



# Hollow Fiber Bioreactors: Single-Use. Perfusion. Scalable. Continuous Manufacturing.

Presented by: **Scott Waniger**  
Vice President, BioServices



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# Cell Culture Company



- **Provider of Bioreactors and BioServices**
  - Manufactures automated single-use perfusion bioreactor systems and disposables
  - Provides custom manufacturing of mammalian and insect cell lines and expressed proteins to producers of human and animal healthcare products
- **Minneapolis-based since 1981**
  - Formerly known as Biovest International, Inc.
  - Pioneered Hollow Fiber Bioreactor platform
  - Spun-off as Cell Culture Company – January 2016
- **33,000 sq. ft. facility**
  - Corporate Offices
  - Bioreactor system and disposables manufacturing operations
  - GMP BioServices compliant manufacturing

# Cell Culture Company

## Our Mission:

*Cell Culture Company strives to revolutionize the quality, scalability and predictability of diagnostic and therapeutic mammalian protein production. We provide automated single-use perfusion bioreactor systems and custom development and manufacturing (CDMO) services for the human and animal healthcare industry.*

*Delivering innovative and scalable GMP custom mammalian cell and protein production solutions and services from pre-clinical to commercial in mg to kg quantities. We're about achieving superior results for our customers. Delivering value through the:*

- *Improvement of cell health quality, resulting in homogeneous protein expression produced through the use of a homeostatic system*
- *Decreasing production risk as a result of using closed, parallel systems*
- *Increasing speed to market due to reduced upstream validation requirements*
- *Reducing capital expenditures, labor, facilities and materials costs delivering increased protein per square foot of production space*

# Hollow Fiber Cell Culture Bioreactors

## Key Initiatives:

- **“Cell culture on artificial capillaries: an approach to tissue growth in vitro”** October 1972 [Knazek RA](#), [Gullino PM](#), [Kohler PO](#), [Dedrick RL](#).
- **Scalable technology from R&D to Manufacturing Scale Single Use Automated Perfusion Bioreactor Systems**
- **Licensed FDA Injectable Diagnostic Imaging Biologic (ProstaScint®)**
- **Clinical trial Support**
  - 4 Phase III Clinical Trials (currently an anti-PD-1 mAb)
  - 23 Phase I/II Clinical Trials
- **Primary upstream manufacturing platform for large scale( >2 gram) IVD products**



# Hollow Fiber Cell Culture Bioreactors

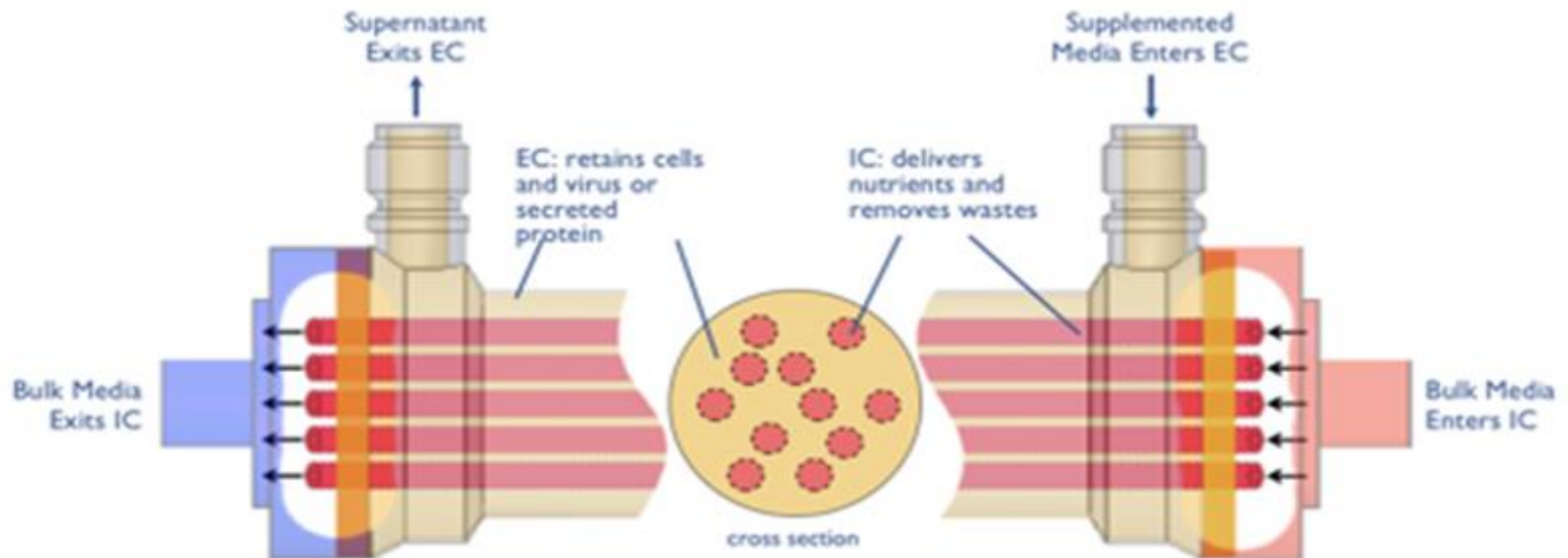
## Separate Compartments Provide Production Advantages

