Optimizing culture of suspension cells using the G-Rex

CAGT
CENTER FOR
CELL AND GENE THERAPY

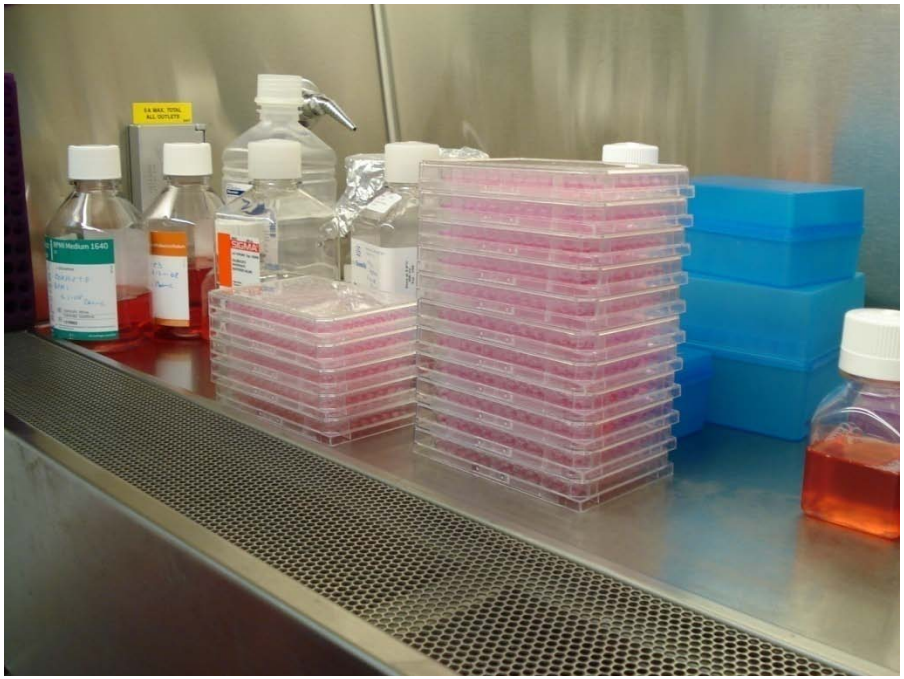
Methodist The Methodist Hospital System

BCM®
Baylor College of Medicine

Texas Children's Hospital

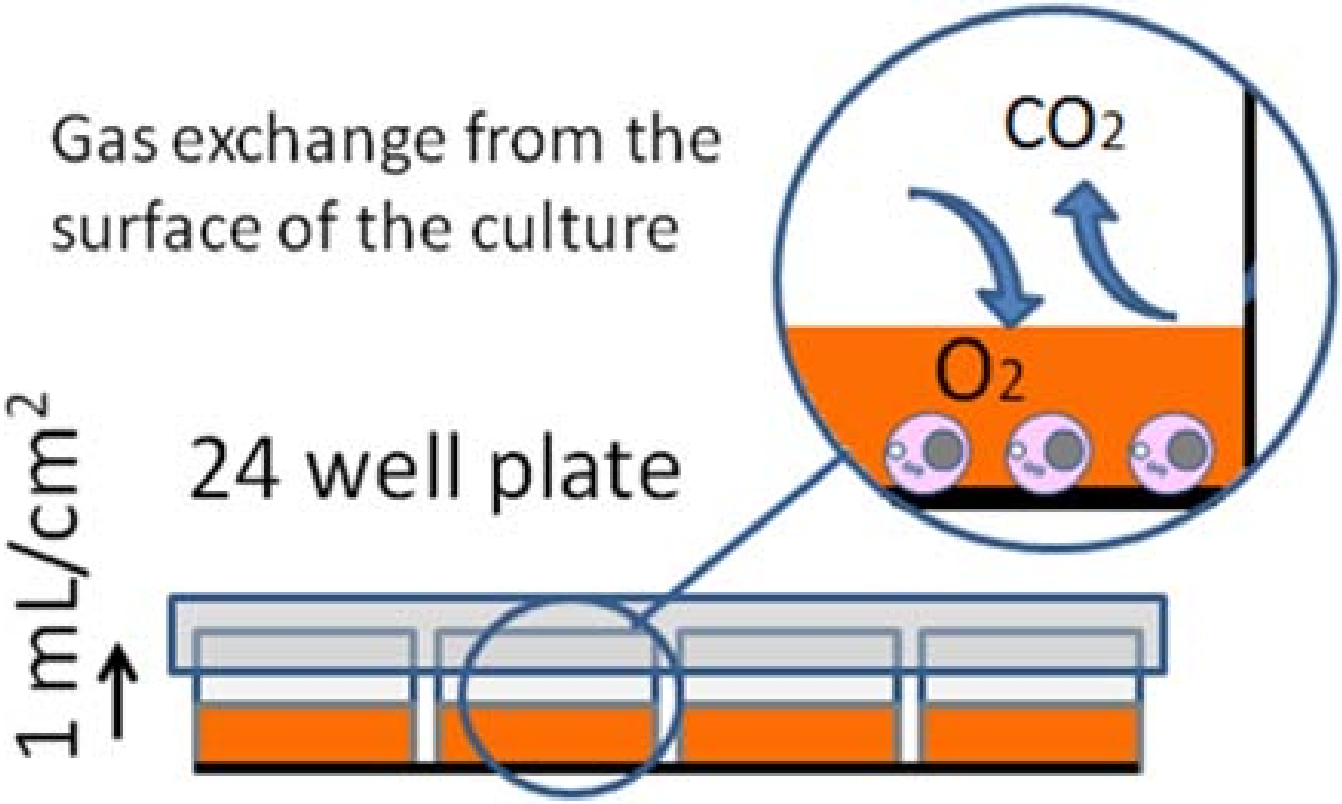
Limitations of conventional suspension cell culture methods

- Prolonged culture period
- Extensive manipulation - risk of contamination
- Labor intensive
- Require highly trained personnel
- Excessive use of reagents

