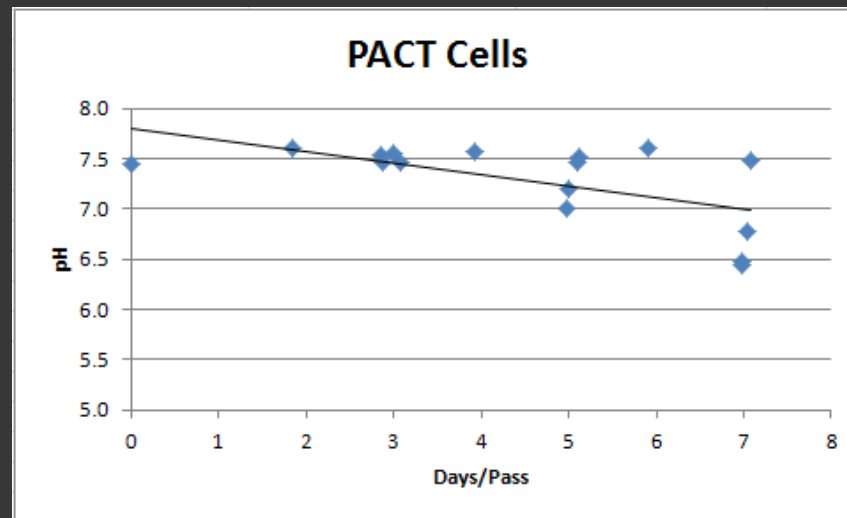
- ⦿ Optimization work required - dependent on cell types / load concentration

# Equipment



## ● BioProfile 400\*

- pH, pO<sub>2</sub>, pCO<sub>2</sub>, Na<sup>+</sup>, K<sup>+</sup>, Gluc, Lac, NH<sub>4</sub><sup>+</sup>, Glu, Gln, Osm



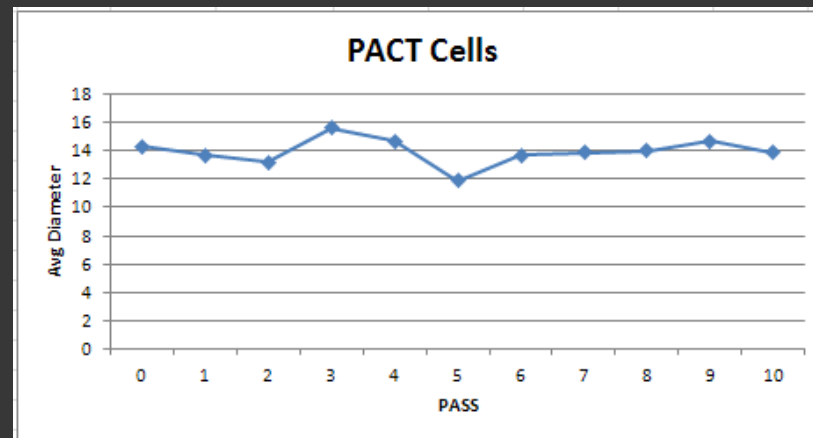
\*Information obtained from Nova Biomedical

# Equipment



## ● Vi-Cell XR\*

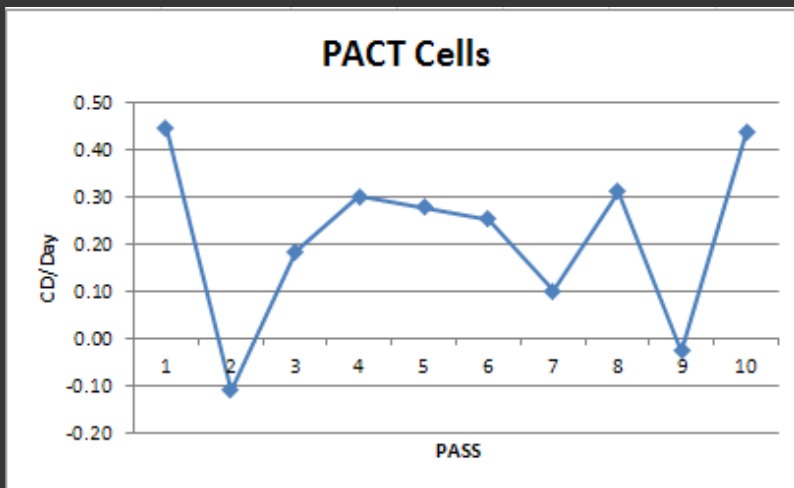
- Automation of the standard trypan blue assay
- % Viability
- Total cell concentration
- Total viable cell concentration
- Mean cell size



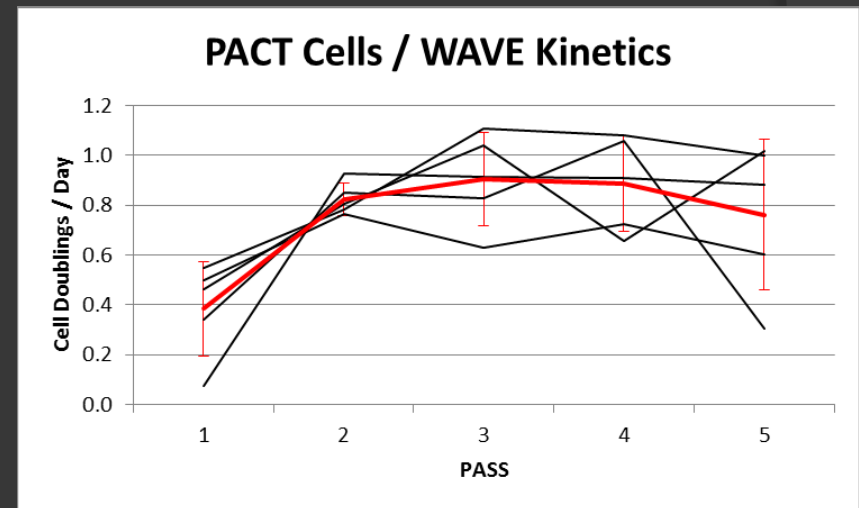
\*Information obtained from Beckman Coulter

# Final Result - Optimized Procedure

## Initial cell culture protocol



## Optimized cell culture protocol



# Lessons Learned

- Historical bottleneck = generating cells
- shift production bottleneck to final formulation and media management (including waste)
- Immediate need for media companies to supply base media in bags as a catalog item (2L / 5L / etc.)
- Budget for supporting equipment

# Special Thanks!

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