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(71) Applicant: **MAXCYTE, INC.** [US/US]; 9713 Key West Avenue, Suite 400, Rockville, Maryland 20850 (US).

(72) Inventors: **ABAD, Joseph Dizon**; 9701 Fields Rd., Unit 1800, Gaithersburg, Maryland 20878 (US). **MENDOZA, Jeraldine Casino**; 11070 Natural Drive, Rancho Cucamonga, California 91730 (US). **HIDAJAT, Matthew Rachmat**; 1118 Agnew Dr, Rockville, Maryland 20851 (US). **ZHU, Yuqi Wu**; 304 Rock Run Cir, Broomall, Pennsylvania 19008 (US).

(74) Agent: **GRINGAUZ, Jordan M.** et al.; 901 New York Avenue, N.W., Washington, District of Columbia 20001-4313 (US).

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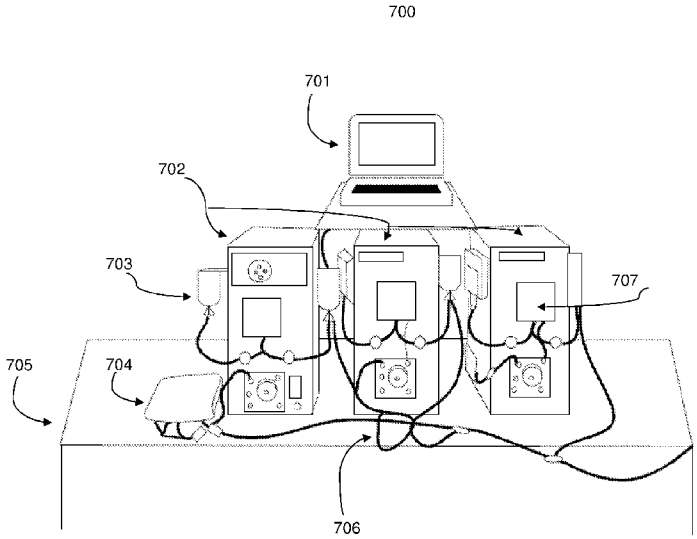

FIG. 7

(57) Abstract: The present disclosure relates generally to a system for electroporating large volumes of cells simultaneously using multiple electroporation systems simultaneously.

[Continued on next page]

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A MULTISYSTEM ELECTROPORATION METHOD

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present application claims priority to U.S. Provisional Application No. 63/483,180 filed February 3, 2024, which is herein incorporated by reference.

[002] The present disclosure relates generally to a system for electroporating large volumes of cells simultaneously using multiple electroporation systems simultaneously.

BACKGROUND

[003] Electroporation is a well-known method of introducing compositions into Cells. Those of skill in the art are familiar with methods of electroporation. The electroporation may be, for example, flow electroporation or static electroporation. Methods and devices for electroporation are also described in, for example, published PCT Application Nos. WO 03/018751 and WO 2004/031353; U.S. patent application Ser. Nos. 10/781,440; 10/080,272; and 10/675,592; and U.S. Pat. Nos. 5,720,921; 6,074,605; 6,773,669; 6,090,617; 6,485,961; 6,617,154; and 5,612,207, all of which are incorporated by reference.

[004] The prior art does not describe a method or system of electroporating large volumes of cells in a semi-closed system that results in bioproduction using multi-electroporation systems in parallel. Current techniques use systems that are prone to human error, contamination, and reduced cell viability. Furthermore, current systems

that can electroporate large volumes of cells in a single run are expensive. Therefore, there remains a need in the art for a method of running multiple smaller electroporation machines in parallel to electroporate a large number of cells while reducing human error and maintaining cell viability.

[005] The disclosed semi-closed system for electroporating cells and methods of using the same overcome one or more of the problems set forth above and/or other problems of the prior art.

SUMMARY

[006] According to some disclosed embodiments, the present disclosure provides a semi-closed system for electroporating cells, comprising, (a) an electroporation machine, (b) an electroporation processing assembly, (c) a cell resting bag; and (d) an electroporation buffer or cell media bag, wherein (a) to (d) are all connected together by silicone-plated tubing manifold.

[007] According to some embodiments, there is disclosed a semi-closed system for electroporating cells, comprising: (a) at least one scalable electroporation machine configured to enable up to 20 billion cell transfection runs for the production of recombinant proteins, antibodies, virus-like particles (VLPs), virus-replicon particles (VRPs), and toxic and apoptotic proteins; (b) a processing assembly; (c) a cell resting bag; and (d) at least one bag comprising therein at least one electroporation buffer, at least one cell media, or combinations thereof, wherein (a) to (d) are all connected together by silicon-plated tubing manifold.

[008] According to some embodiments, there is disclosed a semi-closed system for electroporating cells, comprising: (a) an electroporation machine, configured to enable up to 200 billion cell transfection runs for the production of recombinant proteins, antibodies, virus-like particles (VLPs), virus-replicon particles (VRPs), and toxic and apoptotic proteins; (b) a processing assembly configured to work with the electroporation machine in (a); (c) a cell resting bag; and (d) at least one bag comprising therein at least one electroporation buffer, at least one cell media, or combinations thereof, wherein (a) to (d) are all connected together by silicon-plated tubing manifold.

[009] According to some disclosed embodiments, the system and methods disclosed herein can enable up to 200 billion cell transfection runs for the production of biological products such as, but not limited to, recombinant proteins, antibodies, virus-like particles (VLPs), virus-replicon particles (VRPs), and toxic and apoptotic proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate some disclosed embodiments and, together with the description, serve to explain the disclosed embodiments. The particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the present disclosure. The description taken with the drawings makes apparent to those skilled in the art how embodiments of the present disclosure may be practiced.

[0011] **FIG. 1** is an embodiment of a tube used to connect the electroporation processing units to the manifold.

[0012] **FIG. 2** is an embodiment of the MaxCyte CL2 processing assembly.

[0013] **FIG. 3** Is an embodiment of the complete multi-system (MaxCyte ExPERT STx/Gtx) using CL-2 processing assemblies connected by a manifold.

[0014] **FIG. 4** Is an embodiment of the complete MaxCyte R1-L processing assembly using multiple MaxCyte ExPERT VLx electroporation systems.

[0015] **FIG. 5** is an embodiment of the tubing assembly sets that may be used with the invention.

[0016] **FIG. 6** is an alternative embodiment of the tubing assembly that may be used with the invention.

[0017] **FIG. 7** is an embodiment of 3 MaxCyte ExPERT STx/GTx electroporation devices in parallel with 3 processing assemblies connected to a tubing set.

[0018] **FIG. 8** is an embodiment of 3 MaxCyte ExPERT VLx electroporation devices in parallel with 3 R-1L processing assemblies connected to a tubing set.

[0019] **FIG. 9** is a graph showing viable cell density numbers using the claimed method as detailed in example 2 on a 50 liter and 10 liter bioreactor.

[0020] **FIG. 10** is a graph showing cell viability numbers using the claimed method as detailed in example 2 on a 50 liter and 10-liter bioreactor.

[0021] **FIG. 11** is a graph showing the glutamine concentration level of the bioreactors used in example 2.

[0022] **FIG. 12** is a graph showing the glucose concentration level of the bioreactors used in example 2.

[0023] **FIG. 13** is a graph showing the lactate concentration level of the bioreactors used in example 2.

[0024] **FIG. 14** is a graph showing the ammonia (NH4+) concentration level of the bioreactors used in example 2.

[0025] **FIG. 15** is a graph showing the titer concentration level of the bioreactors used in example 2.