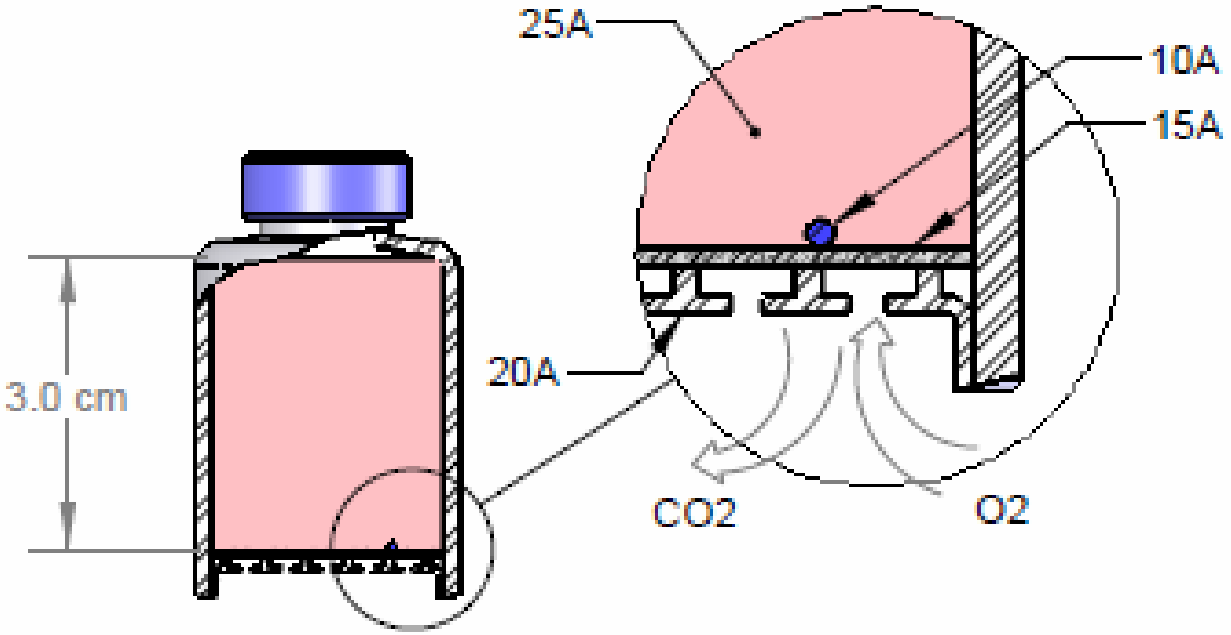


Conventional Cultureware

- Limited volume of media and gas exchange



Wilson Wolf Manufacturing Gas Permeable Devices (G-Rex)

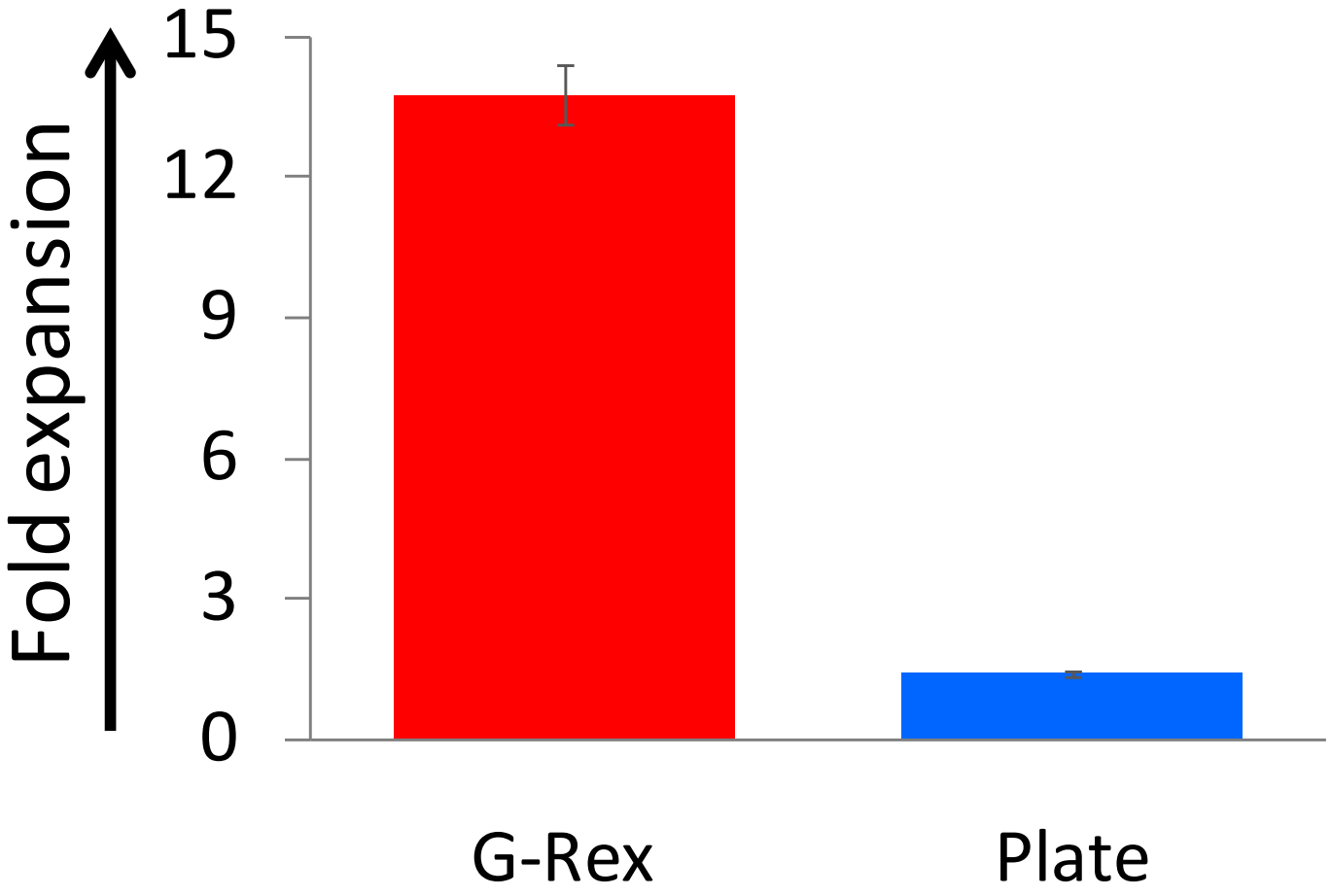


- Gas permeable membrane allows exchange of CO_2 and O_2
- Supports cell growth with large volumes of media
- Reduces feeding frequency and manipulation
- No rocking or stirring

Vera JF et al. JIT. 2010


G-Rex 100L
SA: 100 cm2 Vol: 2000 ml
G-Rex 100
SA: 100 cm2 Vol: 500 ml
G-Rex 10
SA: 10 cm2 Vol: 40 ml

G-Rex vs Conventional cultureware



Questions poll #1

Have you heard about the G-Rex?

- Yes
- No

Have you used the G-Rex?

- Yes
- No

What cells have you expanded in the G-Rex

- T cells
- Regulatory T cells
- NK cells
- Cell lines
- Murine cells

Questions poll #2

What applications do you use your cells for?

- Preclinical/Basic research
- Clinical
- Both

How many cells do you require for your application?

- ≤5x10e8
- 5x10e8 to 1x10e10
- ≥1x10e10

1st – What is the optimal seeding density?

2nd – What is the optimal volume of media to use?

3rd – How can cell expansion be monitored?