### ExtraCapillary ~ EC

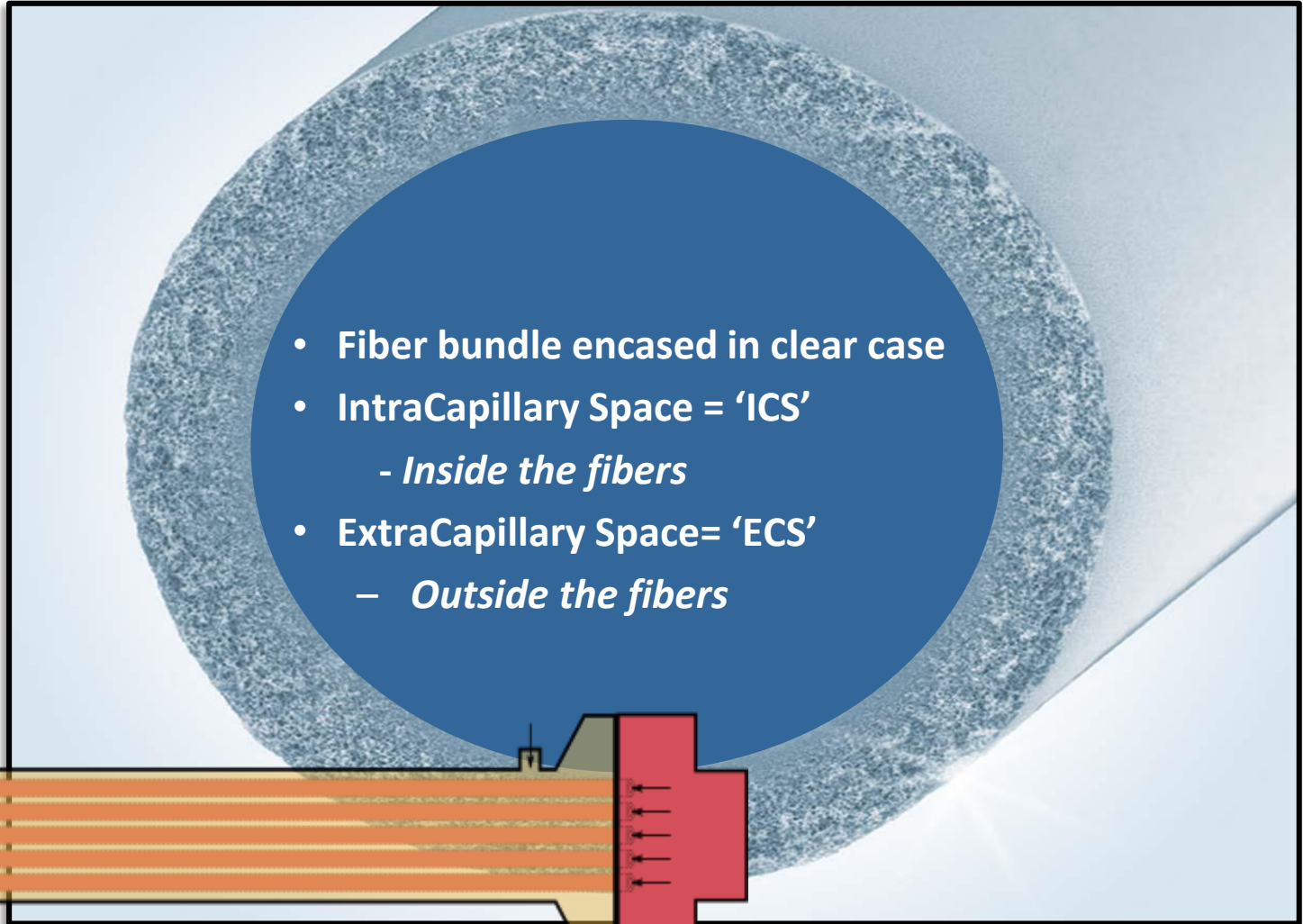
- Cell Compartment
- Secreted Product

### IntraCapillary ~ IC

- Media Perfusion & Nutrient Delivery
- Removal of Metabolic Waste



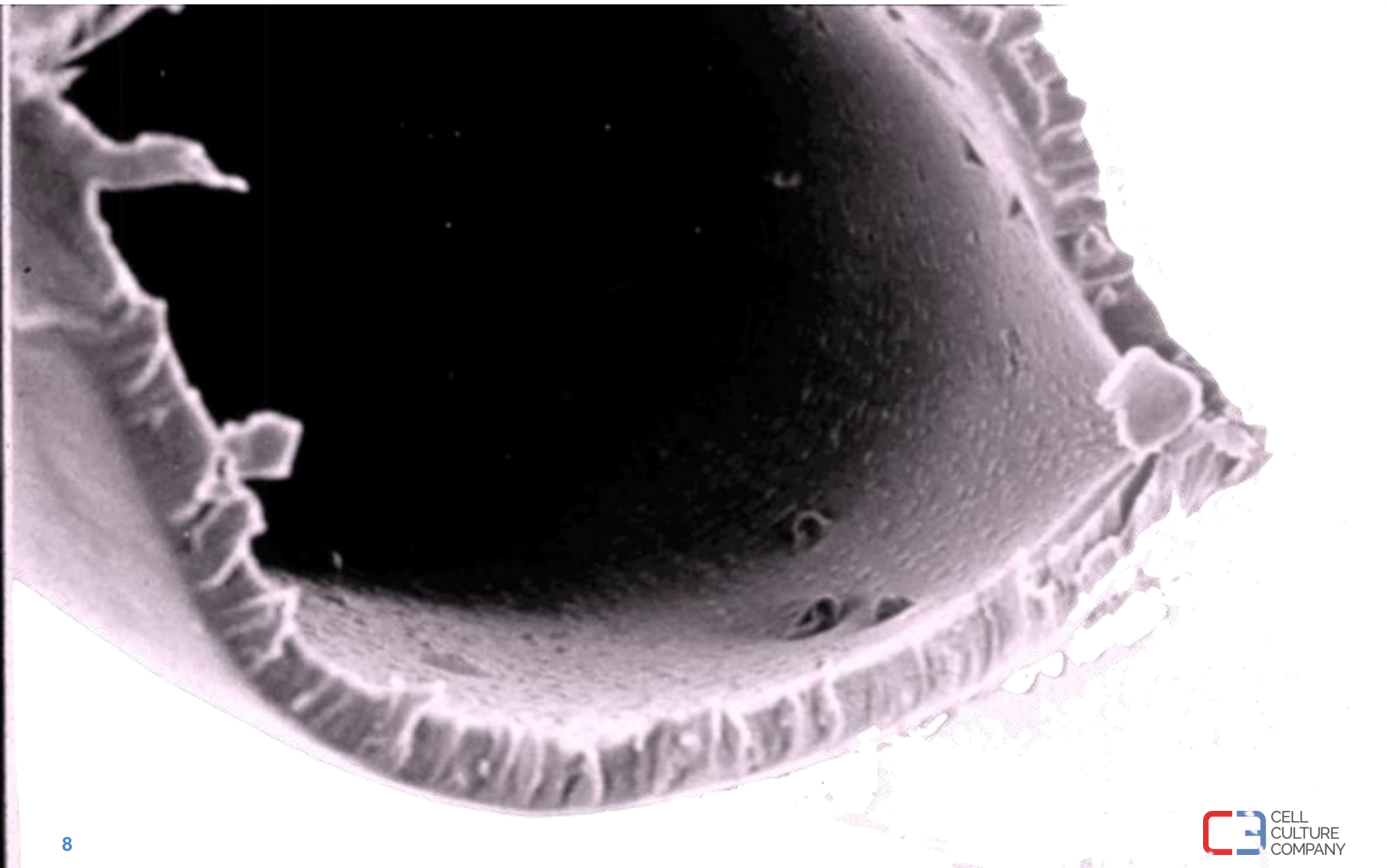
# Hollow Fiber: Perfusion Cell Culture



- Fiber bundle encased in clear case
- IntraCapillary Space = 'ICS'
  - *Inside the fibers*
- ExtraCapillary Space= 'ECS'
  - *Outside the fibers*