[0026] **FIG. 16** is a collection of graphs analyzing glycan profiles of the bioreactors used in example 2.

[0027] **FIG. 17** and **FIG.18** are a collection of graphs analyzing the change variant of the bioreactor used in example 2.

[0028] **FIG. 19** and **FIG.20** are a collection of graphs analyzing the size exclusion chromatography (SEC) of the bioreactor used in example 2.

[0029] **FIG. 21** and **FIG. 22** are a collection of graphs analyzing the change variant of the bioreactor used in example 2.

DETAILED DESCRIPTION OF THE INVENTION

[0030] One embodiment of the present invention is a semi-closed system for electroporating cells, comprising, (a) an electroporation machine, (b) an electroporation processing assembly (c) a cell resting bag; and (d) an electroporation buffer or cell media ban, wherein (a) to (d) are all connected together by silicone-plated tubing manifold and allows for the transfection of up to 200 billion cells per electroporation machine.

[0031] Those skilled in the art understand that a wide range of biological products can be grown and harvested using the claimed system and method, such as antibodies and proteins.

[0032] As used herein, the term “semi-closed system” refers to a system in which the seed train expansion step is an open step but the seed train bioreactor, cell concentration buffer exchange, electroporation, cell resting, and bioreactor production steps are closed. The disclosed system and method can be used on a wide range of cell types, including but not limited to primary cells, CHO cells, mammalian cell lines, insect cell lines, iPS cells, and other stem cells.

[0033] As used herein, the term “processing assembly” refers to the MaxCyte products that are used on the MaxCyte STx/GTx and VLx systems. The processing assembly for CL-2 and R-1L processing assemblies comes with sample and collection bags and the process assembly housing that is inserted into the system. Cells that are mixed with the plasmid DNA of choice in an electroporation buffer and loaded into the sample bag, processed in the housing containing electrodes that deliver an electronic pulse, and then collected into the collection bag.

[0034] As used herein, the term “air” refers to compressed air that is of standard atmospheric makeup.

[0035] As used herein, the term “cell resting vessel” refers to any vessel used to rest the cells directly after electroporation. Examples of suitable cell resting vessels include, but are not limited to, a 22L Meissner biocontainer, a 50L biocontainer, and a 5L multi-port Thomson Optimum Growth® shake flask.

[0036] According to some embodiments, the disclosed system is designed to be compatible with at least one scalable electroporation machine configured to enable up to 20 billion cells to be transfected for the production of recombinant proteins, antibodies, virus-like particles (VLPs), virus-replicon particles (VRPs), and toxic and apoptotic proteins. In some embodiments, the scalable electroporation machines described herein are sold by MaxCyte under the tradename “ExPERT STx”, or “ExPERT GTx”, electroporation systems. The present invention is also designed to be compatible with at least one large scale electroporation machine used for protein production in any desired cell type, including CHO and Vero. These large-scale electroporation machines are configured to enable up to 200 billion cells to be transfected for the production of recombinant proteins, antibodies, virus-like particles (VLPs), virus-replicon particles (VRPs), and toxic and apoptotic proteins. In some embodiments, the very large scale electroporation machine described herein is sold by MaxCyte under the tradename “ExPERT VLx”.

[0037] In one embodiment, a MaxCyte ExPERT STx, GTx, or VLx electroporation system is used for the electroporation step. However, the claimed method and individual components can be used with any electroporation platform. Furthermore, each electroporation system uses a unique assembly. For example, a CL-2 processing assembly is configured for an ExPERT STx or GTx whereas an R-1L processing assembly is configured for the ExPERT VLx. It is understood that any assembly can be used if it is compatible with the selected electroporation platform.

[0038] In one embodiment, the MaxCyte CL-2 and R-1L processing assemblies allow for a direct cell resting step into a cell culture vessel post-electroporation, without

the use of a syringe or the need to bring the electroporated cell into a biosafety cabinet to transfer the cells to a resting bag. The processing assembly is a solution for the aseptic integration of the CL-2 harvest bag to an appropriate cell culture vessel for the cell resting step. The CL-2 connection assembly reduces contamination risk and human error during the cell resting step and could be configured in several ways, allowing process flexibility without the use of a biowelder.

[0039] In one embodiment, the CL-2 connection assembly is configured for the ExPERT GTx or STx by attaching a CL-2 processing assembly and sample bag to the tubing manifold. The sample bag holds the cell before electroporation and the collection bag collects the cells after they undergo electroporation. The collection bag is further connected to a male lure lock with 1/4 inch ID (internal diameter), the male lure lock is attached to thermoplastic elastomer tubing or a silicon-plated tube with 1/4 inch ID. A clamp can be placed on the silicon-plated tube to control the flowthrough rate. The silicon-plated tube can be connected to a wide range of third-party sterile connectors for downstream vessel integration including but not limited to, a Male Kleenpack sterile connector, a CPC AseptiQuick S Connector, a male quick disconnect, or a male lure lock. Multiple CL-2s can be placed on multiple electroporation systems so that a large volume of cells can undergo electroporation at the same time with all the electroporated cells from each machine ending up in the same resting vessel. Each individual system can be connected to a single cell resting bag through a tubing manifold further attached to a cell media bag used to recover cells stuck in the manifold during cell resting.

[0040] In one embodiment a R-1L processing assembly is configured for electroporation using an ExPERT VLx electroporation system. Unlike the CL-2

processing assembly, the R-1L assembly is configured by connecting the collection line directly to the manifold instead of connecting it to a collection bag.

[0041] In one embodiment, the collection bag of the CL-2 is connected directly to the tubing manifold.

[0042] In one embodiment, cells undergo a seed train expansion train to reach the desired number of cells. The media used to help grow the cells and maintain cell viability is any commercially available media that is chemically defined and animal-origin-free.

[0043] In one embodiment, the cell passaging schedule to scale up the cells prior to electroporation for all cell types is as follows:

Passage	Vessel size/ working volume	Inoculation seeding density
P1	125mL SF/30mL	$\geq$ 3.0 x 10^5 cells/mL
P2	1000mL SF /300mL	~3.0 x 10^5 cells/mL
P3	1000mL SF /300mL	~3.0 x 10^5 cells/mL
P4	2 x 5L SF/2L	~3.0 x 10^5 cells/mL

[0044] In one embodiment, the flask that contains the cells during each cell passage is incubated with a vent cap in a shaking incubator at 37°C with 130 rpm, 5% CO_2, and 80% relative humidity for up to 3 days.

[0045] In one embodiment, multiple stirred bioreactors can be set up and inoculated simultaneously to reach the desired number of cells.

[0046] In one embodiment, the cell culture from the bioreactor(s) is transferred to multiple 20L, 2D bags through gravity flow via biowelding, KleenPak™, or CPC AseptiQuick® S Connector sterile connectors.