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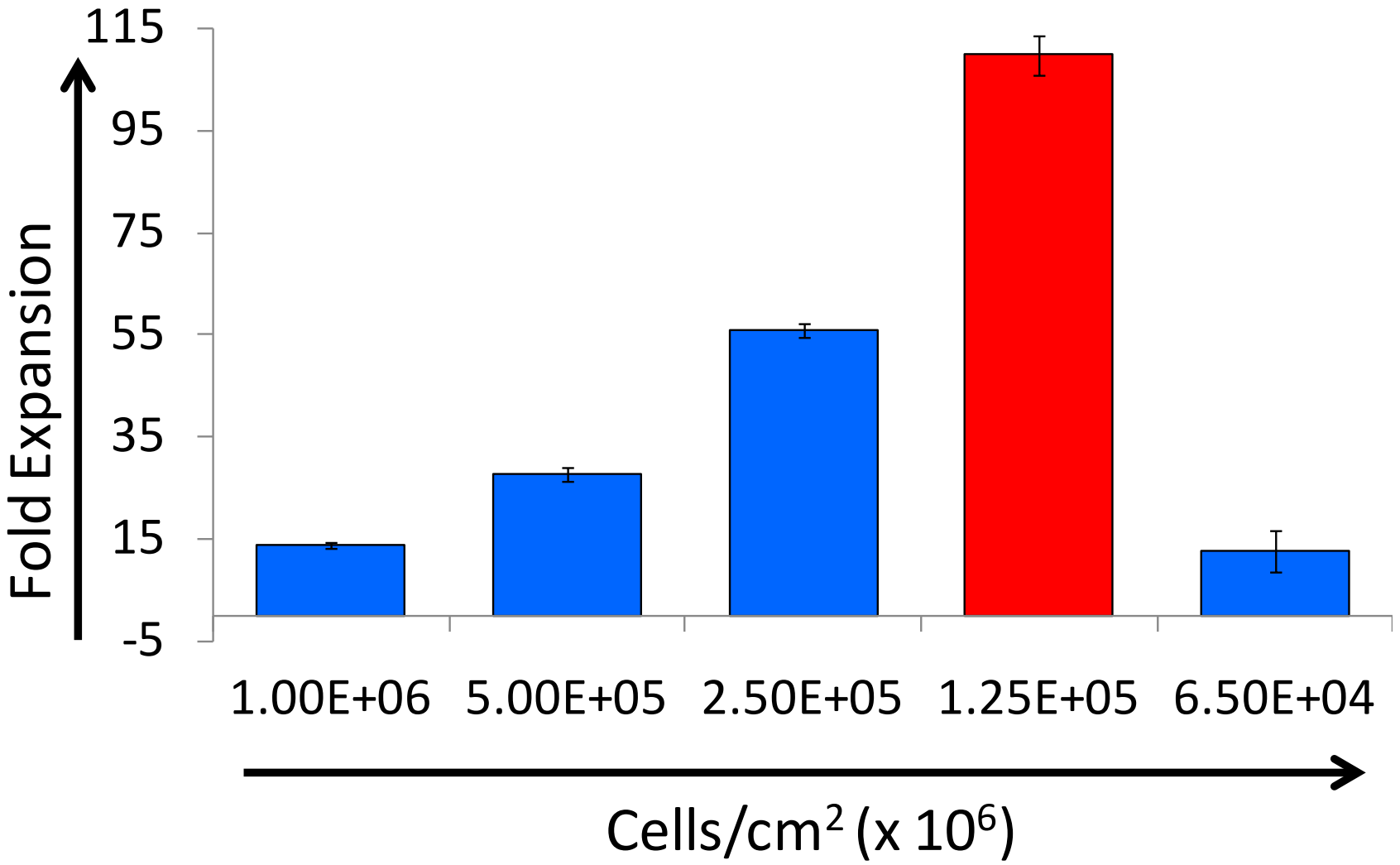
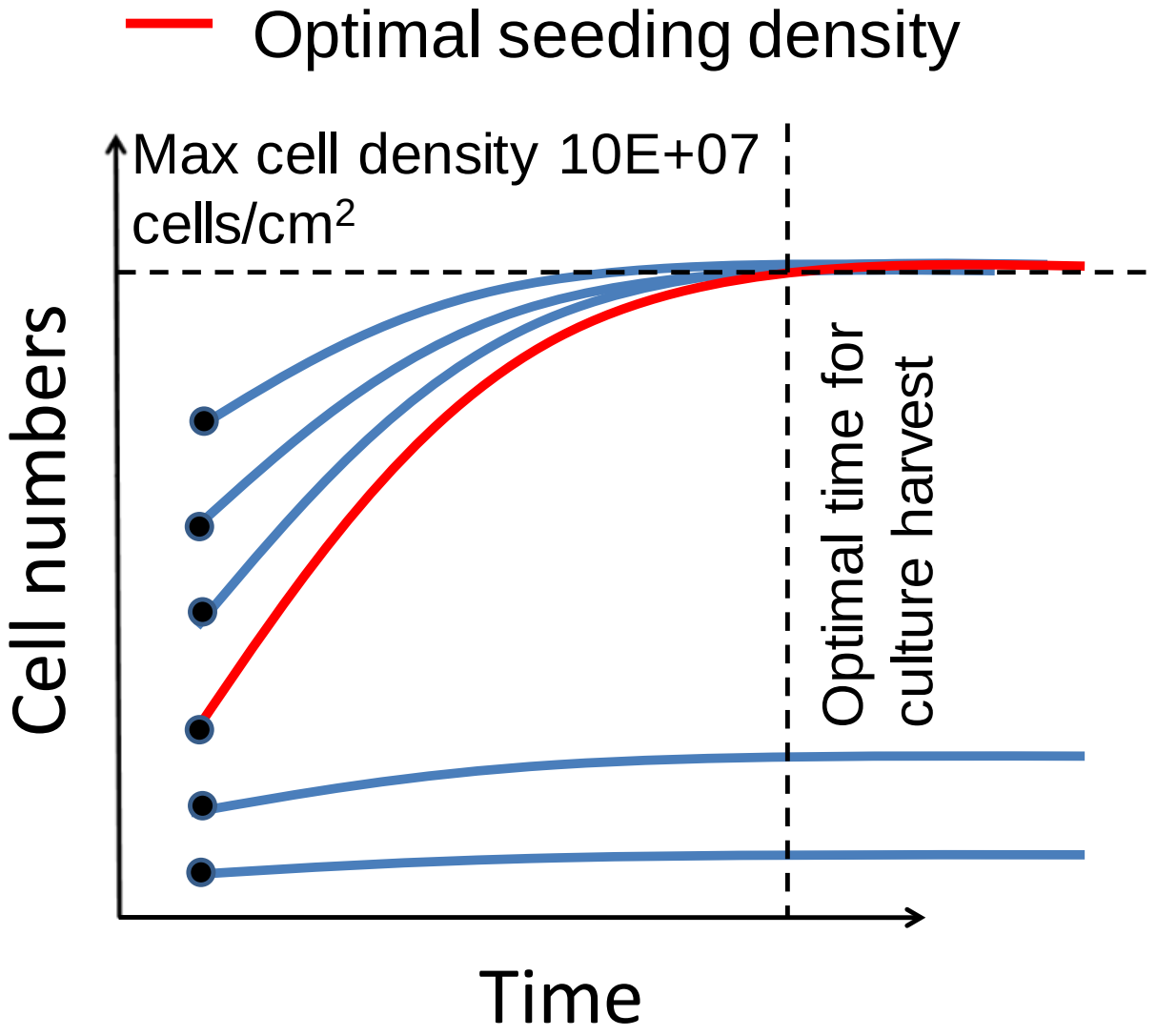
Low seeding density results in greater fold expansion



