G-Rex Instructions for Use

NOTE: This document is for guidance only. You should optimize G-Rex for your particular application.

7/29/2014

Frequently Asked Questions for G-Rex

1. **What is "G-Rex"?** G-Rex is gas permeable rapid expansion culture technology that overcomes the limits of traditional plates, flasks, and bags.
2. **What cell type is G-Rex designed for?** Any non-adherent cells. It has been shown to be a powerful tool for culture of antigen presenting T cells, NK, TIL, Treg, CIK, and even hybridoma, HeLa, CHO, SF9 and others.
3. **What advantages does G-Rex provide compared to other culture device options?**
    - Saves money and time
    - Very simple and practical
    - Much more efficient use of medium
    - Greatly reduced feeding frequency
    - Far less labor
    - Cells can be recovered from very small volumes of medium
    - Can decrease the overall culture duration
    - Occupies very little space
    - No need for perfusion, mixing, or shaking
4. **What is the key to the superiority of G-Rex?**
   It allows cells to reside on a highly gas permeable membrane submerged under an unconventionally high column of medium. No pumps, mixing, or shaking is needed for cells receive a virtually unlimited supply of oxygen and nutrients on demand. **Appendix 1** provides more detail.
5. **Do I need special equipment to operate G-Rex?** No – just place it in a standard cell culture incubator.
6. **Is there a basic protocol or do I need to customize it for each cell type?** A basic protocol that is very effective for any non-adherent cell I shown in **Appendix 2**. We recommend users start with that protocol and make slight modifications to capture advantages that they deem most important.
7. **Are there any basic expectations for the number of cells each device can produce?** Yes. Cell capacity is a function of device surface area. Typically, G-Rex provides about 1 to 3 billion cells per 100 cm^2 of surface area.
8. **How do I know how many cells are in the device at any given time?** There are two ways to accomplish that:
    A. As further detailed in **Appendix 2**, count the cells withdrawing the majority of medium from the device, swirling the device to move the cells from the gas permeable membrane into suspension in the remaining medium and then taking a sample to count.
    B. As further detailed in **Appendix 3**, take a glucose sample. Measuring glucose depletion has been shown to be an excellent surrogate measure of cell number.
9. **Is there a typical feeding strategy?** Yes, as further described in **Appendix 2**.
10. **Can G-Rex simplify the cell recovery process?** Yes, cells can be recovered from just a fraction of the volume they must be recovered from in flasks and bags. **Appendix 2** provides details.

11. **Should the cap be tightened?** Yes, gas transfer occurs via the bottom of the device so there is no need to leave the cap loose.
12. **Are there any nuances about the device that I should know about?**
    A. Unlike flasks, the medium color does not change color quickly so pH cannot be determined by simply looking at the medium. The feeding strategies described in **Appendix 2** will ensure pH is acceptable at all times.
    B. If using the open system version of G-Rex, do not stab at the bottom of the device with a pipette. The bottom is comprised of a thin silicone membrane and, although very resilient, attempting to scrap it or rest your arm by leaning on it with a pipette can cause damage. To remove cells, dislodge them by swirling the medium in the device as described in **Appendix 3**.
13. **Is G-Rex producing cells for use in clinical applications?** Yes, contact us to discuss your application.
14. **Are other versions of G-Rex going to be available?** Yes – contact us for details.
    A. Closed system version are on the way
    B. Larger devices are on the way
15. **Can I reuse G-Rex?** No, G-Rex is a gamma irradiated single use device.
16. **How do I optimize G-Rex for the best expansion of cells in the least amount of time?** Refer to **Appendix 4**.
17. **Other than reduced feeding frequency, what advantages does G-Rex100M provide?** Refer to **Appendix 5**.
18. **How can I avoid counting cells to know how many cells existing in my device?** Refer to **Appendix 6**.

APPENDIX 1: What is the key to the superiority of G-Rex?

A cross-section of the G-Rex device is shown in **Figure 1A** relative to a cross-section of a conventional flask, shown in **Figure 1B**. In G-Rex, cells [**10A**] reside upon a gas permeable membrane [**15A**]. A gas membrane support [**20A**] holds the membrane in a horizontal position so that cells distribute uniformly upon it while simultaneously allowing gas to contact the bottom of the membrane. Media [**25A**] resides at a height far beyond the 3 mm limit of flasks and the typical 1.0 cm limit of existing gas permeable approaches like cell culture bags. Oxygen moves from the ambient incubator environment across the gas permeable membrane in response to cellular demand. Due to the large source of nutrients in the device and the proximity of cells to ambient gas, cells have virtually unlimited access to nutrients and oxygen on demand.

The conventional flask is shown in **Figure 1B**, where cells [**10B**] reside on its **non**-gas permeable plastic bottom [**15B**]. Media [**25B**] resides at its maximum height of just 3 mm so that oxygen can get to the cells. Gas [**30B**] resides above the media and moves into the media in response to cellular demand. Note that only a small amount of media is available to cells relative to the overall height of the flask and nearly 95% of the flask is wasted space. In contrast, none of the space is wasted in the novel device.