[0047] In one embodiment, multiple ExPERT STx or GTx electroporation instruments can be assembled to electroporate a large volume of cells at the same time by preparing a multi-CL-2 electroporation tubing manifold with the appropriate amount of branches to support the number of CL-2 processing assemblies needed. The manifold refers to the part of the system that connects the electroporation processing assembly to the cell resting bag. At one end of the manifold there is a 50L cell resting vessel inflated with 1:1 air:O_2 gas for cell resting and the other end will be connected to a bag containing electroporation (EP) buffer or medium to wash the manifold lines after electroporation. For each CL-2 processing assembly to be used, the CL-2 connection assembly can be attached to the female lure lock of the collection bag. A gas line can be connected to the inlet gas line of the 50-L cell resting vessel with 1:1 air/O_2 gas at 0.3 standard liters per minute (SLPM) flow for cell resting. When the mixing of DNA and cells is sufficient, one can load up to 100mL to a MaxCyte CL-2 processing assembly. One can then install the C-L2 processing assembly onto the ExPERT STx or GTx instrument and connect the C-L2 connection assembly to a multi-CL-2 EP tubing manifold via the CPC AseptiQuick® connector. In some embodiments, this is done for all the CL-2 processing assemblies that are intended to be run. During electroporation ensure that the electroporated cells are flowing through the CL-2 collection line to the multi-CL-2 EP tubing manifold to the 50L cell resting vessel. After allowing the electroporated cells to rest for 30 minutes, the manifold lines can be washed with the

EP buffer or media connected to the end of the manifold to recover additional cells to the 50L cell resting vessel.

[0048] In one embodiment, multiple ExPERT VLx electroporation instruments are used to electroporate a large volume of cells at the same time using multiple R-1L processing assemblies. To do so, one can place the appropriate amount of VLx machines next to each other and prepare a multi-R-1L EP tubing manifold with the appropriate amount of branches to support the number of R-1L processing assemblies needed. One end of the manifold will be connected to a 50L cell resting vessel inflated with 50:50 air: O_2 gas for cell resting and the other end will be connected to a bag containing EP buffer or medium to wash the manifold lines after electroporation. To an inflated 50L cell resting vessel, sterile connect one end of a multi-EP tubing manifold and connect a bag of EP buffer or media to the other end. Connect a gas line to the inlet gas line of the 50-L cell resting vessel with 1:1 Air/O_2 gas at 0.3 SLPM flow for cell resting. When the mixing of DNA and cells is sufficient, load up to 1L to a MaxCyte R-1L processing assembly. Fill in the desired amount of R-1L processing assemblies. Install the R-1L processing assembly onto the VLx instrument. Connect the R-1L collection line to a multi-R-1L EP tubing manifold via the CPC AseptiQuick connector. Do this for all the R-1L processing assemblies that are intended to be run. During electroporation ensure that the electroporated cells are flowing through the R-1L collection line to the multi-R1-L EP tubing manifold to the 50L cell resting vessel. After allowing the electroporated cells to rest for 30 minutes, wash the manifold lines with the EP buffer or media connected to the end of the manifold to recover additional cells to the 50L cell resting vessel.

[0049] In one embodiment, the rested cells are placed into a production bioreactor to produce the desired target, such as, but not limited to, a protein or antibody.

Examples

[0050] The following non-limiting examples, which are intended to be exemplary, further clarify the present disclosure.

Example 1 - Electroporation of CHO cells using multiple STx/GTx and VLx systems in parallel

[0051] A vial of CHOZN cells was thawed and maintained in EX-CELL Advanced CHO Fed-batch Medium supplemented with 12mM L-glutamine and an additional 4g/L glucose. The cells were incubated at 37°C, 5% CO_2, with 80% humidity. Working volume and agitation speed varied based on vessel size (see Table 1 below). Cells were passaged every 2 to 3 days at an initial cell density of 3e5 cells/mL and viabilities >98%. The cells were scaled up in volume as needed.

Table 1.

Erlenmeyer flask with vent cap Vessel Size	**Working Volume (mL)**	**Agitation RPM (25mm orbital throw)**
125 mL	30-40	140
250 mL	60-80	140
1000 mL	250-300	140

3 L	1000	90

[0052] A 50L bioreactor (BioBLU® 50c Single-Use Bioreactor, macrosparger, 1 pitched-blade impeller, optical pH) was used as a seed train bioreactor to grow a large number of cells for electroporation. The bioreactor was batched 1 day prior to inoculation with 18L of EX-CELL® Advanced CHO Fed-batch Medium supplemented with 12mM L-glutamine and an additional 4g/L glucose After the parameters were stable, the bioreactor was inoculated with CHOZN cells at a seeding density of 5x105 cells/mL and final volume of 20L. The cells were cultivated in the seed train bioreactor for 3 days. On day 3 (day -1 from electroporation), an additional 20L of EX-CELL Advanced CHO Fed-batch Medium supplemented with 10mM L-glutamine and an additional 4g/L glucose was added to split the culture to reach a final working volume of 40L. Tubing sets for Multi ExPERT STx/GTx and Multi VLx systems were created to connect to the collection lines of the CL-2 and R-1L processing assemblies, respectively. Completed tubing sets were autoclaved for sterility. FIG. 5 and FIG. 6 below show drawings and descriptions of the tubing assembly sets.

[0053] On the day of electroporation cells from the seed train bioreactor were concentrated to a density of 2e8 cells/ml and spent media was exchanged with MaxCyte EP Buffer using a Sartorius Ksep® 400 system. For each cycle of the Ksep® 400 system, 220mL of concentrated cells were collected into an R-1L sample bag bio welded onto the Ksep® 400 harvest line. The R-1L sample bag containing cells was then removed and transferred into the biosafety cabinet and a new R-1L sample bag

was connected to the Ksep® 400 harvest line. A total of 4 cycles of concentrated cells were collected.

[0054] For the antibody production, 3 plasmids (light chain, heavy chain, and EBNA) were mixed together with equal weight of light chain and heavy chain plasmids for a total of 95% of the total target weight of plasmid DNA. The EBNA plasmid was added to the mix in the amount of 10% of the total target weight of plasmid DNA. The plasmid mix was then added to the concentrated cells prior to electroporation.

[0055] To 1 R-1L sample bag containing 220mL of concentrated cells from 2.4, the calculated amount of DNA was added and a quantity sufficient volume of medium was added. Each of the 3 CL-2 sample bags was filled with a volume of 100 mL. The collection lines of the CL-2 processing assemblies were connected via lure lock to the tubing of the ExPERT STx/GTx tubing set. The CL-2 processing assemblies were then removed from the biosafety cabinet and loaded onto 3 parallel ExPERT STx/GTx systems. The lines from the Multi ExPERT STx/GTx tubing set were biowelded to corresponding positions in the manifold. The upstream line was biowelded to a bag containing medium and the downstream line was biowelded to a 5L Thomson shake flask for cell resting post electroporation.

[0056] For each ExPERT STx/GTx, the CL-2 processing assemblies were set up using the prompts from the on-screen or computer systems. The cells were electroporated using the CHO EP protocol. The electroporated cells were allowed to flow into the tubing manifold and collect in the 5L Thomson shake flask. Once the electroporation was completed for all CL-2 processing assemblies, the cells were rested

for 30 minutes at room temperature. The set up of 3 ExPERT STx/GTx systems in parallel with 3 CL-2 processing assemblies connected to the Multi GTx tubing set is shown in FIG. 7.

[0057] The calculated amount of DNA was added to each of the 3 R-1L sample bags filled with a volume of 220 mL of concentrated cells in the biosafety cabinet (final volume ~250mL). Plasmid DNA mixture was added to the R-1L sample bags and thoroughly mixed. The sample bags were taken out from the biosafety cabinet and were then connected via CPC AseptiQuik connector to the sample line of the R-1L. The collection lines of the R-1L processing assemblies were connected to a multi VLx tubing set by using Biowelder. The upstream line was biowelded to a bag with medium and the downstream line was biowelded to a 22L bioprocess container filled with 1:1 air:O_2 for cell resting post electroporation.