Low cell density = Higher cell expansion

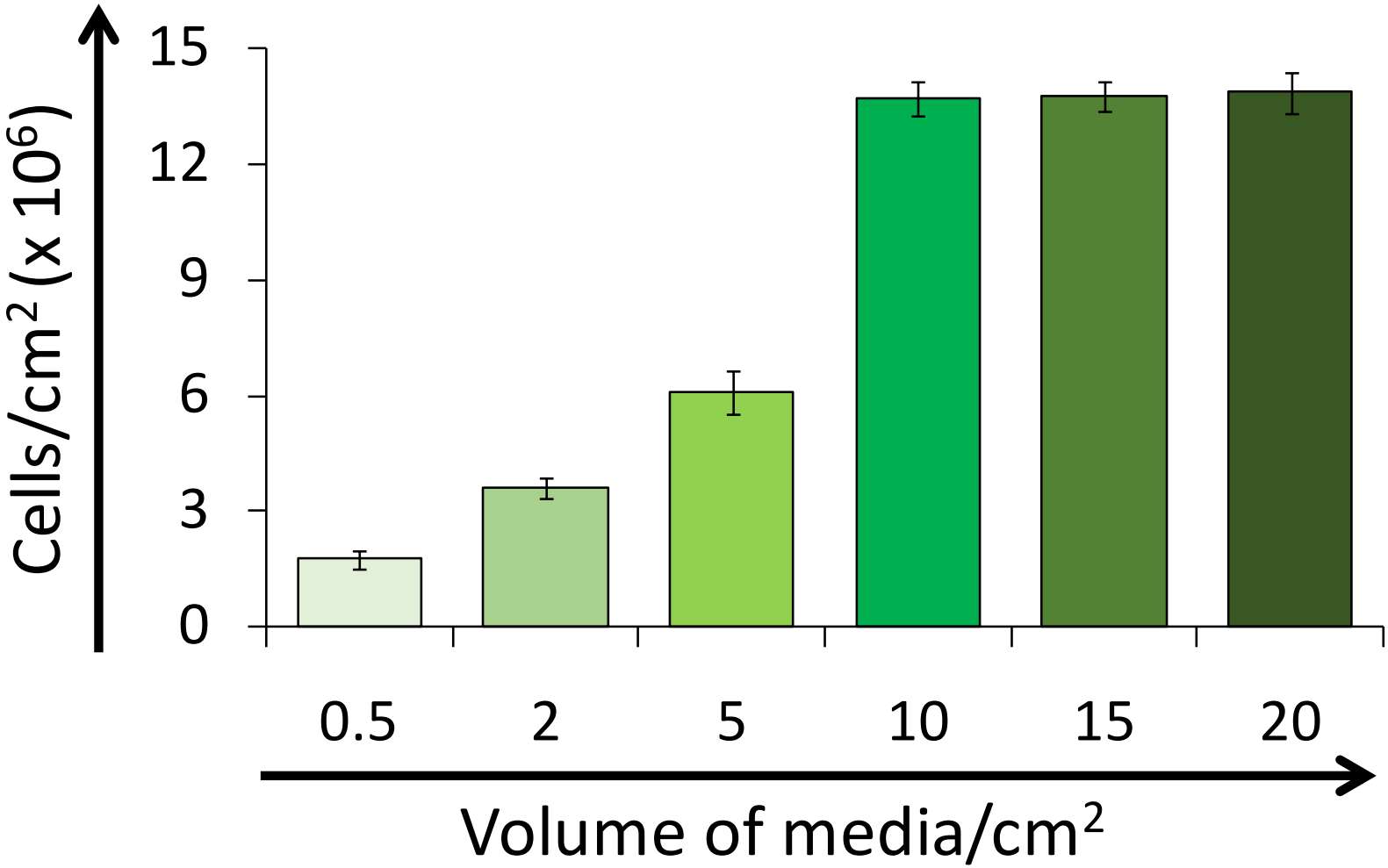


1st – What is the optimal seeding density = 1.2E+05 cells/cm^2

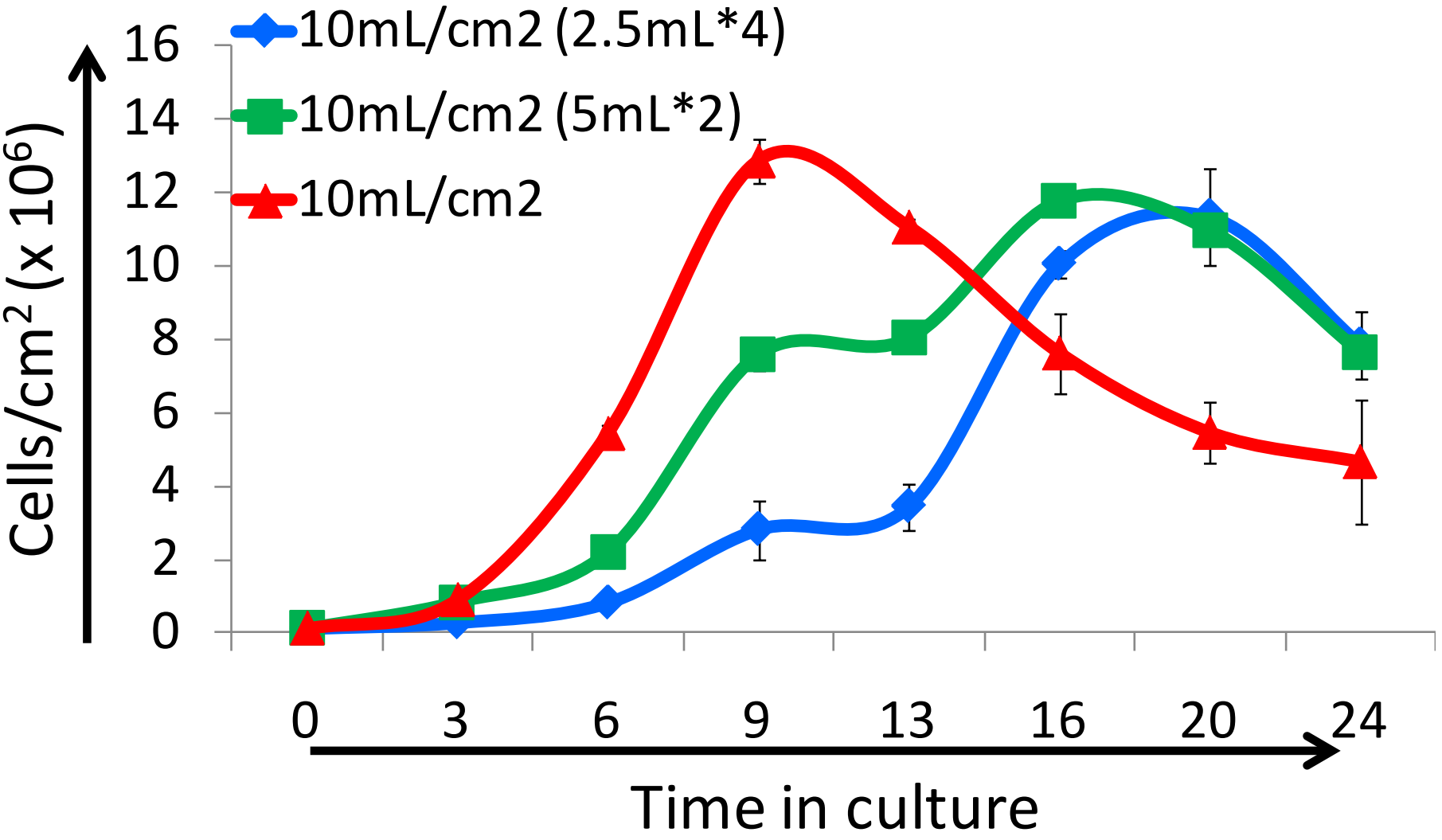
2nd – What is the optimal volume of media to use?