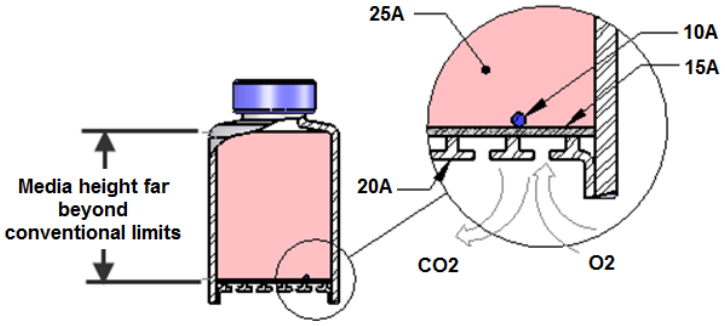


Figure 1A: *Novel device*

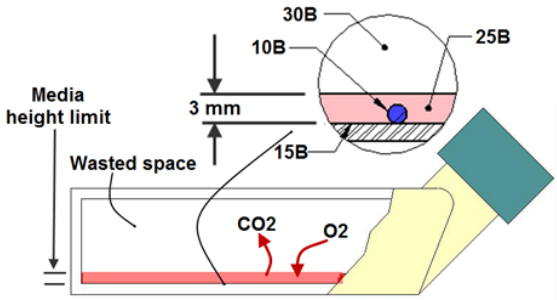


Figure 1B: *Conventional flask*

The G-Rex also allows media to be removed without disturbing cells. Since there is no need for a gas-media interface, G-Rex can be moved without media sloshing about and dispersing cells throughout the media. Instead, cells remain in a quiescent state on the gas membrane during routine handling. When secreted products are desired, cells remain in G-Rex when supernatant is removed, eliminating need of cell separation. Likewise, when cells are desired, excess media can be removed prior to cell recovery leaving the cells to be removed from just the small volume of media that remains, which greatly simplifies cell recovery.

APPENDIX 2: Basic G-Rex Protocol

Key Points:

- "Surface density" is the most critical variable in getting optimum performance. We refer to cells per cm^2 as "surface density". Stated differently, cells per cm^2 of membrane, **not** cells per ml of medium, are the most important factors on G-Rex performance.

- Cell expansion typically begins when cells are at 500,000 per cm^2.

- Cell expansion typically ends when cells are at between 10 -30 million cells per cm^2.

- G-Rex10 and G-Rex100 are typically fed only once every 5 days so long as cytokines (typically IL-2) are added at the same frequency as your standard protocols require (typically every 2-3 days).

- G-Rex100M typically doesn't need to be fed at all. Usually 1000 ml of medium is enough to last until the G-Rex has hit maximum cell capacity, which takes about 9 to 13 days. Cytokines (typically IL-2) must be added at the same frequency that your standard protocols require (typically every 2-3 days) and in a quantity that assumes all prior IL2 has been depleted.

- Once you establish conditions that work within the guidelines stated above, optimize your application by:

    - ❖ Reduce cell surface density at the onset of culture. Culture expansion often can start with only 125,000 cells per cm^2.

    - ❖ Alter the feeding frequency until you attain the steepest population growth curve and the minimal feeding frequency.

    - ❖ Take glucose measurements. Glucose depletion has been shown to be a surrogate measure of the number of cells present in the device at any time (see **Appendix 5** for details).

- Feed cells by aspirating 75% of the medium from the device and replacing it with fresh medium. Cells will remain on the gas membrane during medium removal so long as the aspirating pipette remains near the top of the medium during this process. 75% of the medium is 30ml in G-Rex10, 300 ml in G-Rex100, and 750ml in G-Rex100M.
- The most accurate cell count is obtained by aspirating 75% of the medium, swirling the medium to dislodge cells from the gas membrane and to suspend them in the remaining medium. Then take a sample.

- Harvest cells at the end of culture by aspirating the maximum amount of medium without disturbing the cells on the gas permeable membrane. Typically, this can be accomplished at a rate of 75% of the medium in the G-Rex10 (30ml medium collection) and the G-Rex100 (300 ml medium collection). With the added height and visibility of the medium in the G-Rex100M, users can collect up to 90% (or 900 ml) of the medium before cell harvest. Cells will remain on the gas membrane during medium removal so long as the aspirating pipette remains near the top of the medium during this process. Next, swirl the medium to dislodge cells from the gas membrane and to suspend them in the remaining medium. Then remove the medium and cells. Thus, the final cell population can be recovered in G-Rex10 in just 10 ml of medium (typically 100-300 million cells in just 10 ml) and in G-Rex100 and G-Rex100M in just 100 ml of medium (typically 1-3 billion cells in just 100 ml).

APPENDIX 3: Expansion of primary T cells transduced with a chimeric antigen receptor

Day 0: Place 1E+06 PBMCs per well in a non-tissue treated (NT) 24 well plate (final volume of 2 ml of media per well)

Day 1: Add 50 u of IL2 per well

Day 3: Coat a NT 24 well plate with RetroNectin for 3 hours at 37C and add the vector and activated T cells. Perform the transduction of T cells using the conventional method

Day 6: Phenotype the expression of the transgene (if transduction is satisfactory continue to cell expansion)

Day 7: Initiate cell expansion transferring the transgenic T cells to a G-Rex at a minimal seeding density of 0.5E+06 cells per cm^2 of gas permeable surface area and adding medium as specified below.

NOTE: Cells per device will be as follows based on the surface area of each device

- G-Rex10 gas permeable surface area = 10 cm^2. Therefore, place about 5E+06 T cells in the device.
- G-Rex 100 and G-Rex100M gas permeable surface area = 100 cm^2. Therefore, place about 50E+06 T cells in the device.

Depending on the device, add the following amounts of media:

- 40 ml in the G-Rex 10
- 400 ml in the G-Rex100
- 1000 ml in the G-Rex100M

Normalize the cytokine concentration accordantly to the volume of media present in the G-Rex.

NOTE: Based on user feedback, the G-Rex10 will typically deliver 100 - 300 million cells and the G-Rex100/100M will typically deliver 1 -3 billion cells. The duration of culture and the amount of feeding needed to achieve these cell quantities may vary. The time sequence below is our best estimate.