[0058] For each VLx, the R-1L processing assemblies were set up using the prompts from the on-screen systems. The cells were electroporated. The electroporated cells were allowed to flow into the tubing manifold and were collected in the 22L bioprocess container. Once the electroporation was completed for all R-1L processing assemblies, the cells were rested for 30 minutes at room temperature with 0.1 SLPM of 1:1 air:O_2 flowing into the bag. The set up of 3 VLx systems in parallel with 3 R-1L processing assemblies connected to the Multi VLx tubing set is shown in FIG. 8.

[0059] Cells electroporated from Multi ExPERT STx/GTx and Multi VLx systems were rested in a 5L shake flask and a 22L bioprocess container respectively for 30 minutes at room temperature. After the resting period, media from the bags attached

upstream of the tubing assemblies was used to wash the lines and collect the remaining cells into the resting vessels.

[0060] A 10L bioreactor (BioBLU® 10c Single-Use Bioreactor, macrosparger, 1 pitched-blade impeller, optical pH) was used as a production bioreactor. The bioreactor was batched 1 day prior to inoculation with EX-CELL® Advanced CHO Fed-batch medium supplemented with 12mM L-glutamine, an additional 4g/L glucose, and 30mL per liter of 20% Kolliphor® P188 as production media. The bioreactor was inoculated with the electroporated cells from the 5L shake flask to a density of $6.7x10^6$ cells/mL and viability of 97.5%. For production, the pH was maintained at 7.0 with a dead band of +/-0.2, and the dissolved oxygen (DO) setpoint was 30%. On day 1 post inoculation, the temperature was shifted from 37°C to 32°C, and sodium butyrate was supplemented to a final concentration of 2mM. Additionally, feeding of EX-CELL® Advanced CHO Feed 1 from day 1 to the end of the process (day 12) is detailed in Table 2 below.

Table 2.

Day	Percent Feed (%)
0	0
1	5
2	0
3	3
4	0
5	3
6	0
7	3
8	0

9	3
10	0
11	3
12	0

[0061] Samples were taken daily for cell count and nutrient and metabolite concentration. Glutamine and glucose were supplemented to maintain a concentration above 4mM and 2g/L, respectively. Samples for titer were taken starting on day 3. Samples for product quality were taken on day 9. Production was ended on day 12 when viability dropped below 50%.

[0062] A 50L bioreactor (BioBLU® 50c Single-Use Bioreactor, macrosparger, 1 pitched-blade impeller, optical pH) was used as a production bioreactor. The bioreactor was batched 1 day prior to inoculation. The bioreactor was inoculated with the electroporated cells from the 5L Thomson shake flask to a density of $6.4x10^6$ cells/mL and viability of 98.3%.

[0063] The bioreactor was batched 1 day prior to inoculation with EX-CELL® Advanced CHO Fed-batch medium supplemented with 12mM L-glutamine, an additional 4g/L glucose and 30mL per liter of 20% Kolliphor® P188 as production media. The bioreactor was inoculated with the electroporated cells from the 5L Thomson shake flask to a density of $6.7x10^6$ cells/mL and viability of 97.5%. For production, the pH was maintained at 7.0 with a dead band of +/-0.2, and the DO setpoint was 30%. On day 1 post inoculation, the temperature was shifted from 37°C to 32°C, and sodium butyrate was supplemented to a final concentration of 2mM. Additionally, feeding of EX-CELL®

Advanced CHO Feed 1 from day 1 to the end of process (day 15), is detailed in Table 3 below.

Table 3.

Day	Percent Feed (%)
0	0
1	5
2	0
3	3
4	0
5	3
6	0
7	3
8	0
9	3
10	0
11	3
12	0
13	3
14	0
15	0

[0064] Samples were taken daily for cell count and nutrient and metabolite concentration. Glutamine and glucose were supplemented to maintain a concentration above 4mM and 2g/L, respectively. Samples for titer were taken starting on day 3. Samples for product quality were taken on day 9. Production was ended on day 15 when viability dropped below 50%.

[0065] viable cell densities FIG. 9 and viabilities FIG. 10 were measured daily using a ViCell Blu automated cell counter. The bioreactors were inoculated at ~6e6 cells/mL at high viabilities above 97% post transfection. The 50L bioreactor grew to a peak density of around $15x10^6$ cells/mL. The 10L bioreactor grew to a peak density of around $11x10^5$ cells/mL. Foaming issues in the 10L bioreactor on day 1 impacted cell growth. The trends for cell growth and viability were as expected based on historical data.

[0066] Nutrients and metabolites were measured daily using a Nova® Biomedical Flex2 Analyzer. Glutamine concentration FIG. 11 and glucose concentration FIG. 12 were monitored closely to ensure that concentrations remained above 4mM and 2g/L respectively. Lactate concentration FIG. 13 and ammonia concentration FIG. 14 were tracked to ensure that the buildup of cell culture byproducts was within tolerable process ranges.

[0067] Titer was measured using the Biolayer Interferometry method using a GatorBio instrument. Titer in the 50L and 10L bioreactors demonstrates that the cells were successfully electroporated and are producing the intended product FIG. 15.

[0068] Product quality was sent to a third-party vendor for Pro-A purification and analysis. Below are the results of the product quality analysis for glycan profile FIG. 16, charge variant (FIG. 17 and FIG. 18), size exclusion chromatography (SEC) (FIG. 19-FIG. 20), and LabChip® gel (FIG. 21 and FIG. 22) compared to previous studies using the same process on a single electroporation system. executed in the process development lab. The previous studies were run using a single ExPERT STx/GTx or

VLx. The product quality profiles are similar for all studies suggesting that multisystem electroporation and single system electroporation are equivalent.

[0069] Experimental data was compared to studies 8, 9, 10, and 11. These studies were run using the same protocol above but on a single electroporation device. Studies 8, and 10, were run on a single ExPERT VLx using a 50L bioreactor for production post EP and studies 9 and 11 were run on a single ExPERT STx using a 10L bioreactor for production post EP.

[0070] FIG. 1 is an embodiment of tube 100 used to connect the electroporation processing units to the manifold. The figure demonstrates that a male lure lock 101 is attached to the top of the tube which connects to either a collection bag or the output line of the electroporation processing unit. Tube 102 is silicon plated with an internal diameter of 0.25 inches. A clamp 103 is placed on the tube to control the flowthrough rate. The bottom of the tube is attached to a CPC AseptiQuick® S Connector 104 which can be attached to the manifold.

[0071] FIG. 2 Is an embodiment of the CL2 processing assembly 200. The figure demonstrates that a CL2 electroporation processing unit 201 is connected to an airbag 202, a sample bag 203 to hold the cells before electroporation, and a collection bag 204 used to capture the cells immediately after electroporation. The figure further demonstrates that the collection bag 205 can be connected to tube 206, which is also shown in FIG. 1, through a male lure lock. The bottom of the processing assembly is attached to CPC AsepticQuick S connector 207 which is used to connect the processing assembly to a tubing manifold. The connector shown in 207 can be substituted for a

male Kleenpack® sterile connector, a male disconnect, or a male lure lock shown in 208.

[0072] FIG. 3 Is an embodiment of the complete CL2 processing assembly 300 wherein the CL-2 processing assembly 301, also shown in FIG. 2, is attached to multiple ExPERT GTx electroporation systems 302. The figure demonstrates that the assembly 301 is installed onto the GTx 302 and further connected to the manifold line 304 by using CPC AsepticQuick® S connectors 303. The manifold line 304 is attached to a cell media bag 303 and a cell resting bag 305.