# Electron Micrograph of a single fiber



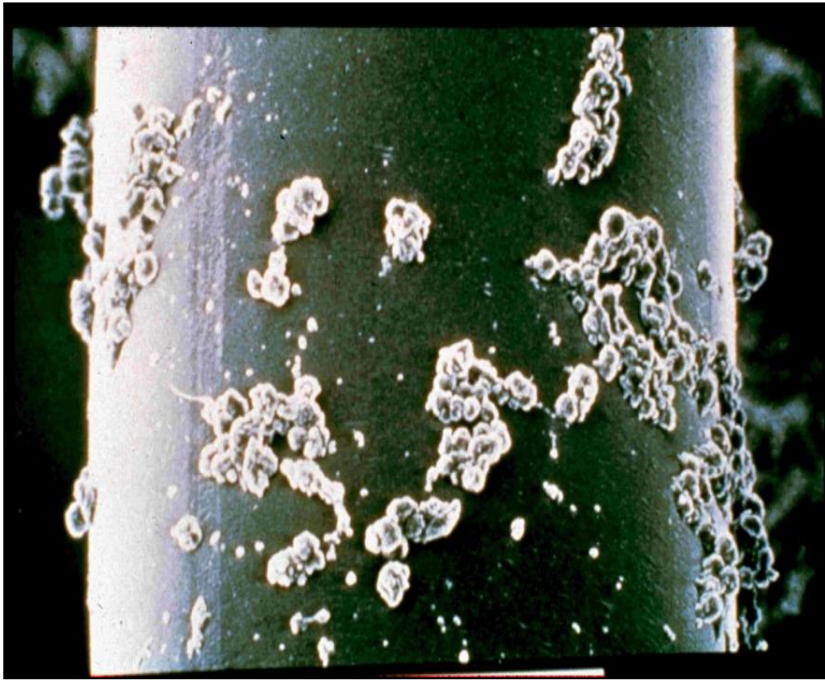


# Hollow Fiber: Perfusion Cell Culture

- Hollow fiber material is a semi-permeable membrane composed of cellulose acetate: 35-60 kDa MWCO
- Small nutrients freely exchange between ICS & ECS
- Cells and products are retained in ECS volume