Day 10: Add cytokine

Day 13: Add cytokine

Day 15: Count cells and add fresh medium. The easiest way to do this is to carefully move the G-Rex from the incubator to the flow hood so that cells remain at the bottom of the device, aspirate the majority of medium from the device (remove 30 ml from the G-Rex10, remove 300 ml from the G-Rex100 and 750 ml from the G-Rex 100M), and swirl the device to distribute the cells into

the remaining medium. Take a sample for a cell count, but prior to counting replace the medium, add cytokine, and return the device to the incubator. Count the cells. Estimate the doubling time of the cells based on the number used to initiate culture and the number currently present. Use that information to estimate when the culture will reach the target numbers of at least 100 million cells in the G-Rex10 and 1 billion cells in the G-Rex100/100M. What follows is our best estimate based on data we have seen to date.

Day 19: Add cytokine

Day 22: The G-Rex devices should be at maximum capacity by now. But if they are slow growing, you may have to feed again as previously described for the steps on Day 15 and continue the culture.

Repeat medium exchanges and cytokine addition as needed until the cells have reached a maximum number at high viability.

Collect the cells from the G-Rex: This can be done most easily by carefully moving the G-Rex from the incubator to the flow hood so that cells remain at the bottom of the device. Then aspirate medium from the device to a level that leaves the cells in the device but minimizes the medium volume (typically about 10 ml in the G-Rex10 and 100 ml in the G-Rex100/100M should remain in the device). Then swirl the device to suspend the cells in the small volume of medium remaining in the G-Rex, and collect the medium and cells.

NOTE: Please report your results to Argos Technologies, so that we can assist in determining if more efficiency can be brought to your process and so we can learn more about your experience. Any advice for device modifications and improvements would be most appreciated. Our goal is to use our platform technology to create devices that are optimized for the field of adoptive cell therapy.

APPENDIX 4: QUICK GUIDE TO G-REX OPTIMIZATION

Definitions

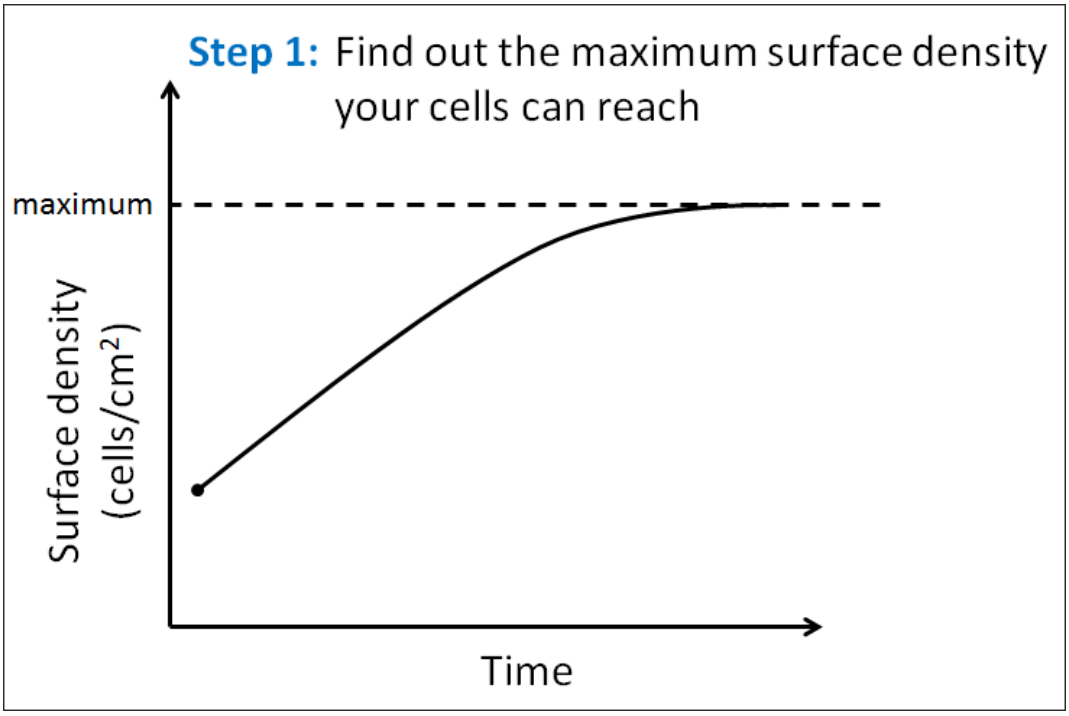
Surface Density: The number of cells residing on the gas permeable G-Rex bottom when normalized to units of cells/cm^2.

Maximum surface density: The maximum number of cells, normalized to cells/cm^2, that can reside on the gas permeable G-Rex bottom before a drop in viability occurs.