[0073] FIG. 4 Is an embodiment of the complete R1-L processing assembly 400 using multiple ExPERT VLx electroporation systems 404. The figure shows an R-1L electroporation processing wherein an R-1L electroporation processing unit 401 is attached to a sample bag 402 that holds the cell before electroporation and is further attached to the CPC AsepticQuick S Connector 403. The R-1L is attached to the manifold line 407 by connecting adjoining CPC AsepticQuick S Connectors 405. The manifold line is further attached to a cell media bag 406 and a cell resting vessel 408.

[0074] FIG. 5 shows the tubing sets for Multi ExPERT STx/GTx systems that were created to connect to the collection lines of the CL-2 processing assembly.5(a) is the tubing line that connects the female lure lock on the CL-2 collection bag processing assembly. 5(b) is the manifold tubing used to connect up to 3 CL-2 processing assemblies together. 501 is the male lure that is connected to the CL-2 processing assembly, 502 is the silicon plated tubing with 1/8 inch internal diameter x 1/4 inch outer diameter, 503 is the filter part of the tube that is welded to 506 which is 1/8 inch internal

diameter x 1/4 inch outer diameter. 504 is the part of the tube that is welded to the media bag and is 1/4 inch internal diameter x 3/8 inch outer diameter, 506 demonstrates that the entire tube is made of silicone, 507 is that part of the tube that is welded to either a shake flask or cell resting vessel and is 1/4 inch internal diameter x 7/16 inch outer diameter.

[0075] FIG. 6 shows the tubing sets for multi ExPERT VLx electroporation systems that were created to connect to the collection lines of the R-1L processing assembly. 601 is the part of the tube that is welded to the media bag and 1/4 inch internal diameter x 3/8 inch outer diameter, 602 shows that the tube is made of silicone, 603, is that part of the tube assembled to the R-1L processing assembly, 604 is the part of the tube that connects to the cell resting vessel and is 1/4 inch internal diameter x 7/16 inch outer diameter.

[0076] FIG. 7 is a drawing of the set up used in example 2 using 3 MaxCyte ExPERT STx/GTx systems in parallel with 3 CL-2 processing assemblies connected to the Multi GTx tubing set. 701 is a depiction of a computer used to control the electroporation systems, 702 is a depiction of the three electroporation systems set up in parallel, each containing a processing assembly, 707, connected together by the tubing manifold 706, all placed on a flat surface or table, 705. The system further depicts a cell resting bag, 704, and a cell loading bag, 703.

[0077] FIG. 8, 800. is a drawing of the set up used in example 2 using 3 MaxCyte ExPERT VLx systems in parallel with 3 R-1L processing assemblies connected to the Multi VLx tubing set. 801 depicts the three MaxCyte ExPERT VLx systems all set on a

flat surface or table, 801. 802 depicts the sample bag used to load the cells into the system, and 806 depicts the R-1L processing assembly connected together by the tubing manifold of 804. 805 depicts the cell resting bag used to collect cells post electroporation.

[0078] FIG. 9 is a measure of viable cell densities over time in each of the bioreactors used in example 2. The data were measured daily using a ViCell Blu automated cell counter. The bioreactors were inoculated at ~6e6 cells/mL at high viabilities above 97% post transfection. The 50L bioreactor grew to a peak density of ~15e6 cells/mL. The 10L bioreactor grew to a peak density of ~11e5 cells/mL.

[0079] FIG 10. Is a measure of cell viability over time in each of the bioreactors used in example 2. The data were measured daily using a ViCell Blu automated cell counter and shows that the cell viability in each reactor was maintained above 40% from inoculation to harvest.

[0080] FIG. 11 is a graph showing the measurement of glutamine in both bioreactors used in example 1. The data was measured using Biomedical Flex2 Analyzer and shows that the glutamine concentrations were maintained above 4mM throughout the experiment in both bioreactors.

[0081] FIG. 12 is a graph showing the measurement of glutamine in both bioreactors used in example 1. The data was measured using Biomedical Flex2 Analyzer and shows that the glutamine concentrations were maintained around 2g/L throughout the experiment in both bioreactors.

[0082] FIG. 13 is a graph showing the measurement of lactate in both bioreactors used in example 1. The data was measured using a Biomedical Flex2 Analyzer and shows that the lactate concentrations were maintained below 4g/L throughout the experiment in both bioreactors.

[0083] FIG. 14 is a graph showing the measurement of ammonia (NH4+) in both bioreactors used in example 1. The data was measured using Biomedical Flex2 Analyzer and shows that the ammonia concentrations were kept below 9.2 g/L throughout the experiment in both bioreactors.

[0084] FIG. 15 is a graph showing titer concentrations in both bioreactors used in example 1. The data was measured using the BLI method using a GatorBio instrument. Titer in the 50L and 10L bioreactors demonstrates that the cells were successfully electroporated and are producing the intended product.

[0085] FIG. 16 is a collection of graphs comparing glycan profiles of the bioreactor used in example 1 in the “Experimental Data” box to control data in the “Historical Data” box. The data shows the identification and quantitation of different glycoforms in the product. Studies 8, 9, 10, and 11 in the “Historical Data” box are control studies showing the glycan profiles of a bioreactor from experiments running the same protocol as example 1 but on a single electroporation device. Comparing the experimental data to the control group, it is clear that there was no significant difference between the groups, demonstrating that the multisystem electroporation results in similar product quality. 16(a) shows the data from the 50L bioreactor used in example 1 and 16(b) shows the data from the 10L bioreactor used in example 1.

[0086] FIG. 17 and FIG.18 are a collection of graphs analyzing the change variant of the bioreactor used in example 1. Studies 8 and 10 were used as control groups and refer to experiments using the same protocol in example 1 on a single electroporation system. The data shows the heterogeneity of the charge variant forms which needs to be characterized and monitored as changes can potentially affect biological activity and safety. Comparing the experimental data to the control group, it is clear that there was no significant difference between the groups, demonstrating that the multisystem electroporation results in similar product quality.

[0087] FIG. 19 and FIG.20 are a collection of graphs analyzing the SEC of the bioreactor used in example 1. Studies 8, 9, 10, and 11 were used as control groups and refer to experiments using the same protocol in example 1 on a single electroporation system. The data shows the distribution of aggregation in the monoclonal antibody/protein. Comparing the experimental data to the control group, it is clear that there was no significant difference between the groups, demonstrating that the multisystem electroporation results in similar product quality.

[0088] FIG. 21 and FIG. 22 are a collection of graphs analyzing the change variant of the bioreactor used in example 1. Studies 8, 9, 10, and 11 were used as control groups and refer to experiments using the same protocol in example 2 on a single electroporation system. Comparing the experimental data to the control group, it is clear that there was no significant difference between the groups, demonstrating that the multisystem electroporation results in similar product quality.

[0089] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

What is Claimed Is:

1. A semi-closed system for electroporating cells, comprising:

(a) an electroporation machine;

(b) an electroporation processing assembly;

(c) a cell resting bag; and

(d) an electroporation buffer or cell media bag,

wherein (a) to (d) are all connected together by silicone-plated tubing manifold.

2. A semi-closed system for electroporating cells, comprising:

(a) at least one scalable electroporation machine configured to configured to enable up to 20 billion cells per machine to be transfected for the production of recombinant proteins, antibodies, virus-like particles (VLPs), virus-replicon particles (VRPs), and toxic and apoptotic proteins;

(b) a processing assembly;

(c) a cell resting bag; and

(d) at least one bag comprising therein at least one electroporation buffer, at least one cell media, or combinations thereof,

wherein (a) to (d) are all connected together by silicon-plated tubing manifold.