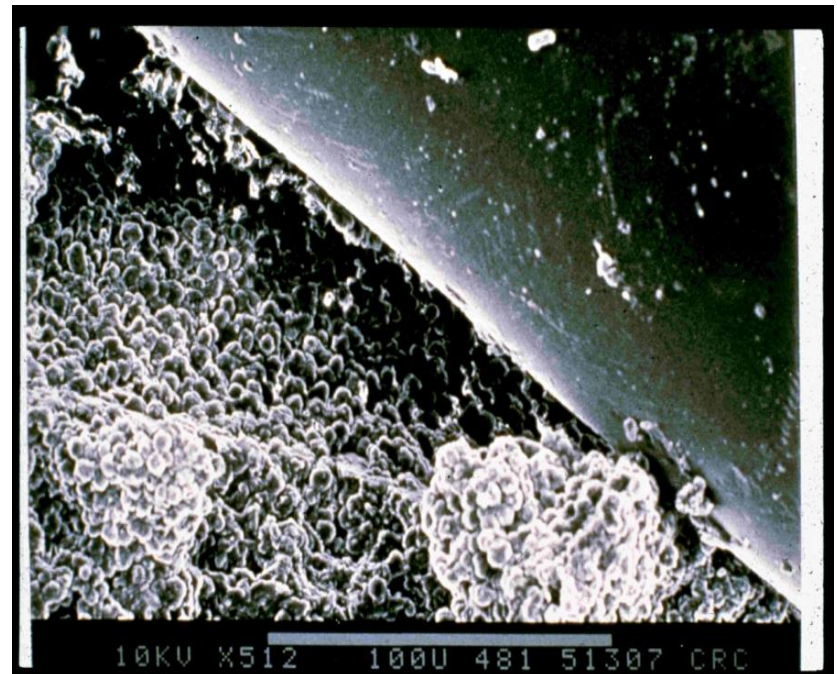


# EM photos of cells on fibers

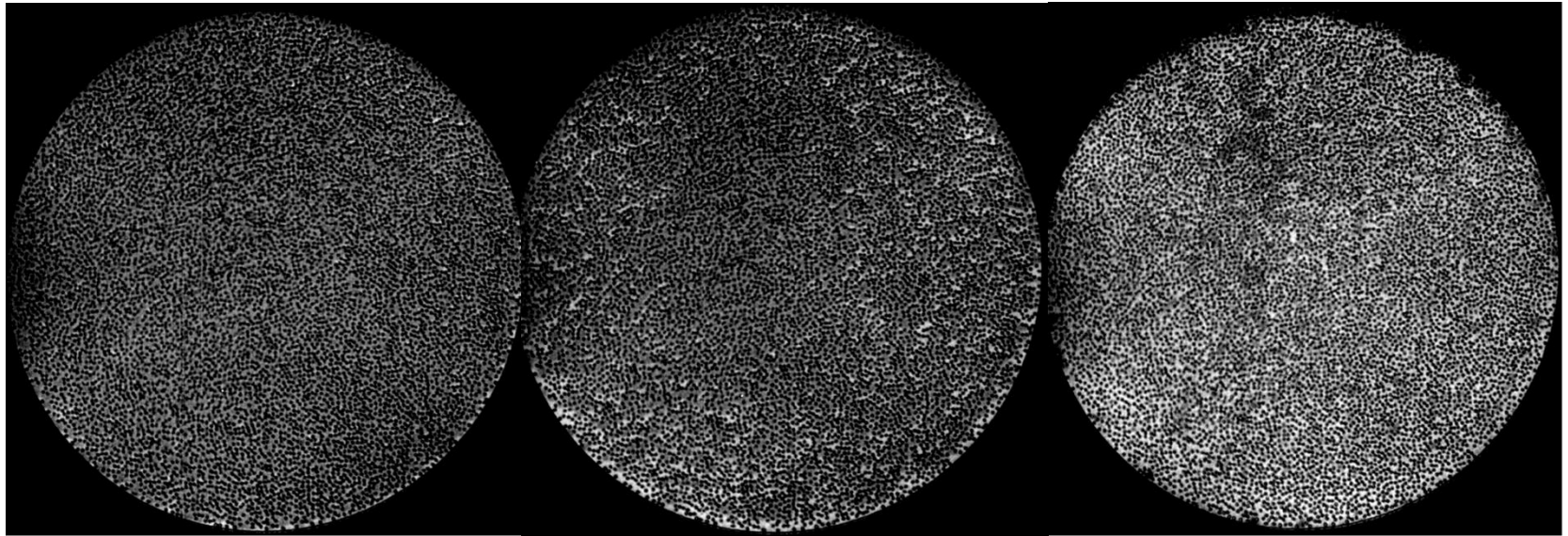


Adherent CHO Culture

Suspension Hybridoma Culture



# Nuclear Magnetic Resolution (NMR) images of cell growth in a Hollow Fiber Bioreactor



Day 1

Day 2

Day 5

A high-resolution, diffusion weighted image of the bioreactor at 1, 2, and 5 days post-inoculation with a hybridoma cell line. The cell mass appears bright/white and shows homogenous distribution.

# C3 Manufactures & Distributes Hollow Fiber Bioreactors

Research Scale Protein	Pilot Scale Protein	Large Scale Protein	Commercial Scale Protein
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HF Primer™



AcuSyst-MAXIMIZER®



AcuSyst XCELLERATOR™



HF MicroBrx



AutovaxID®



AcuSyst XCELLERATOR™



# Scalable Technology

AutovaxID®



1X

ACUSYST-MAXIMIZER®



2X

AcuSyst-Xcellerator



20X

# Case Studies

## Study 1: Scalable Technology that reduces the need to validate during scale up

- C3's Hollow Fiber Perfusion technology allows for a 20-fold expansion of bioreactor capacity
- Cell substrate (cartridge) space does not change in form fit size function or size
- Scale up occurs by connecting multiple cartridges in parallel

## Study 2: Continuous Protein Expression Manufacturing

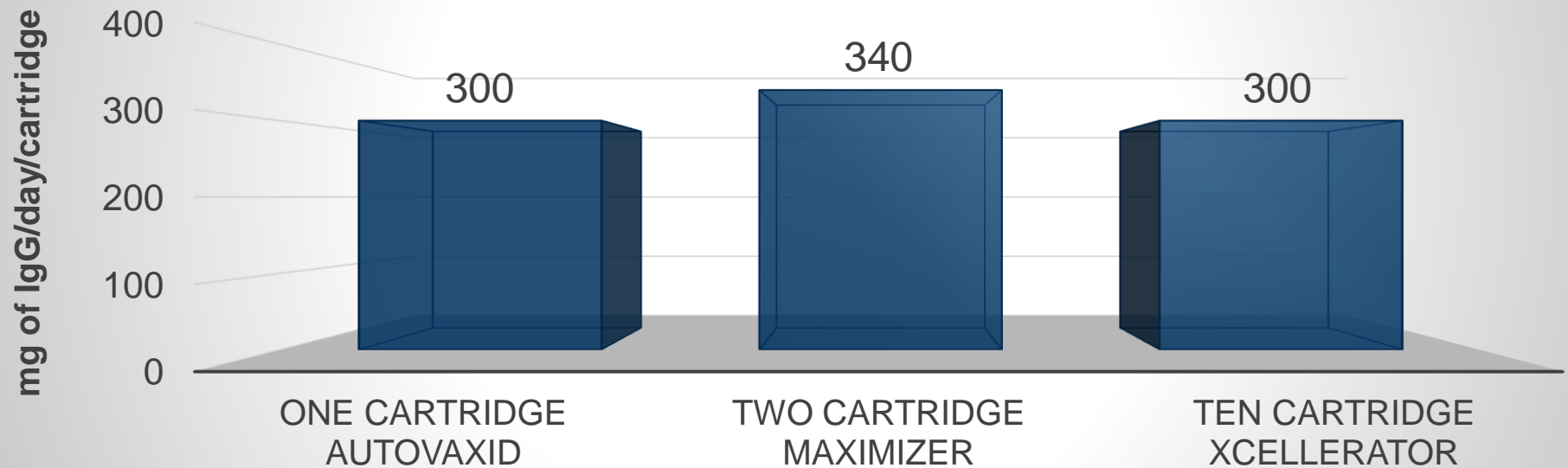
- In vivo-like cell environment supports cells at  $>10^9$  cells per mL of space
- No seed train
- Steady state metabolic activity
- Long lasting protein expression production

# Case Study 1: Data Table

Bioreactor System	No. of 2.1 m <sup>2</sup> Cartridges	No. of Culture Days	Grams of IgG	Liters of Supernatant	Titer g/L	IgG Production mgs/day/cartridge
<b>Cell Line A (Shaker Flask titer 0.02 g/L)</b>						
AutovaxID	1	30	9.0	3.0	3.00	300
Maximizer	2	60	40.8	16.0	2.55	340
Xcellerator	10	37	111.0	43.0	2.58	300
<b>Cell Line B (Shaker Flask titer 0.01 g/L)</b>						
AutovaxID	1	30	3.5	3.0	1.16	116
Maximizer	2	62	13.0	16.0	0.81	105
Maximizer	2	30	6.1	8.0	0.76	102
Maximizer	2	60	17.0	16.0	1.06	142
Maximizer	2	30	7.5	3.3	2.27	125
Maximizer	2	60	14.0	16.0	0.88	117
Maximizer	2	60	17	15.0	1.14	143
Xcellerator	10	60	70	50.0	1.40	117
<b>Cell Line C (Shaker Flask titer 0.005 g/L)</b>						
Xcellerator	10	47	8	32.9	0.25	18
Xcellerator	10	94	21	66.4	0.31	22
Xcellerator	10	51	11	36.7	0.31	22
Xcellerator	10	51	11	36.1	0.30	21
Xcellerator	10	55	10	38.5	0.27	19
Xcell	6	82	12	35.4	0.32	23
<b>Cell Line D (Shaker Flask titer 0.008 g/L)</b>						
Xcellerator	10	85	81	62	1.32	96
Xcellerator	10	69	62	50	1.24	90
<b>Cell Line E (Shaker Flask titer 0.008 g/L)</b>						
Maximizer	2	48	10	11	0.95	104
Xcellerator	10	21	91	38	2.39	433