3rd – How can cell expansion be monitored?

10ml of media/cm^2 resulted in the maximum cell expansion



Addition of media at the culture onset resulted in short culture period

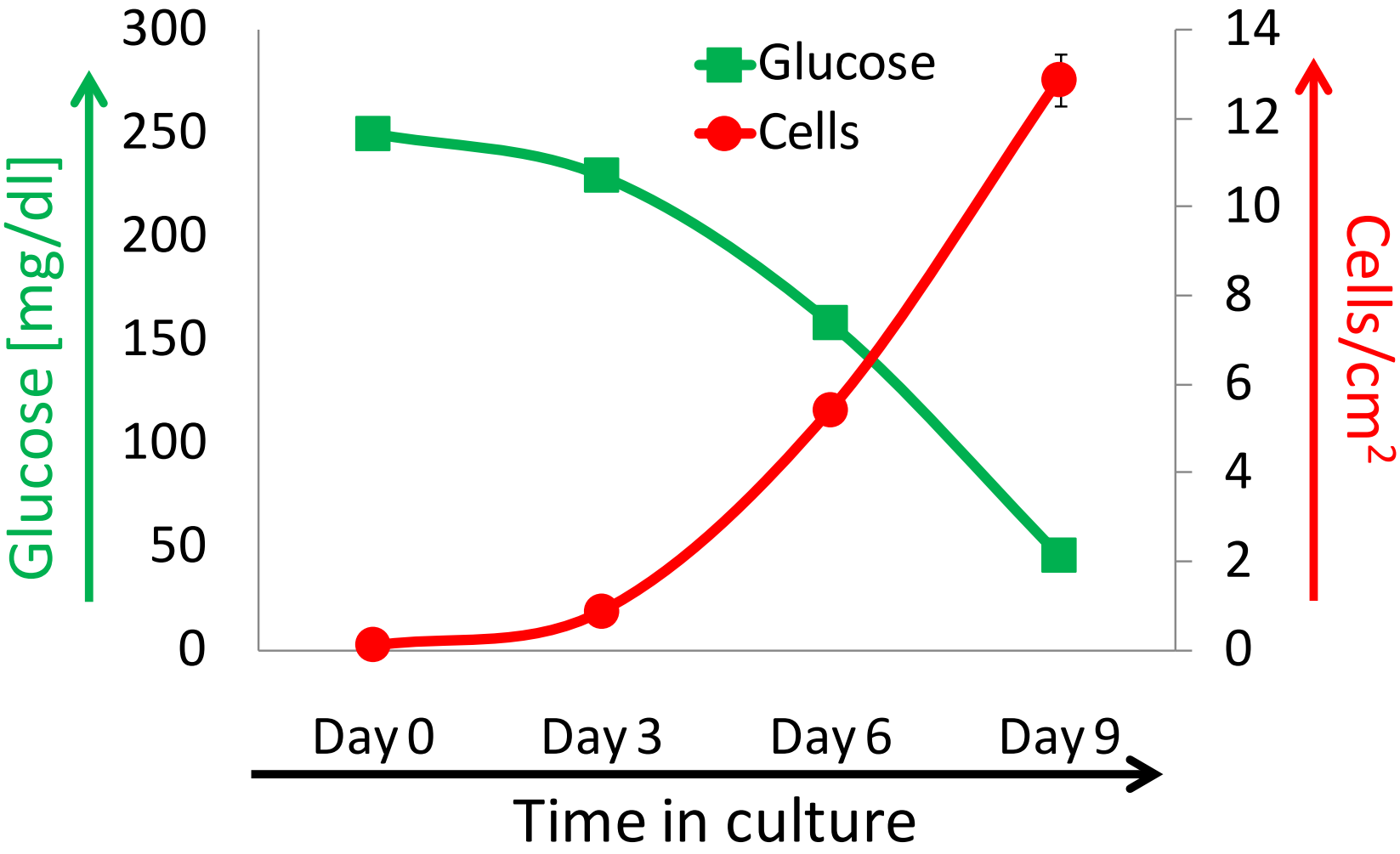


1st – What is the optimal seeding density = 1.2E+05 cells/cm^2

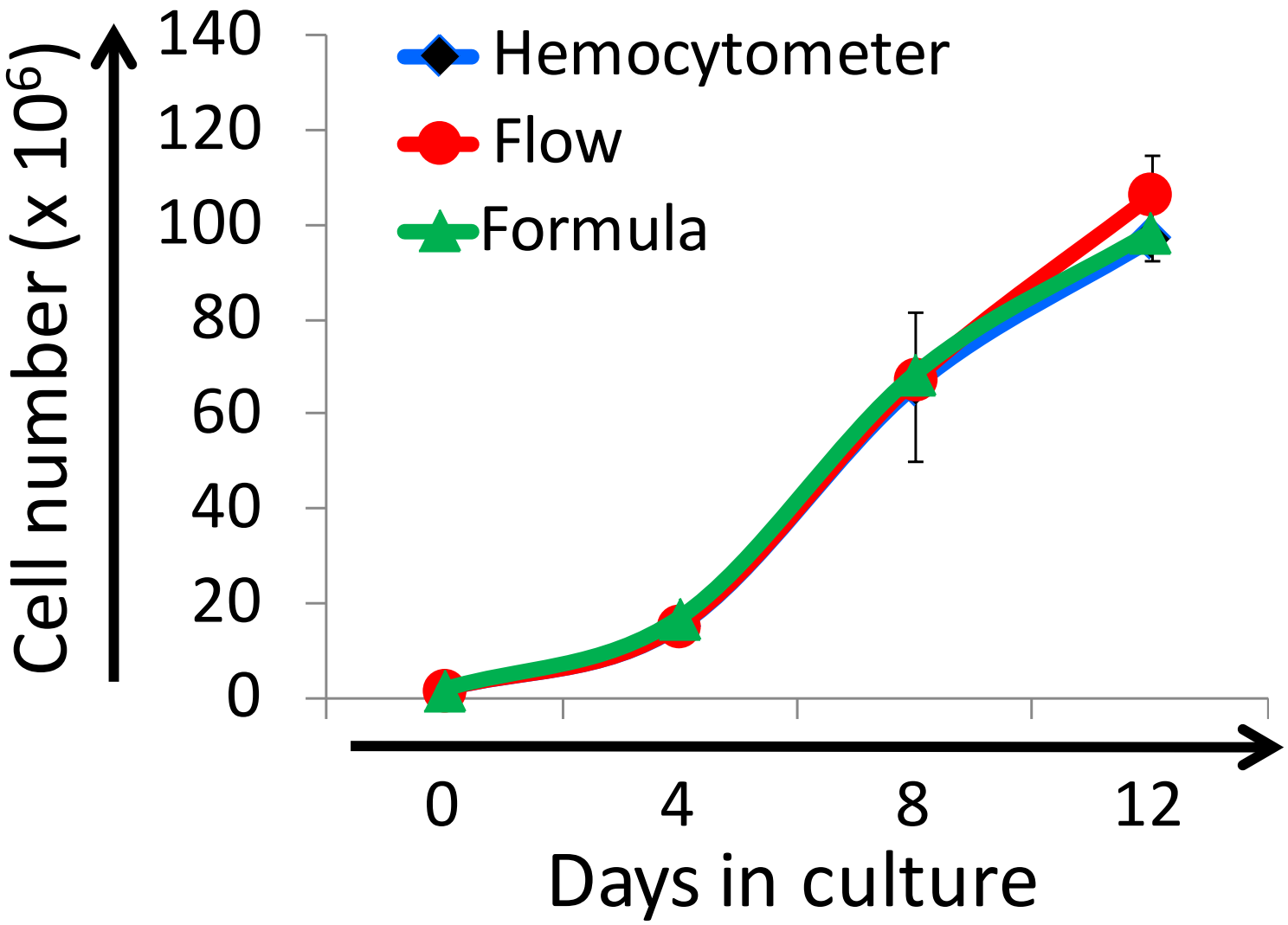
2nd – What is the optimal volume of media = 10ml/cm^2

3rd – How can cell expansion be monitored?

Inverse correlation between cell number and glucose concentration



Glucose assessment can be used to predict cell output



1st – What is the optimal seeding density = 1.2E+05 cells/cm^2

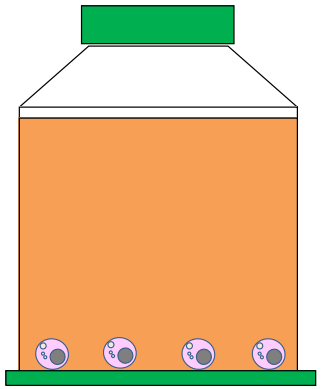
2nd – What is the optimal volume of media = 10ml/cm^2

3rd – How to monitor cell expansion = Glucose consumption

Are this observations reproducible?