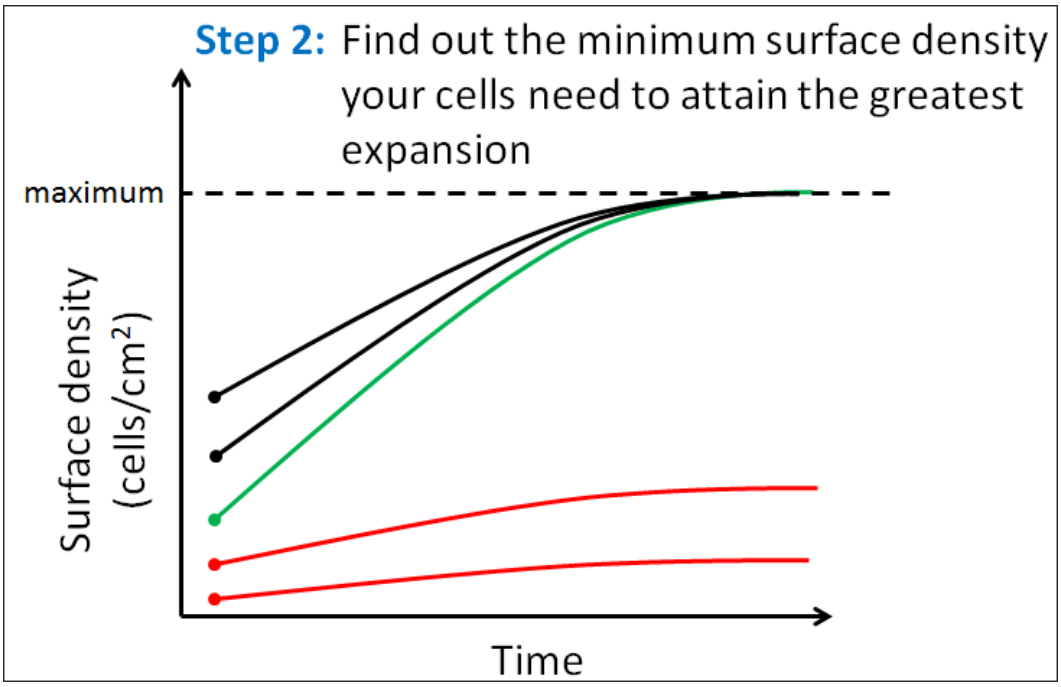
Minimum surface density: The minimum number of cells, normalized to cells/cm^2, that must reside on the gas permeable G-Rex bottom to initiate population expansion.

Fold expansion: The maximum surface density divided by the minimum surface density.

Background: The G-Rex10 is an excellent device for optimizing protocols for your cells. Whatever is learned in G-Rex10 scales directly to G-Rex100 and G-Rex100M. By using G-Rex10, you will use less media and counting cells is very easy (which is frequently required during optimization work).



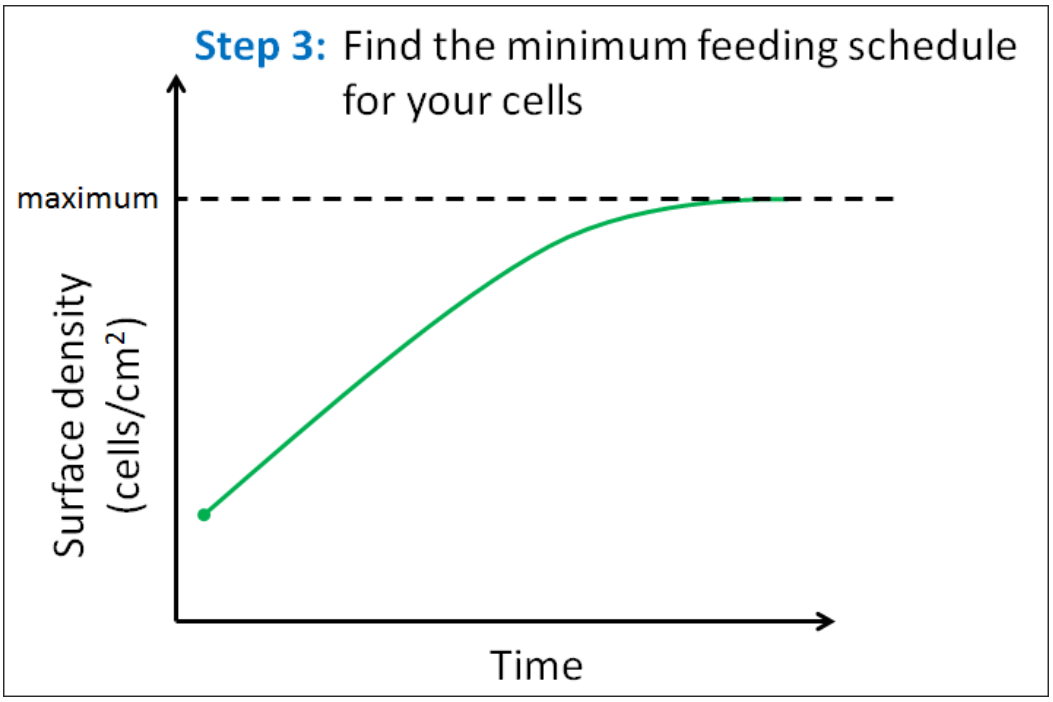
- Add 10 million cells (i.e. 1 x 10^6 cells/cm^2) and 40 ml of medium into each G-Rex10
- Every 3 days, remove and replace 30 ml of medium and count cells
- Continue until cells reach a plateau. That is the maximum cell density your cells can achieve.



Create an experimental matrix that determines the minimum surface density that is needed for your cells to begin growth. Add 40 ml of medium to each G-Rex10 devices along with the following number of cells:

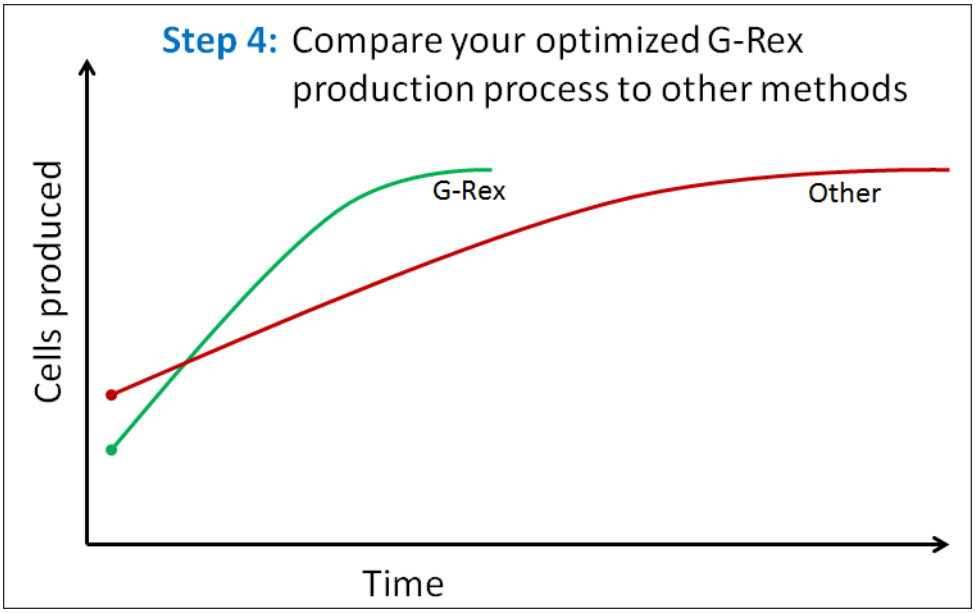
- 10 million (i.e. 1 x 10^6 cells/cm^2)
- 5 million (i.e. 0.5 x 10^6 cells/cm^2)
- 2.5 million (i.e. 0.25 x 10^6 cells/cm^2)
- 1.25 million (i.e. 1.25 x 10^6 cells/cm^2)
- 0.625 million (i.e. 0.0625 x 10^6 cells/cm^2)

About every 4 days, remove and replace 30 ml of medium and count cells to find out if cells are capable of expanding from the starting surface density. **Make sure you add IL2 (or other cytokines) at least every 2-3 days no matter how often you exchange the medium. Add the IL2 at a quantity that assumes it has all been depleted.** You can optimize IL2 frequency later, but we do not want to lack of adequate IL2 to affect your determination of the minimum surface density that is needed for your cells to begin growth.



- Start the cultures with the minimum surface density of Step 2 and with 40 ml of medium.
- Experiment with different feeding schedules to find out the minimum amount of medium needed to reach the maximum surface density of Step 1.
- **Make sure you add IL2 (or other cytokines) at least every 2-3 days no matter how often you exchange the medium. Add the IL2 at a quantity that assumes it has all been depleted.**

Note: Once the maximum and minimum surface density is found in G-Rex10, these values will scale 10-fold into the G-Rex100 and G-Rex100M. After you have determined the minimum feeding frequency, compare your optimized G-Rex production process to any other method.



- Feed G-Rex100 at the optimized schedule found in Step 3.
- Add 1 liter of medium into G-Rex100M at the onset of culture and do not feed. Periodically counts cells. **Make sure you add IL2 (or other cytokines) at least every 2-3 days until cells reach maximum surface density. This will probably occur in around 10 to 14 days.**

We encourage you to compare G-Rex to alternative production methods based upon the following criteria. We are confident a comparison will show the power of G-Rex to simplify and reduce the cost of your cell production process.

	Device Comparison		
Evaluation Criteria	**G-Rex**	**Bag**	**WAVE**
Quantified:			
Minimum starting surface density (cells/cm^2)			
Maximum final surface density (cells/cm^2)			
Media efficiency (ml per 10^6 cells)			
Cost of media per 10^9 cells			
Fold expansion			
Labor (minutes)			
Cost of labor per 10^9 cells			
Downstream processing			
Labor to separate cells from media			
Cost of labor per 10^9 cells			
Cost of centrifuge material per 10^9 cells			
Space required to perform production			
Characterized criteria:			
Practicality			
Scale up potential			
Skill level needed to perform production			
Commercialization practicality potential			
Functional Comparison			
Cell phenotyping comparison			
Cell functionality			

APPENDIX 5: G-Rex100M Advantages

Placing Media at a High Level at the Onset of Culture is Beneficial

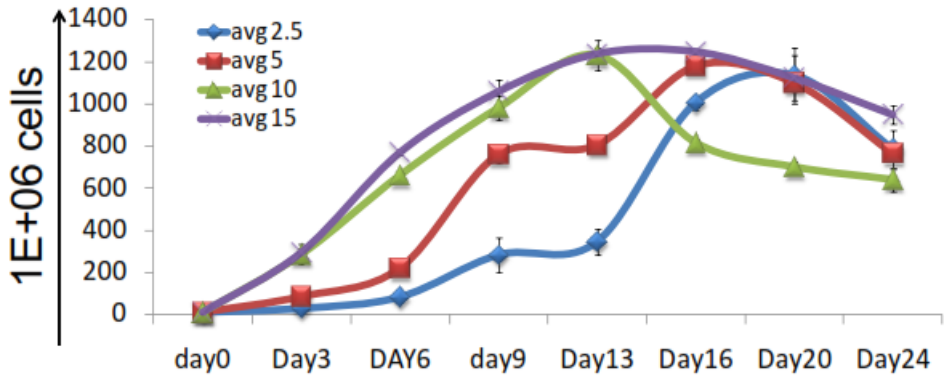
When using flasks and bags to produce cells, periodic media replenishment is needed. However, since media replacement is usually made on the basis of convenience and not exactly at the point when solutes have been depleted to specific level, often population expansion of the cells is slowed (e.g. solutes such as glucose have dropped below needed levels). When fresh media is added, it takes time for the population expansion to reach it optimal rate.

By increasing media height at the onset of culture relative to flasks and bags, G-Rex is designed to minimize or even eliminate the need to perform media exchange, thereby minimizing or even eliminating the number of times cell population expansion slows throughout culture.

The graphs below show an observation about growth curves in G-Rex. GRex100M can facilitate an undiminished rate of culture expansion.