# Case Study 1: Scalable Production

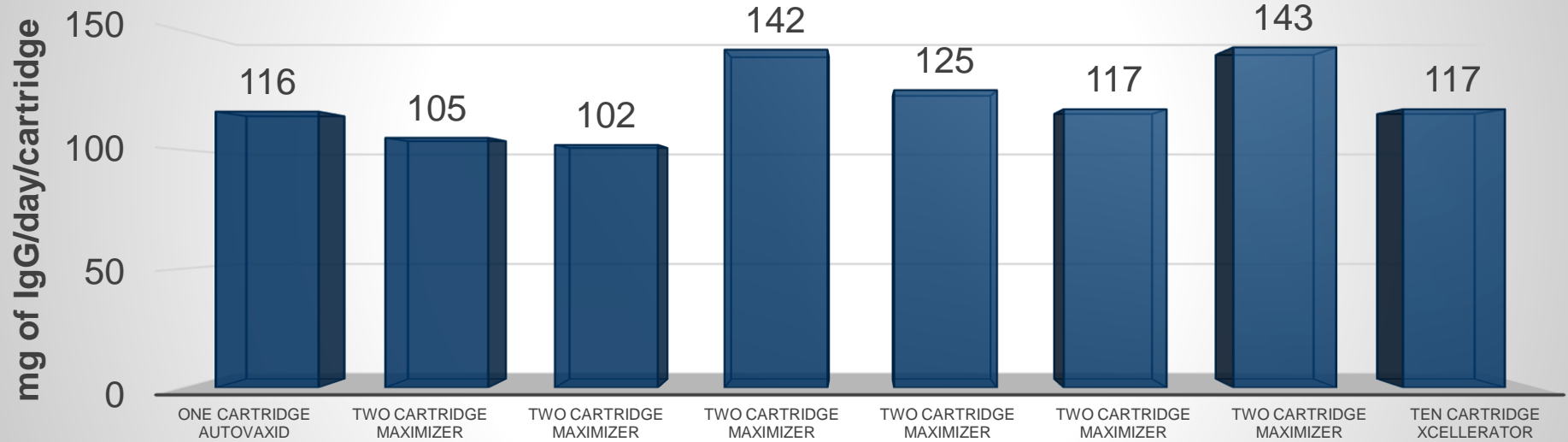
## Cell Line A Normalized Production Rate