Combination of optimal culture conditions using the G-Rex

Day 0



G-Rex 100M

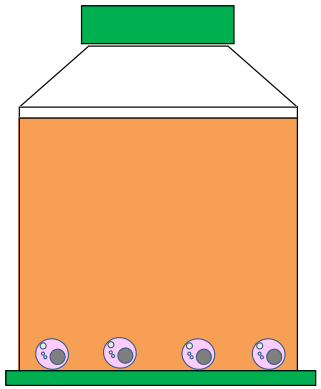
- 25E+06 cells
- 1 L of media

Multi center PACT study

- Celgene
- City of Hope
- CAGT

Combination of optimal culture conditions using the G-Rex

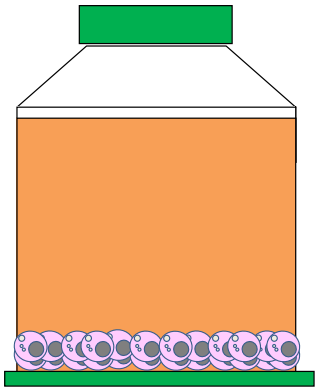
Day 0



G-Rex 100M

- 25E+06 cells
- 1 L of media

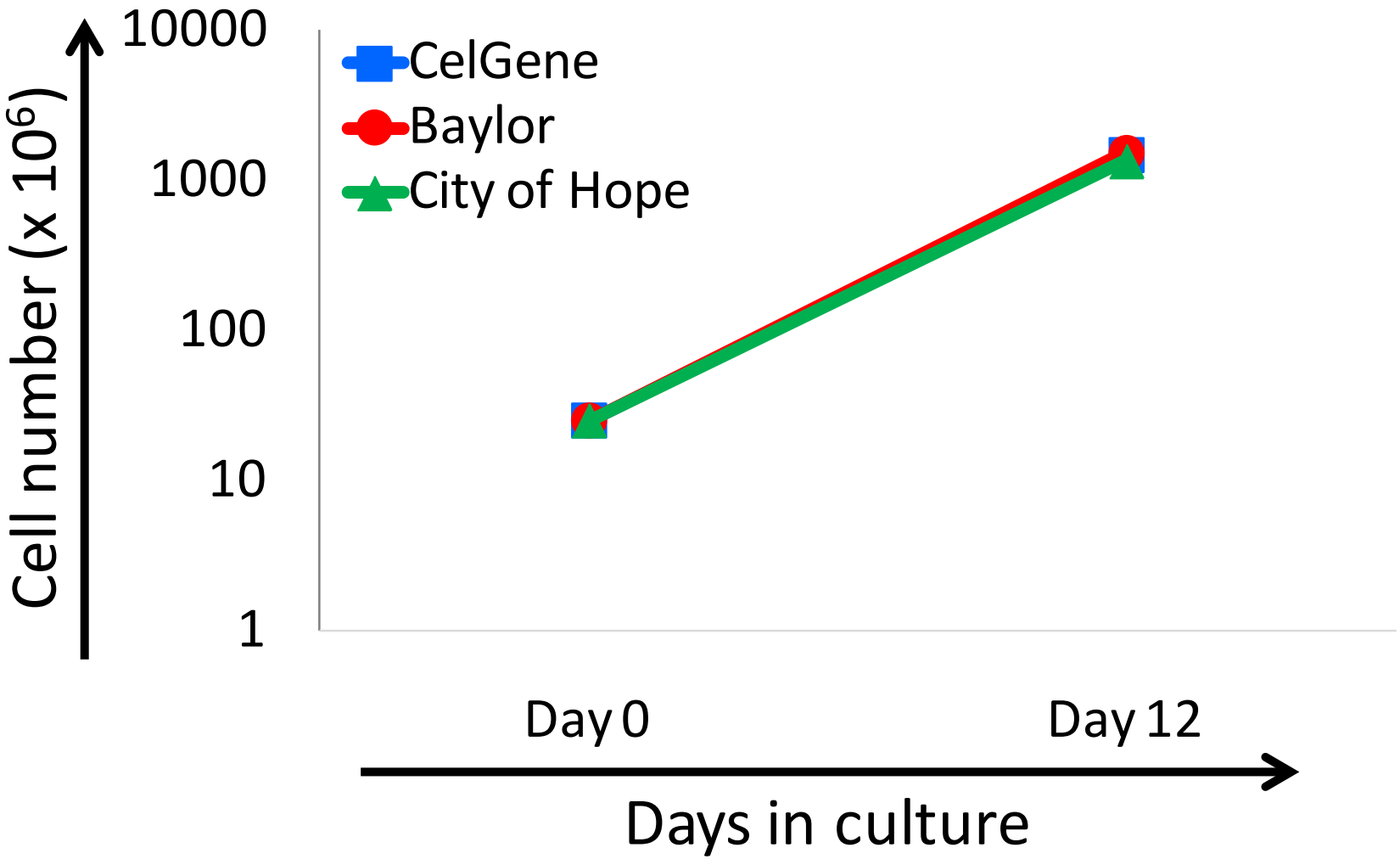
Day 12



G-Rex 100M

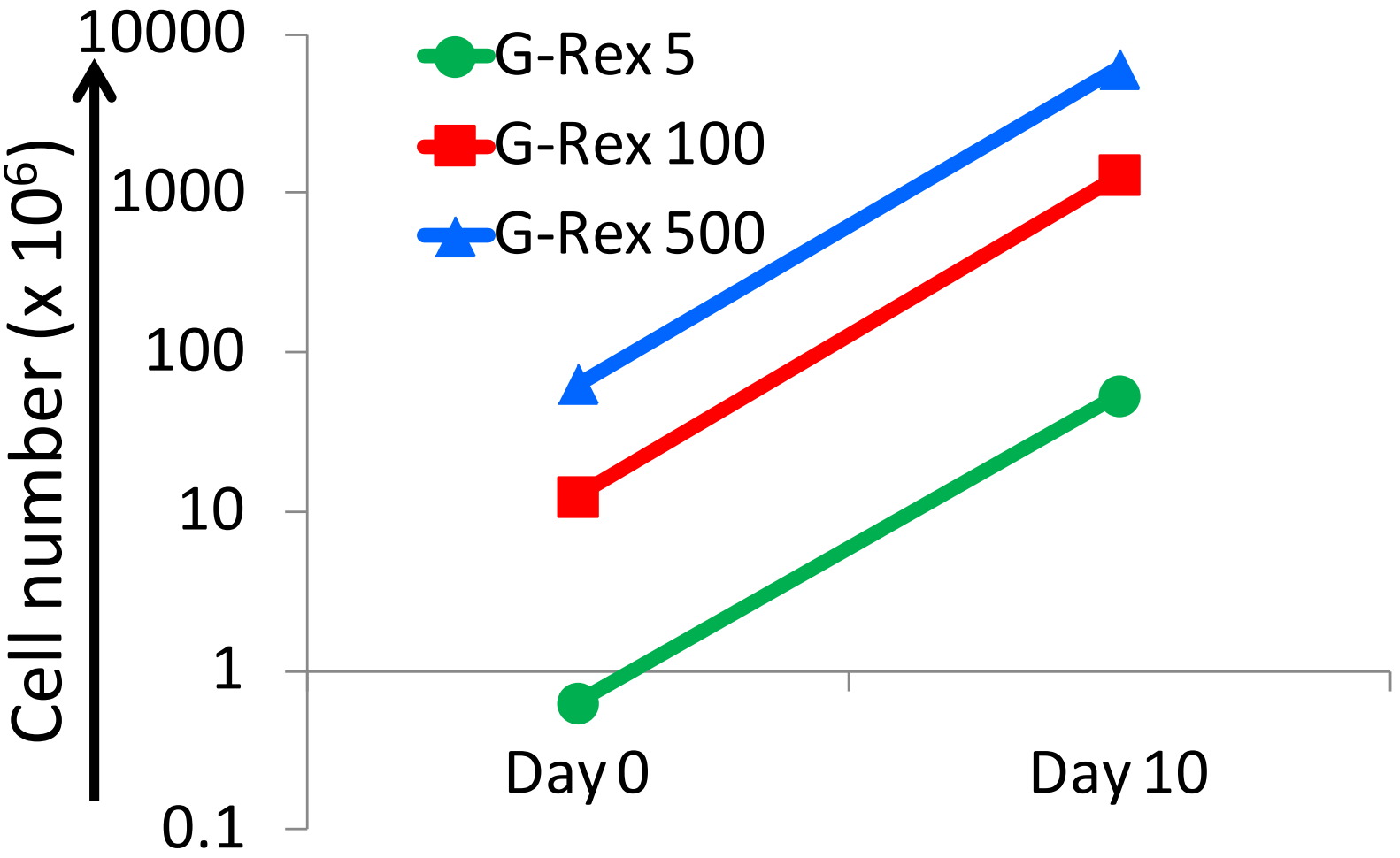
- 1.4E+09 cells

Multi center study of optimal G-Rex culture conditions