3. The semi-closed system of claim 2, wherein the processing assembly contains a processing unit that is connected to an airbag, a sample bag used to contain the cells before electroporation, and a collection bag used to hold the cells after electroporation, which is further connected to a silicon plated tube by a male lure lock.

4. The semi-closed system of claim 2, wherein the processing assembly contains a processing unit that is connected to an airbag, a sample bag used to contain the cells before electroporation, wherein the output line of the electroporation processing unit is attached to the manifold with a silicon plated tube attached by a male lure lock.
5. The semi-closed system of claim 3, wherein the silicon-plated tube is connected to the sample bag on one end and to a device on the other end, wherein the device is selected from the group consisting of a CPC Aspetiquick S Connector, a male Kleenpak sterile connect or a male quick disconnect, or a male lure lock.
6. The semi-closed system of claim 3, wherein the internal diameter of the silicon-plated tube is 0.25 inches.
7. The semi-closed system of claim 3, wherein a clamp is placed on the silicon plated tube to help control flow rate.
8. The semi-closed system of claim 2, wherein in said electroporation step uses multiple systems that are set up at the same time and connected together by a silicone tube manifold that connects each processing assembly to the cell resting bag.
9. A semi-closed system for electroporating cells, comprising:

(a) an electroporation machine, configured to enable up to 200 billion cells per machine to be transfected for the production of recombinant proteins, antibodies, virus-like particles (VLPs), virus-replicon particles (VRPs), and toxic and apoptotic proteins;

(b) a processing assembly configured to work with the electroporation machine in (a);

(c) a cell resting bag; and

(d) at least one bag comprising therein at least one electroporation buffer, at least one cell media, or combinations thereof,

wherein (a) to (d) are all connected together by silicon-plated tubing manifold.

10. The semi-closed system of claim 9, wherein the assembly contains an electroporation unit connected to a sample bag which holds the cell before electroporation, and to the tubing manifold.

11. The semi-closed system of claim 9, wherein the processing unit is connected to the manifold by a silicone tube with a male lure lock on the end that attaches to the processing unit and a sterile connector device that further connects to the manifold that is attached to the cell resting bag.

12. The semi-closed system of claim 11, wherein the sterile connector device is selected from the group consisting of, a CPC Aspetiquick S Connector, a male Kleenpak sterile connect or a male quick disconnect, or a male lure lock.

13. The semi-closed system of claim 11, wherein the manifold is further connected to at least one bag containing either electroporation buffer or cell media.

14. The semi-closed system of claim 9, wherein multiple systems are connected in parallel by a silicone tube manifold that attaches the assembly of each system to a cell resting bag.