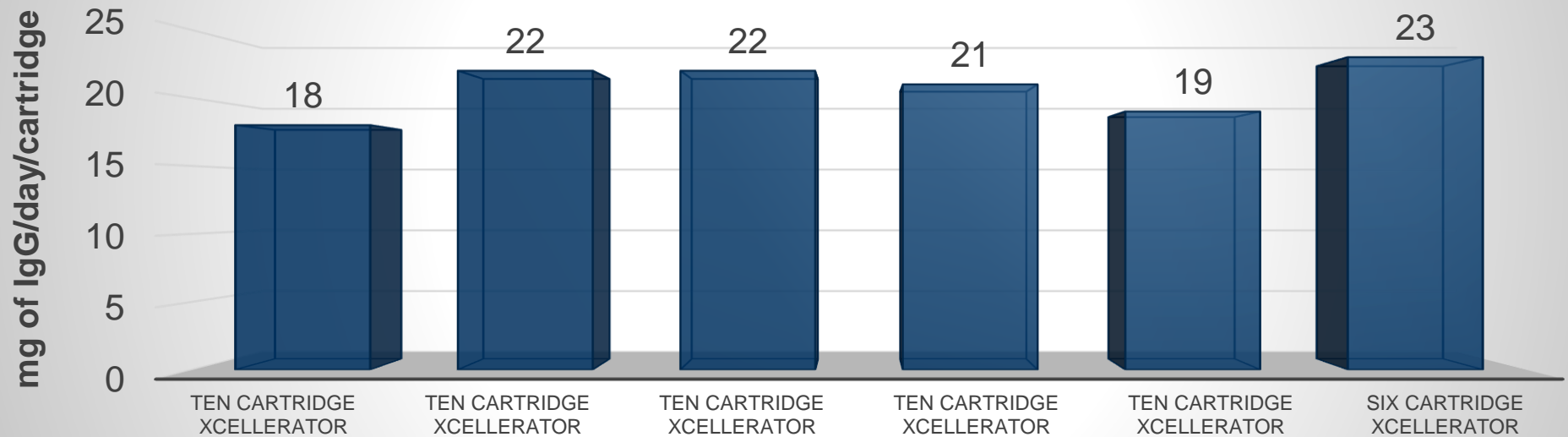
# Case Study 1: Scalable Production

## Cell Line B Normalized Production Rate



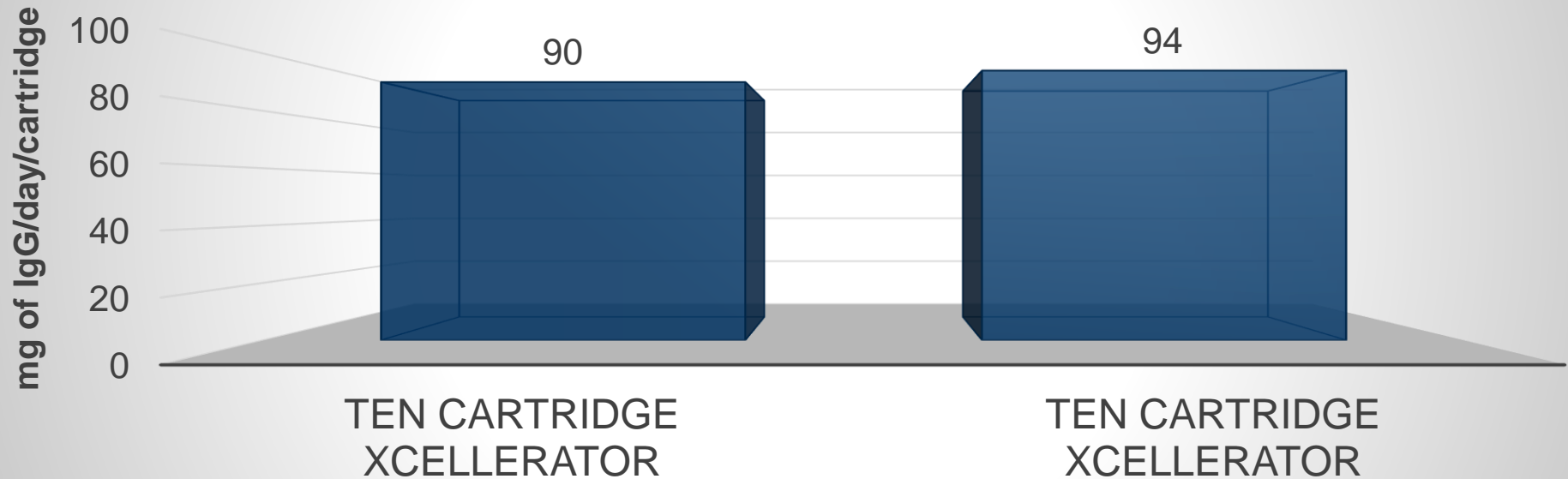
# Case Study 1: Scalable Production

## Cell Line C Normalized Production Rate



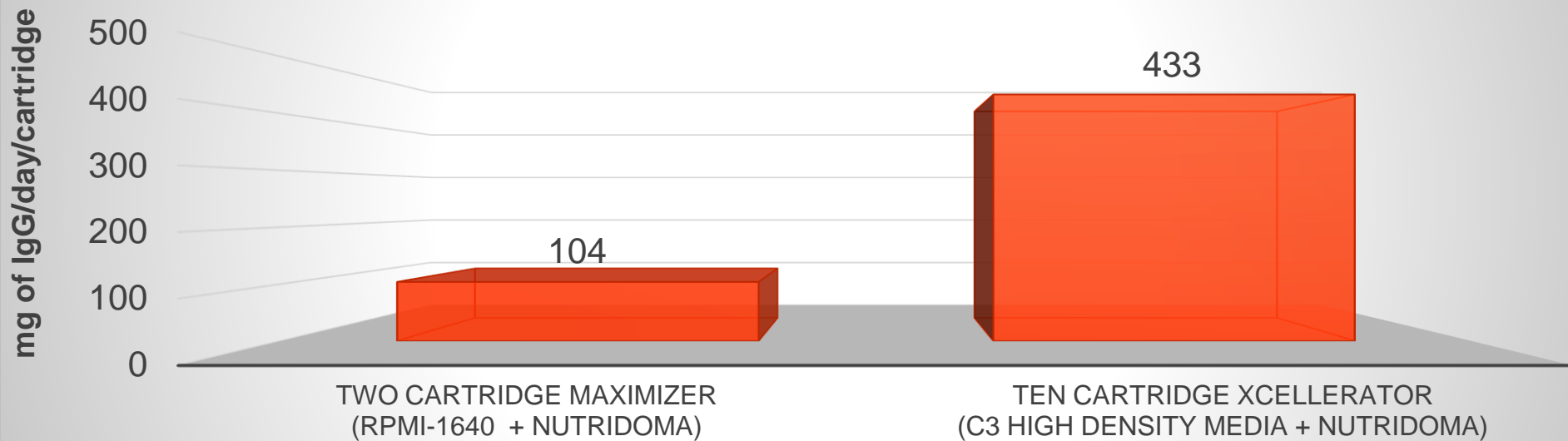
# Case Study 1: Scalable Production

## Cell Line D Normalized Production Rate



# Case Study 1: Media Optimization

## Cell Line E Normalized Production Rate: Tissue Culture Media Comparison of RPMI-1640 vs. C3 High Density Media



# Case Studies

## Study 1: Scalable Technology that reduces the need to validate during scale up

- C3's Hollow Fiber Perfusion technology allows for a 20-fold expansion of bioreactor capacity
- Cell substrate (cartridge) space does not change in form fit size or function
- Multiple cartridges are connected in parallel

## Study 2: Continuous Protein Expression Manufacturing

- In vivo-like cell environment supports cells at  $>10^9$  cells per mL of space
- No seed train
- Steady state metabolic activity
- Long lasting protein expression production

## Case Study 2: Operator Requirements to Support a 90-Day Xcellerator Culture (~ 1,600 L stirred tank equiv.)

- Disposable bioreactor assembly installation = 2 hours
- Inoculation preparation (thaw to seed  $\sim 10^8$ ) = 6 hours
- Daily bioreactor support = 1 hour/day
- Total operator time for 90 day perfusion culture = 98 hours
- Product Output:
  - 100-200x concentration based on standard suspension culture environment (stirred tank requires additional concentration step)
  - Cell culture supernatant passes through a sterilizing grade 0.2 micron filter to yield cell-free supernatant
  - Proceed immediately to downstream processing