Bajgain et al. Mol Therapy – Methods and Development, 2014 In press

The culture conditions in the G-Rex are linear scalable



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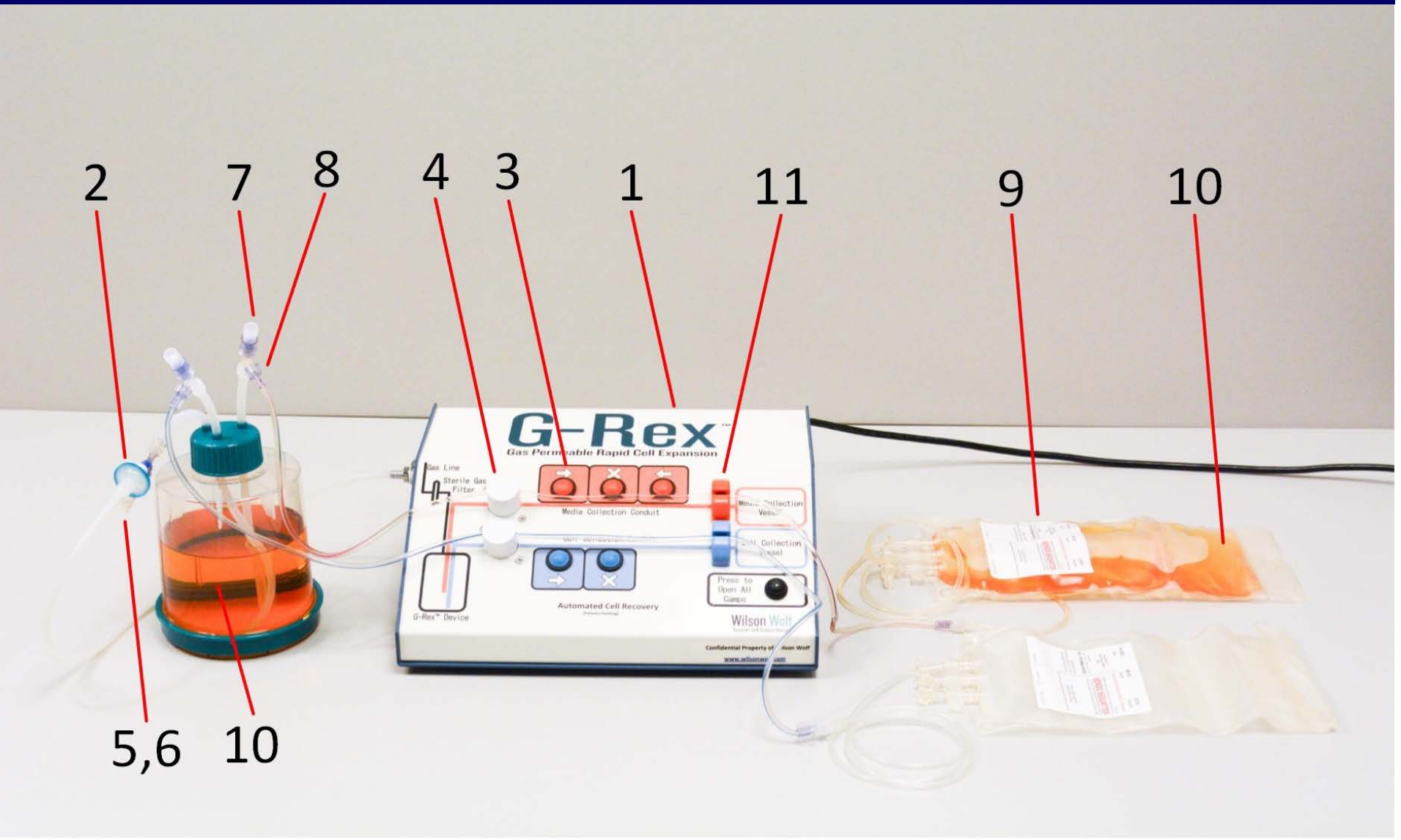
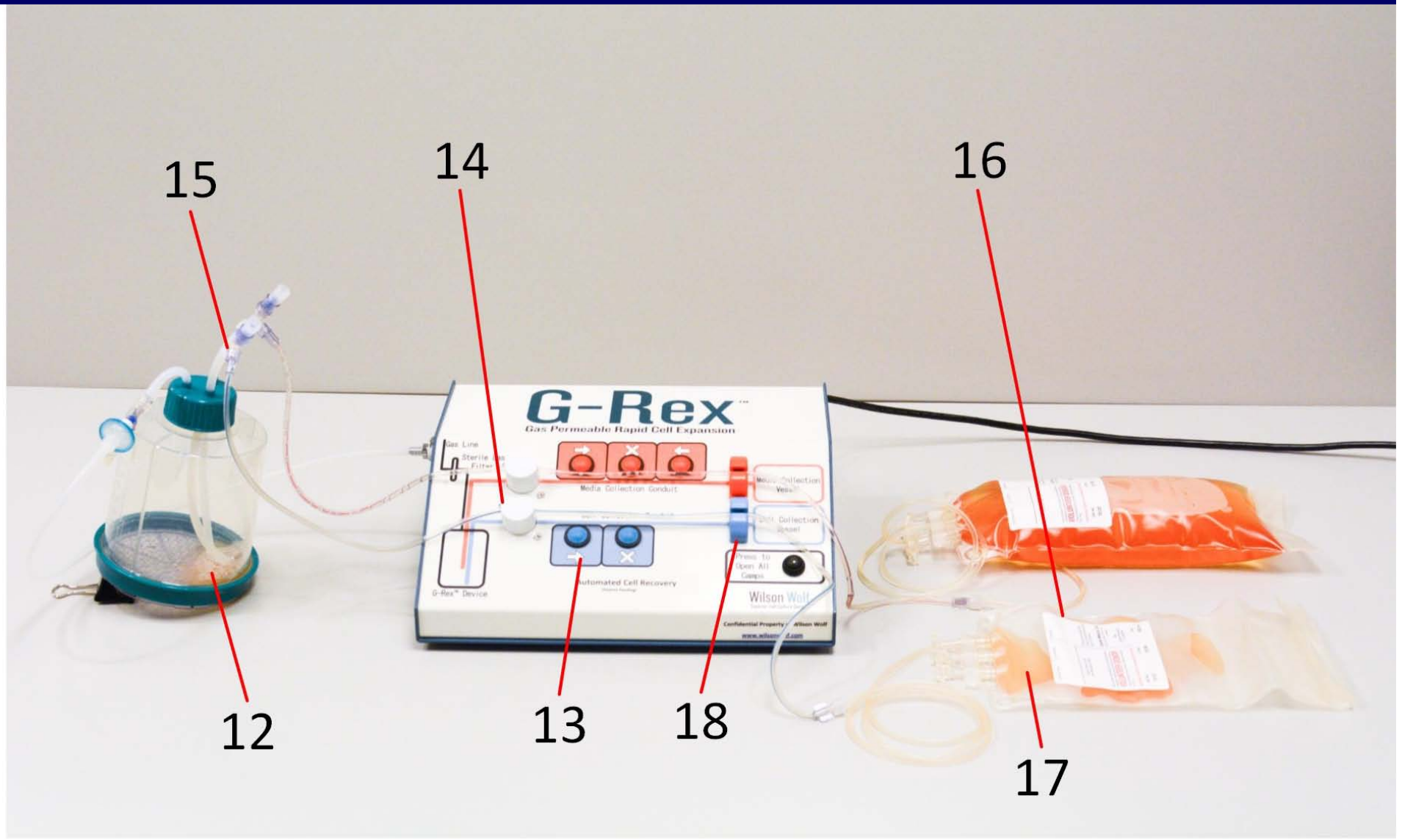
What about the cell harvest?

GatheRex device



GatheRex device



Questions poll #3

How would you like to see the G-Rex develop?

- Closed system devices
- Larger capacity
- Multiwell platform

Conclusions

- G-Rex provides a simple yet highly efficient platform for the expansion of suspension cells

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Optimal G-Rex conditions:

- Seeding density: **1.25E+05 cells/cm^2**
- Media volume: **10mL of media/cm^2**
- Simple culture assessment: **Glucose**
- Over 100 fold expansion in 10 days of culture
- No feeding/manipulation required
- Robust and easily scalable
- Validated by multi-center study
- GatheRex/semi-automatic harvest process

Acknowledgement

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