As expected, the media exchange process of GRex100, although far less frequent than flasks and bags, does alter the rate of culture expansion (red and blue). It makes sense given very few (if any) people actually exchange media based on depletion of nutrients when using static culture devices. However, the population expansion can expand at a uniform rate when media is at a 10 cm height (green) at the onset of culture. When media is at 11 cm height (purple) you also get a little more time to harvest before viability drops off. Thus the G-Rex100M is designed to integrate media height at up to 11 cm should these attributes be needed.

In summary, the time needed to expand a population of cells to any given quantity is reduced by G-Rex. When selecting the particular G-Rex device, one should recognize that G-Rex100M has an advantage over G-Rex100 since it allows media to reside at great height at the onset of culture. Thus, the G-Rex prices are adjusted accordingly to allow you to choose whatever level of efficiency you are comfortable with.



APPENDIX 6: Glucose depletion rate as a surrogate measure of cell quantity

NOTE: Use a value of 250 mg/dl for the value "C" in the formulas shown below.

Thus value, which constitutes "total reduction in glucose concentration" was found by measuring glucose concentration at the onset of culture (the "starting glucose concentration"), and then counting cells and measuring glucose over time until the cells reached maximum surface density (i.e. the maximum number of cells per cm^2). Whatever glucose concentration existed at the time cells reached maximum surface density we call "final glucose concentration." By subtracting final glucose concentration from starting glucose concentration we found that it was typically about 250 mg/dl for a number of cell types. Thus, use that number in your formulas (i.e. the "C" variable) and see if that allows you to predict your cell number by just measuring glucose.

We observed that glucose depletion rates were consistently indicative of the number of cells in a culture despite the culture medium residing in a static state and (other than just routine handling of the device) not subjected to mechanically forced mixing such as by perfusion, shaking, or stirring prior to sampling. This finding opens the door to further simplification in the field of Adoptive Cell Therapy. For example, the act of counting cells to determine how well a culture is progressing is one of many factors that make cell production for Adoptive Cell Therapy impractical. The use of a surrogate measure in lieu of cell counts, combined with the inventive disclosures herein, brings even more simplification to cell production.

We have discovered that it is possible to use glucose concentration of the culture as a surrogate indicator of the population of the culture. For cultures in which cells reside upon a growth surface comprised of a given type of gas permeable material, knowing the minimum total medium volume needed for the culture to reach maximum surface density and the total reduction in glucose concentration needed for the culture to reach maximum surface density and the total reduction in glucose concentration needed for the culture to reach maximum surface density sets the stage for a surrogate prediction of the number of cells in the population of the culture. Equipped with that knowledge, one initiating culture (or a stage of culture) would determine the baseline glucose concentration of medium, the baseline volume of medium, and would keep track of the volume of medium added to the culture prior to taking a measure of glucose concentration at the time of population estimation. The estimated number of cells in the population is a function of the prorated total reduction in glucose concentration needed to reach maximum cell density multiplied by the prorated minimum medium volume needed to reach maximum surface density and multiplied by the maximum surface density possible on the growth surface.

Skilled artisans should be aware that by predetermining the maximum cell density in medium (cells/cm^2) that the specific cell types (s) can attain when residing on the growth surface comprised of a particular type of gas permeable material, an alternative formulaic relationship can be used to estimate the

number of cells in the culture. In that case, the formulaic relationship would be a function of; (the prorated total reduction in glucose concentration needed to reach maximum cell density) x (the volume in medium at the onset of culture plus the volume of medium added to the culture) x (maximum cell density). Be advised that in the event that the cumulative volume of medium exceeds that of the minimum volume medium needed to reach maximum surface density, the minimum volume of medium should be used in place of the cumulative volume (as no extra medium volume will increase the surface density beyond its maximum).

A series of experiments were undertaken that determined minimum volume of medium needed to allow the cells to reach maximum surface density and the corresponding total reduction in glucose concentration. The total reduction in glucose concentration was about 250 mg/dl for a variety of cultures with various cell types including K562, LCL, and T cells. We were able to create formulaic relationships that were predictive of cell number in culture as shown below, where:

A= baseline glucose concentration of medium

B= measure of glucose concentration at the time of population estimation

C= total reduction in glucose concentration needed to reach maximum surface density

D= baseline volume of medium

E= volume of medium added after baseline

F= minimum total medium volume needed to reach maximum surface density

G= maximum surface density

E= surface area of the growth surface

[(A - B)/C] x [(D + E)/F] x G x E = estimated number of cells in the culture population in the device. Note that the prorated minimum medium volume cannot exceed 100%, since additional medium will not increase surface density beyond the maximum capacity. For example, if a culture requires 10ml to reach maximum surface density and the baseline volume of medium plus the volume of medium added exceeds 10ml, one should use 100% as the prorated minimum medium volume.

Note that the predictive formulas require knowledge of the cell culture applications maximum cell density (and/or maximum surface density) under conditions in which cells reside on a growth surface comprised of the particular gas permeable material the artisan has selected. Experiments can be undertaken to make that determination. For example, to determine the maximum cell surface density of K562 cells upon a growth surface comprised of the gas permeable material in our experimental fixtures (dimethyl silicone as described previously), we increased medium height until surface density could increase no more. The minimum volume of medium needed to support a maximum attainable surface density of K562 at about 12.0E+06 cells/cm²was determined to be 10 ml with a corresponding total reduction in glucose concentration of 250 mg/dl.

Illustrative examples of how this information could be used to assess the number of cells in K562 culture follow. For the first example, assume medium is not added after the onset of culture and these conditions exist:

baseline medium volume = 10 ml

baseline glucose concentration = 475 mg/dl

glucose sample = 300 mg/dl

surface area of growth surface = 100 cm^2

Then the calculation would process as follows:

[(475 mg/dl – 300 mg/dl) x (10 ml + 0 ml)/10 ml] x 12E+06 cells/cm^2 x 100 cm^2 = 840 x 10^6 cells.