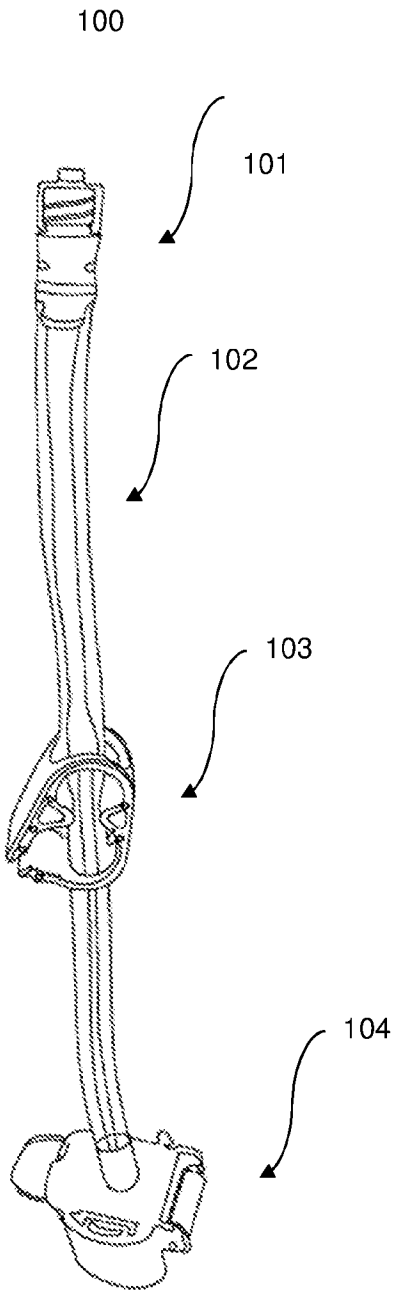


FIG. 1

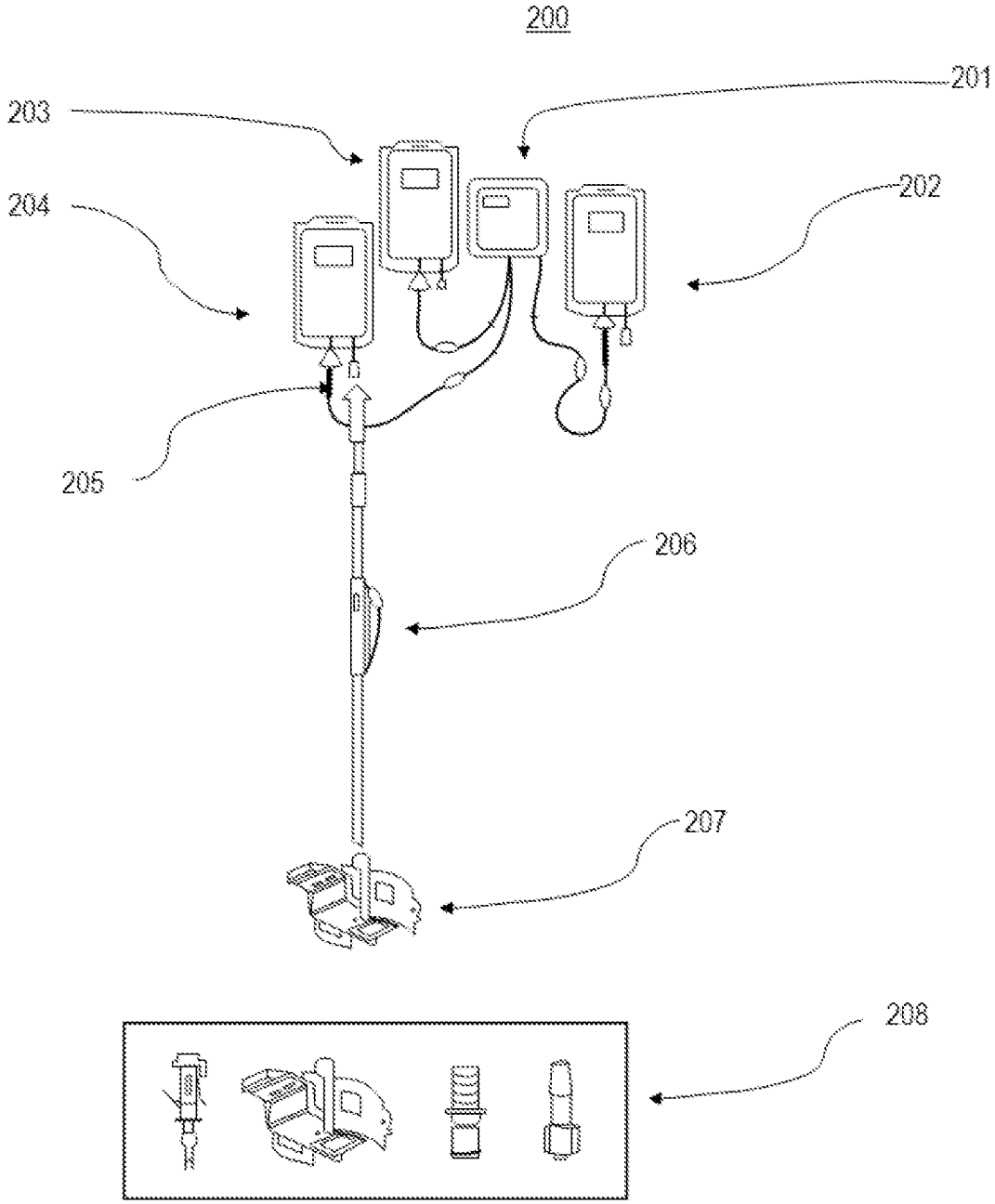


FIG. 2

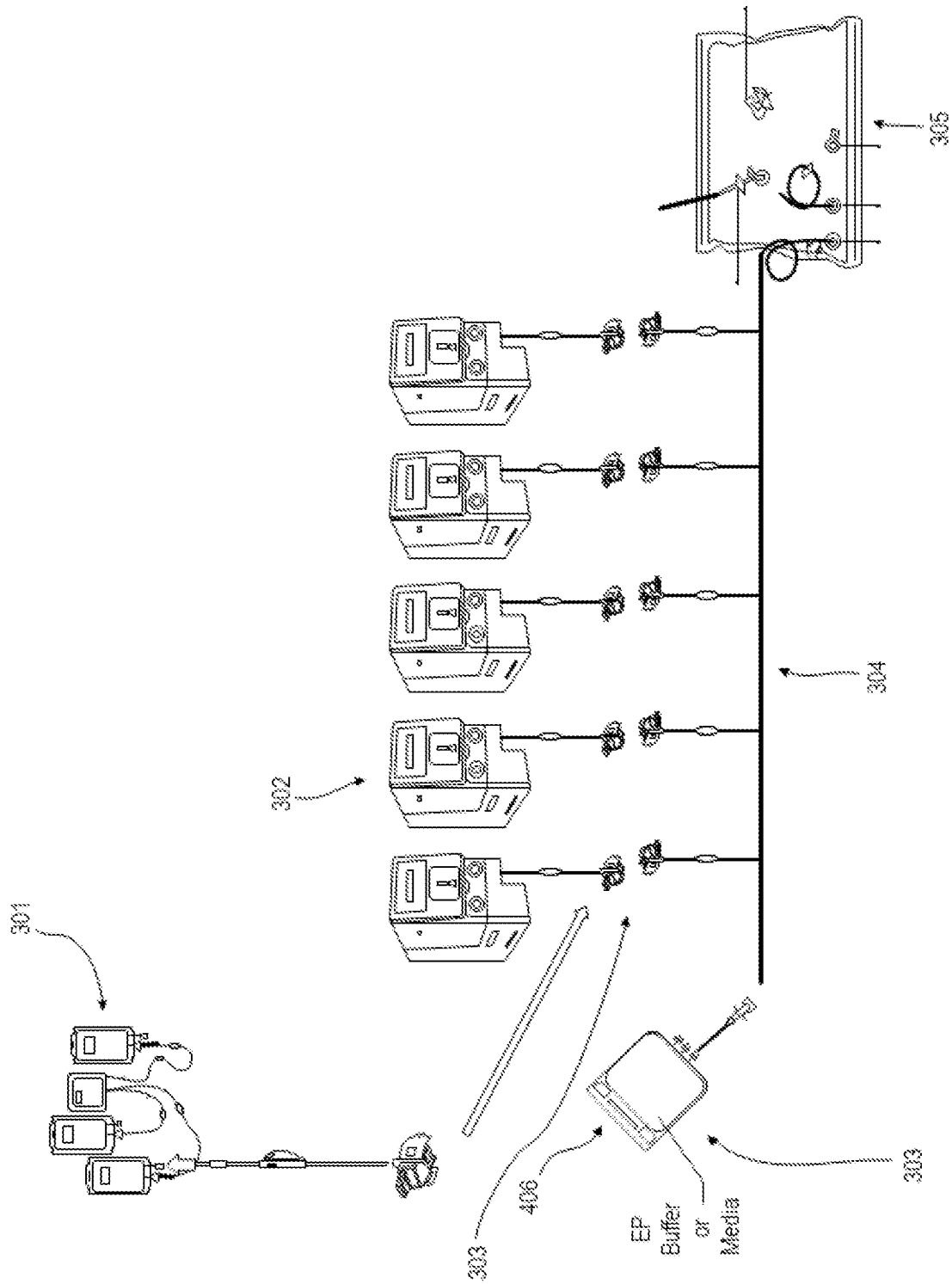


FIG. 3

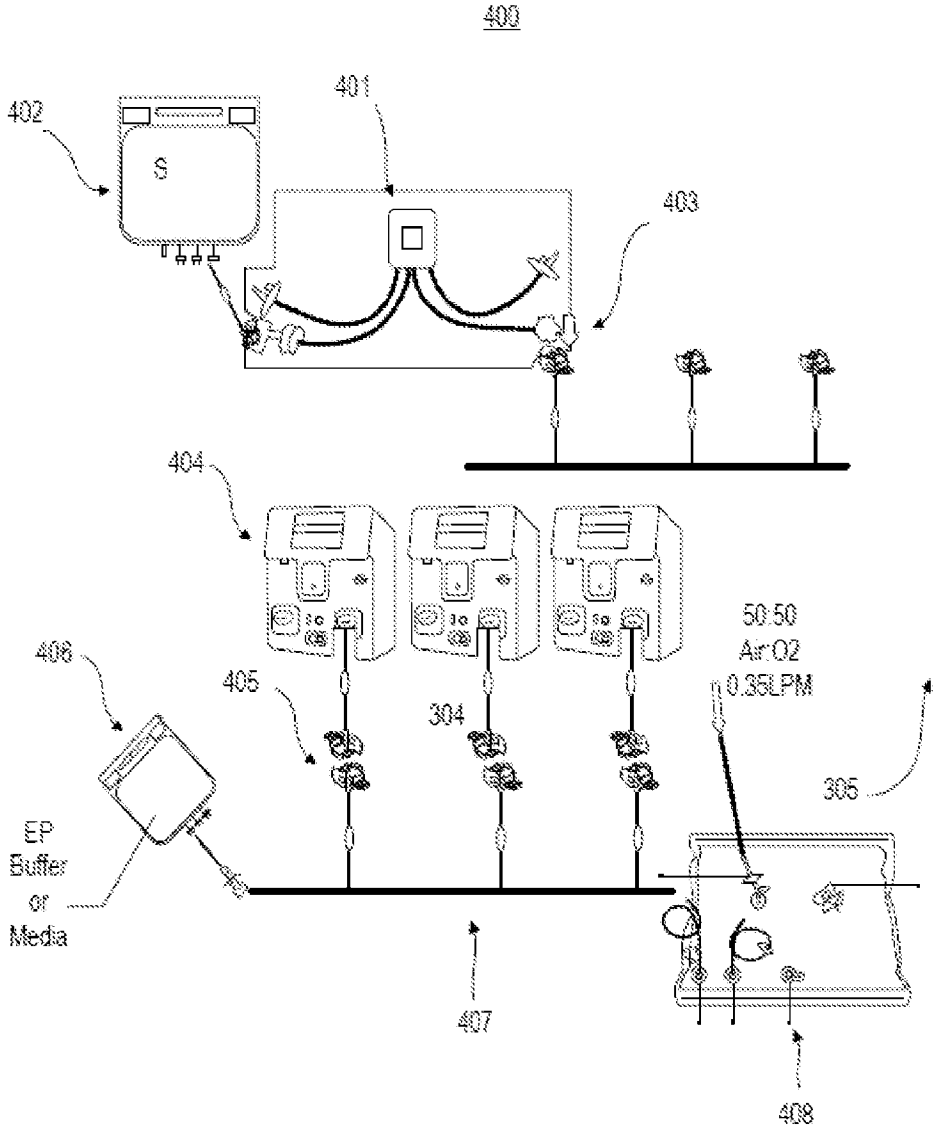

400
401
402
S
403
404
50:50
Air:O2
0.35LPM
406
405
304
305
EP
Buffer
or
Media
407
408