# Case Study 2: No Seed Train



**Culture Day 6**



**Culture Day 8**



**Culture Day 10**



**Culture Day 12**



**Culture Day 14**

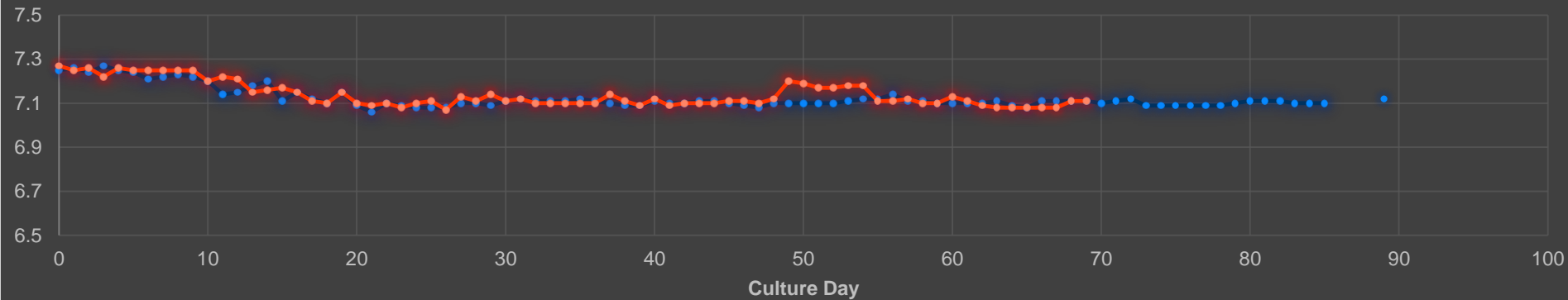


**Culture Day 16**

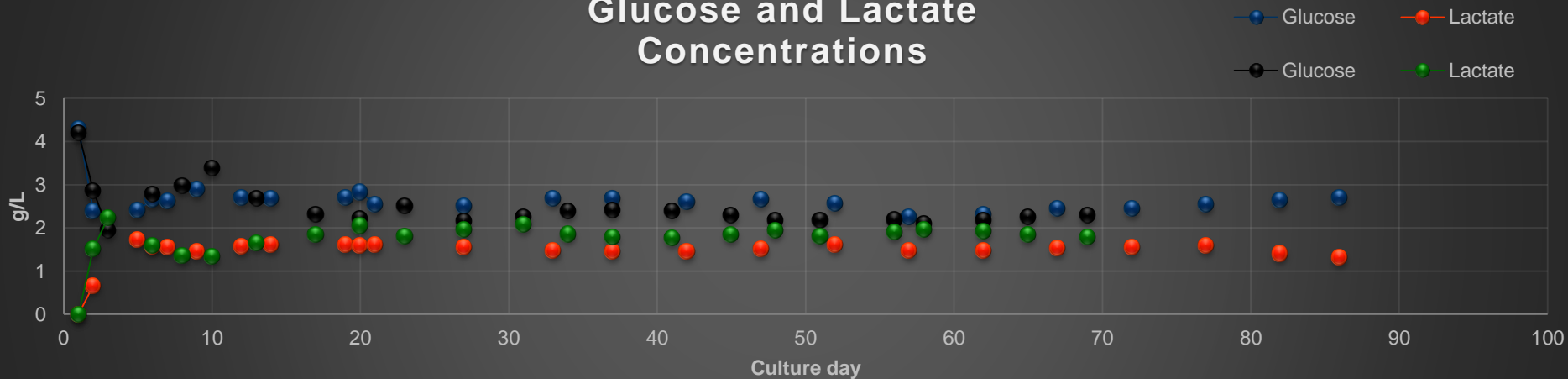
# Case Study 2: Steady State Metabolic Control:

Cell Line D, 84 and 69 day cultures

pH



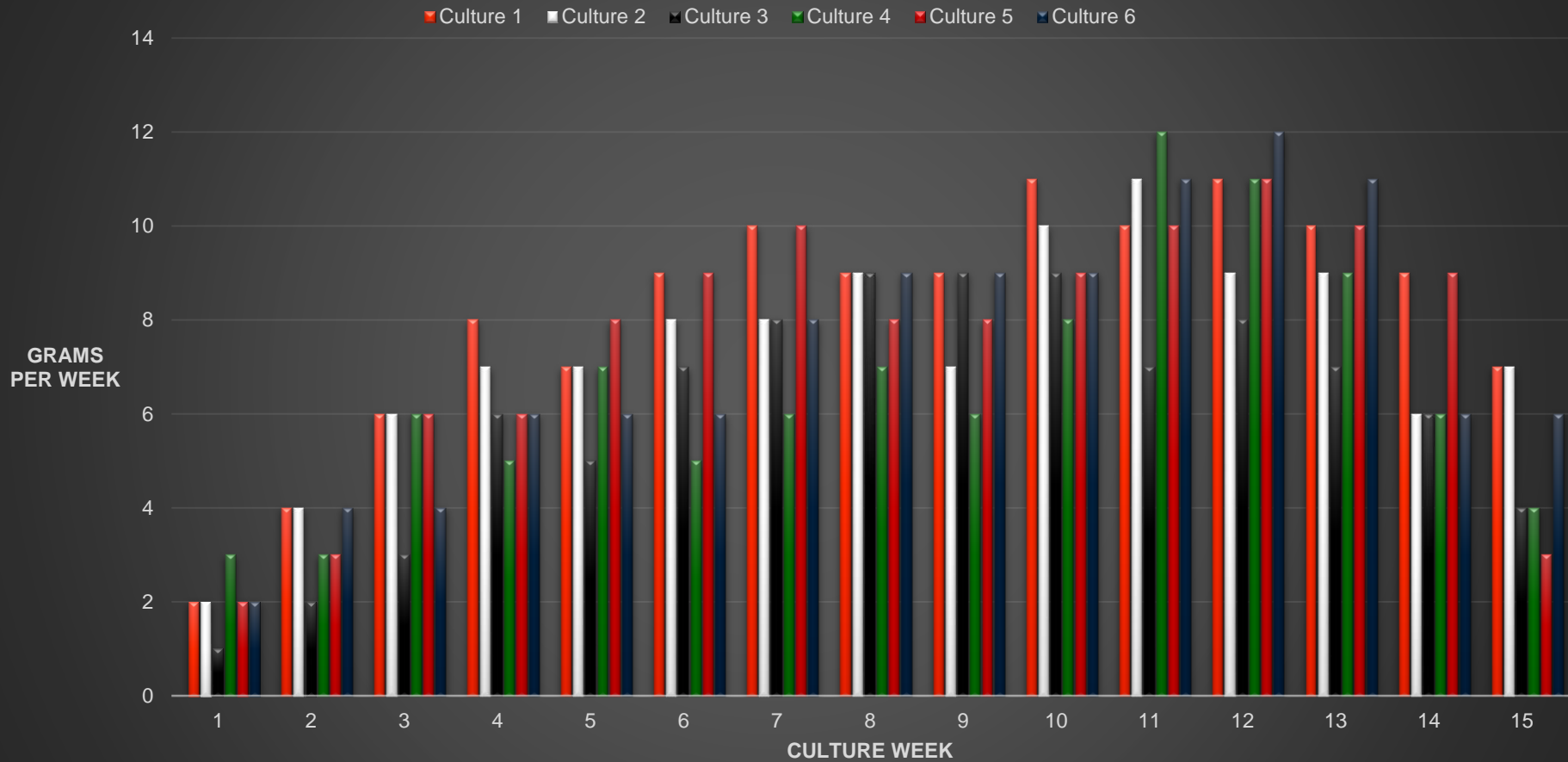
## Glucose and Lactate Concentrations





# Case Study 2: 100+ Day Consistent and Continuous Protein Expression

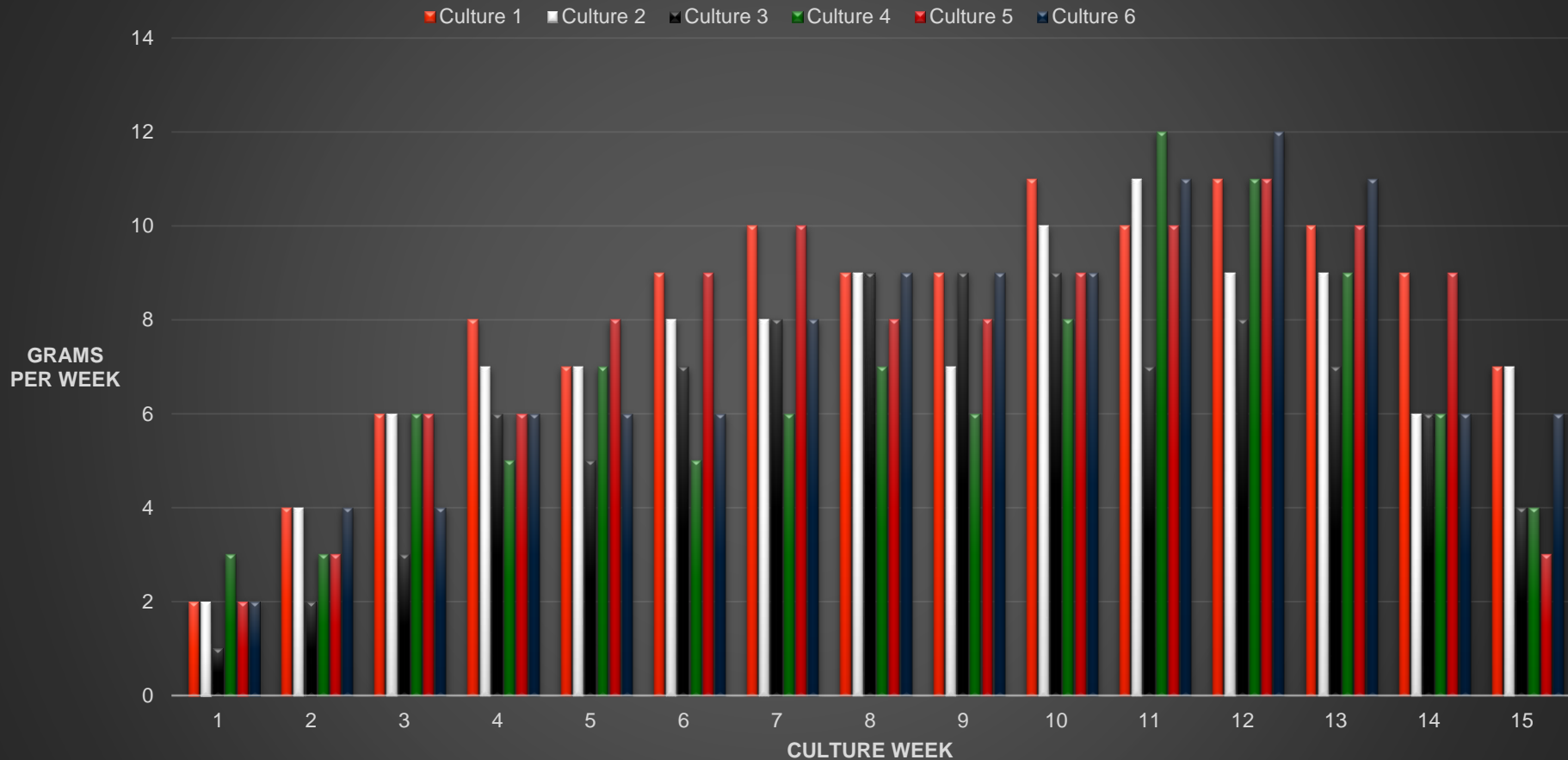
## Perfusion Hollow Fiber Bioreactor; IgG production rate\*



\*Cell Line IgG titer in shake flask = 5 mg/L

# Case Study 2: 100+ Day **Consistent** and **Continuous** Protein Expression

## Perfusion Hollow Fiber Bioreactor; IgG production rate\*



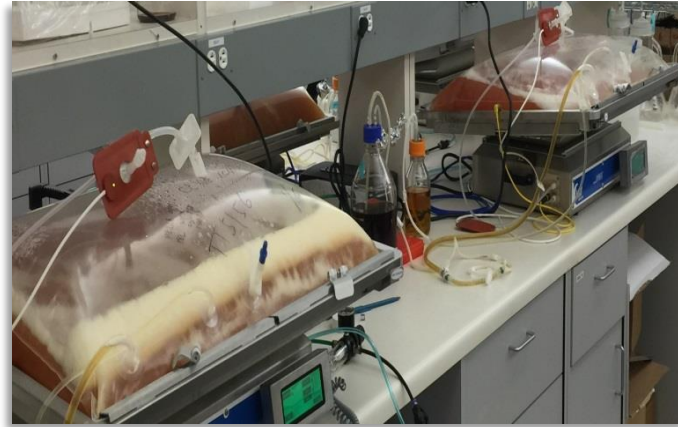
\*Cell Line IgG titer in shake flask = 5 mg/L

# Perfusion Hollow Fiber and Batch/Fed-Batch

System and Process Features	Perfusion Hollow Fiber	Batch/Fed-Batch
Support mammalian cell culture expression system	Yes	Yes
Single Use	Yes	Yes
Closed System	Yes	Yes
Supported in market for 30+ years	Yes	Yes
Maintains Metabolic Homeostasis for 60+ days (pH, glucose, lactate, l-glutamine, oxygen)	Yes	No
Concurrent product concentration	Yes (100-200X)	No (requires further downstream processing)
Continuous Manufacture of Expressed Proteins	Yes	No (requires ATF modification)
Linear Scalability	Yes (same cartridge size in parallel)	No (each scale expansion requires validation)
Seed Train Required	No	Yes

# What environment is best for your cells production?

**Consider Thinking Outside the Wave....**



**Consider Thinking Outside the Tank....**



## ...and consider Automated Perfusion Hollow Fiber Bioreactors for your protein expression needs



- Improved cell health quality, resulting in homogeneous protein expression produced through the use of a homeostatic system
- Decreased production risk as a result of using our closed, parallel systems
- Increased speed to market due to reduced upstream validation requirements
- Reduced capital expenditures, labor, facilities and materials costs delivering increased protein per square foot of production space

# C3's BioServices and Hollow Fiber Perfusion Bioreactor Platform

## Key Initiatives:

- Serviced over 1,700 labs globally
- Expanded and worked with over 2,650 different cell lines
- Manufactured a licensed FDA therapeutic imaging biologic
- Supported manufacturing for 4 phase III clinical trials
  - Currently supporting anti-PD-1 mAb at CMO
  - 23 Phase I/II clinical trials
- Hollow Fiber Perfusion Bioreactors are “The” primary platform for large scale (>2 g/year) IVD mAb products

# Hollow Fiber Perfusion Bioreactors:

Please stop by booth  
#505 for more information

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