As another example, assume medium is added after the onset of culture and these conditions exist:

baseline medium volume = 6 ml

baseline glucose concentration = 475 mg/dl

glucose sample = 300 mg/dl

surface area of growth surface = 100 cm^2

Then the calculation would proceed as follows:

[((475 mg/dl – 300 mg/dl)/250 mg/dl) x (6 ml + 2 ml)/10 ml] x 12E+06 cells/cm^2 x 100 cm^2 = 672 x 10^6 cells.

As yet another example, assume medium is added after the onset of culture and these conditions exist:

- baseline medium volume = 6 ml
- baseline glucose concentration = 475 mg/dl
- glucose sample = 300 mg/dl
- surface area of growth surface = 100 cm^2

Then, since total medium volume added to the culture exceeds the minimum total medium volume needed to reach maximum surface density, prorated minimum medium volume goes to 100% and therefore the prorated value equals 1, and the calculation would proceed as follows:

[((475 mg/dl – 300 mg/dl)/250 mg/dl) x (1)] x 12E+06 cells/cm^2 x 100 cm^2 = 840 x 10^6 cells.

More experiments were undertaken to determine if the formula dictating the relationship between glucose depletion and the number of live cells in the device was accurate when glucose concentration at the onset of cultures varied. For illustrative purposes, **FIG. 19A** shows a representative spreadsheet of the experimental conditions and typical results for the culture of K562 cells under equivalent starting conditions except for the glucose concentration, which was 240 mg/dl vs. 475 mg/dl at the onset of culture.

Fig. 19A

ROW			DAY 0	DAY 4	DAY 8	DAY 11
1	SURFACE DENSITY ON DAY 0 (cells/cm^2)	1.25E + 05				
2	SURFACE AREA OF GROWTH SURFACE (cm^2)		100	100	100	100
3	MEDIUM VOLUME IN ml		1000	1000	1000	1000
4	RATIO OF MEDIUM VOLUME TO GROWTH SURFACE AREA (mls/cm^2)		10	10	10	10
5	MEDIA HEIGHT (cm)		10	10	10	10
6	SURFACE DENSITY (cells/cm^2)		1.25E + 05	2.65E + 06	6.55E + 06	9.88E + 06
7	CELL DENSITY (cells/ml)		1.25E + 04	2.65E + 05	6.55E + 05	9.88E + 05
8	GLUCOSE CONCENTRATION (mg/dl)	240	240	176	111	18
9	GLUCOSE CONSUMPTION (mg/ml)		0	64	129	222
10	PREDICTED CELL NUMBER BY USE OF GLUCOSE		0.00E + 00	3.00E + 08	6.19E + 08	1.07E + 09
11	VIABILITY		100%	100%	100%	98%
12	TOTAL LIVE CELLS		1.25E + 07	2.65E + 08	6.55E + 08	9.88E + 08
ROW			DAY 0	DAY 4	DAY 8	DAY 11
1	SURFACE DENSITY ON DAY 0 (cells/cm^2)	1.25E + 05				
2	SURFACE AREA OF GROWTH SURFACE (cm^2)		100	100	100	100
3	MEDIUM VOLUME IN ml		1000	1000	1000	1000
4	RATIO OF MEDIUM VOLUME TO GROWTH SURFACE AREA (mls/cm^2)		10	10	10	10
5	MEDIA HEIGHT (cm)		10	10	10	10
6	SURFACE DENSITY (cells/cm^2)		1.25E + 05	5.13E + 06	9.26E + 06	1.05E + 07
7	CELL DENSITY (cells/ml)		1.25E + 04	5.13E + 05	9.26E + 05	1.05E + 06
8	GLUCOSE CONCENTRATION (mg/dl)	475	475	374	287	256
9	GLUCOSE CONSUMPTION (mg/ml)		0	107	193	219
10	PREDICTED CELL NUMBER BY USE OF GLUCOSE		0.00E + 00	5.13E + 08	9.20E + 08	1.00E + 09
11	VIABILITY		100%	100%	100%	98%
12	TOTAL LIVE CELLS		1.25E + 07	5.13E + 08	9.26E + 08	1.05E + 09

Results are graphically depicted in **FIG. 19B**, **FIG. 19C**, **FIG 19D**, **FIG. 19E** and **FIG 19F**. Population growth by cell count and as predicted by glucose depletion was normalized for surface density.

FIG 19B shows the population growth rate differed slightly, but arrived at about the same number in 11 days.

Fig. 19B

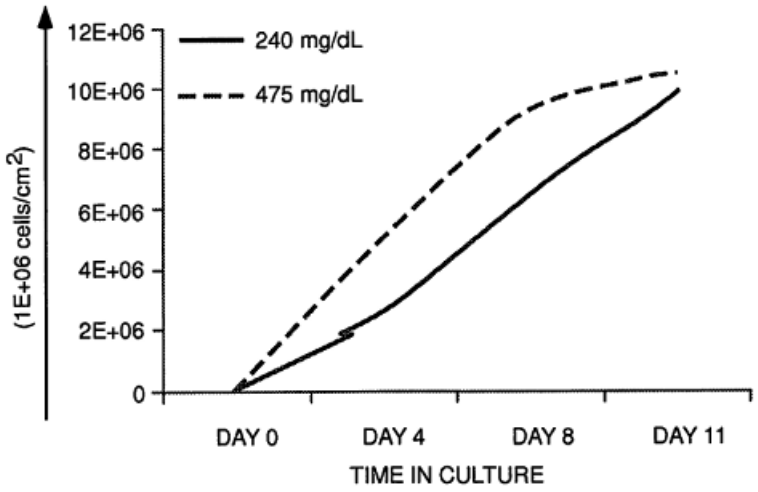


FIG. 19C shows the glucose depletion rate in each future condition.

Fig. 19C

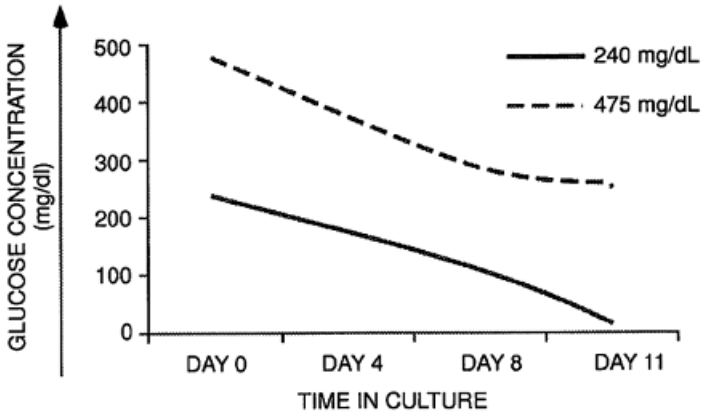


FIG. 19D shows the glucose consumption rate in each culture condition.

Fig. 19D

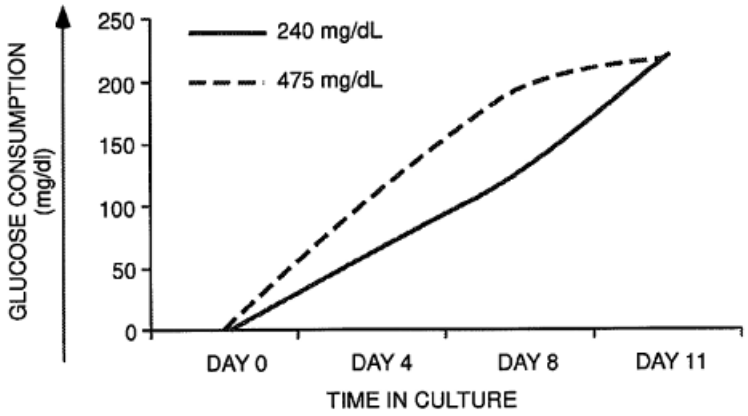


FIG. 19E shows an overlay of the predicted value, using the formulaic calculation of cell number, versus the cell number as determined by manual counts for the culture initiated at a glucose concentration of 240 mg/dl.

Fig. 19E

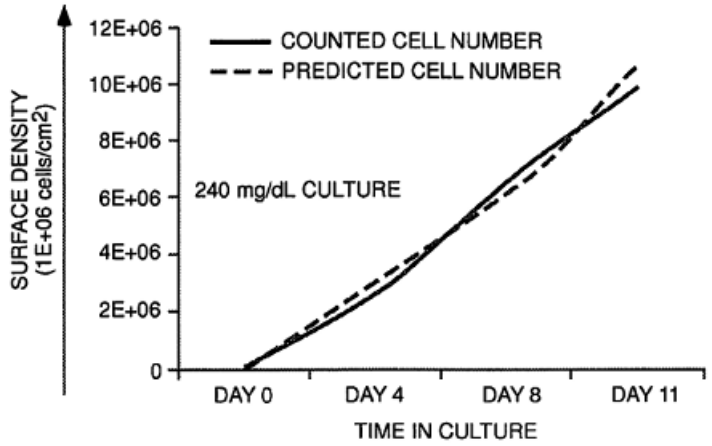
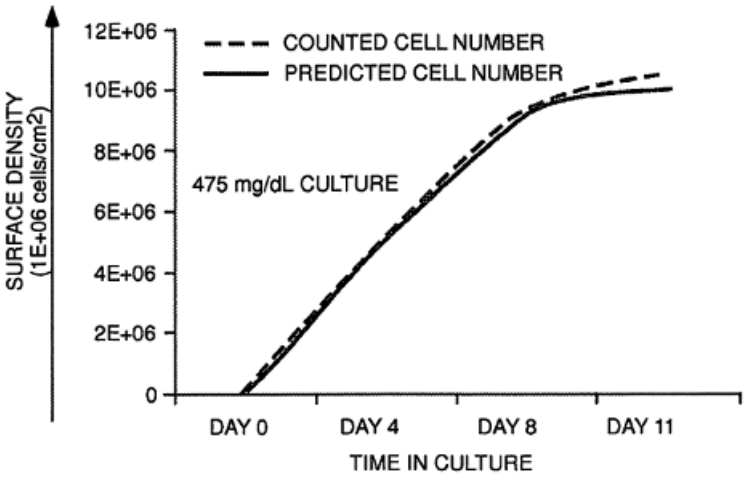


FIG. 19F shows and overlay of the predicted value, using the formulaic calculation of the cell number, versus the cell number as determined by manual counts for the culture initiated at a glucose concentration of 475 mg/dl. Note the predictive capacity of the formulaic approach relative to the method of manual cell counts. This further demonstrates that various embodiments of the present invention can be utilized in conjunction with a method of reducing, or even eliminating, the frequency of cell counts in lieu of a surrogate measure of the concentration of solutes in the medium.

Fig. 19F


12E+06
10E+06
8E+06
6E+06
4E+06
2E+06
0
SURFACE DENSITY
(1E+06 cells/cm2)
COUNTED CELL NUMBER
PREDICTED CELL NUMBER
475 mg/dL CULTURE
DAY 0
DAY 4
DAY 8
DAY 11
TIME IN CULTURE