FIG. 4

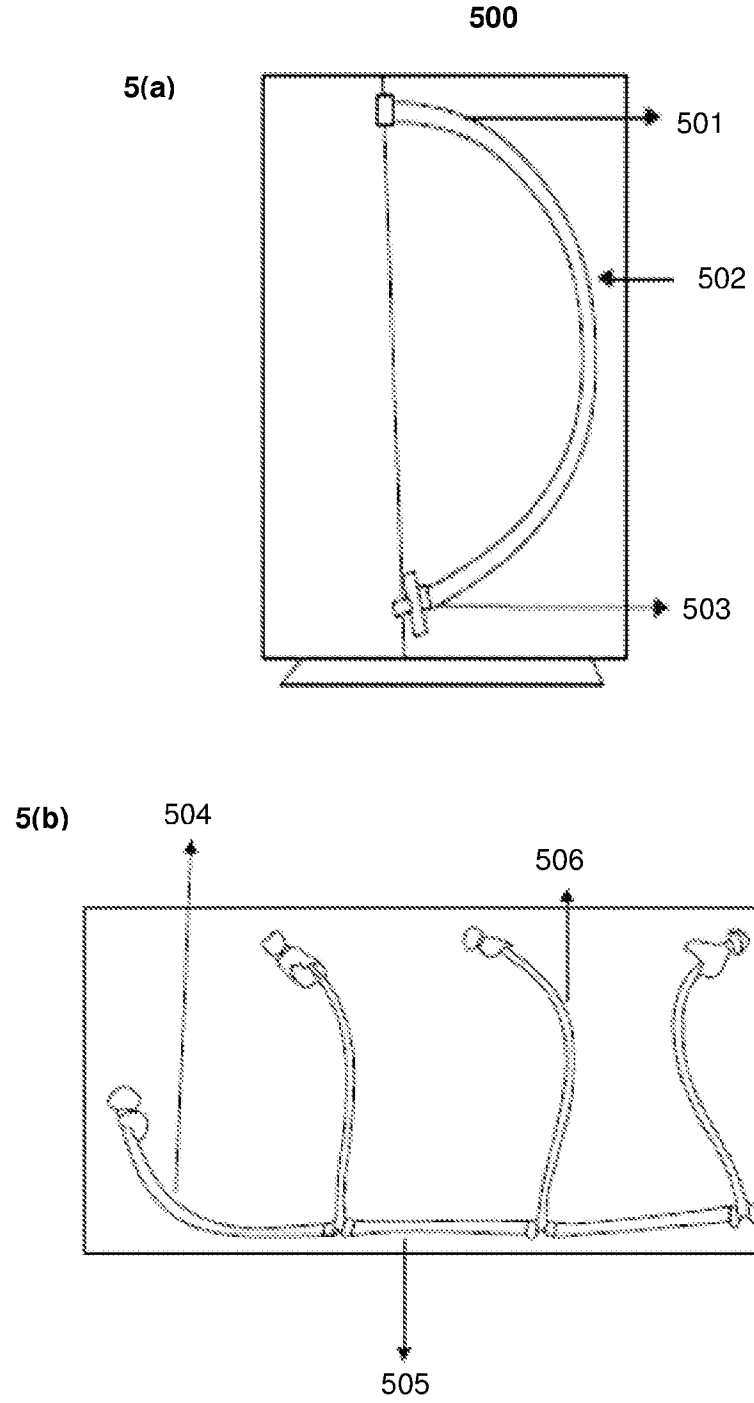


FIG. 5

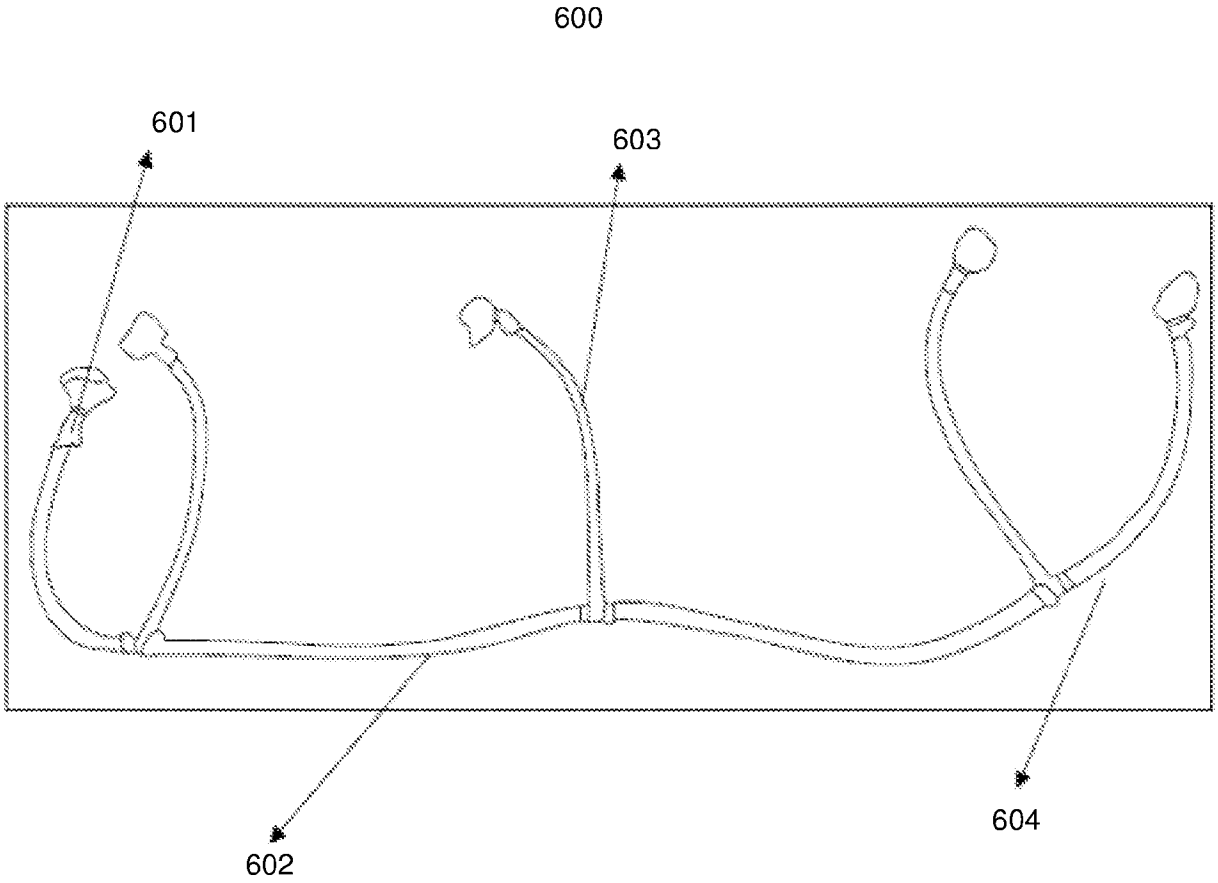


FIG. 6

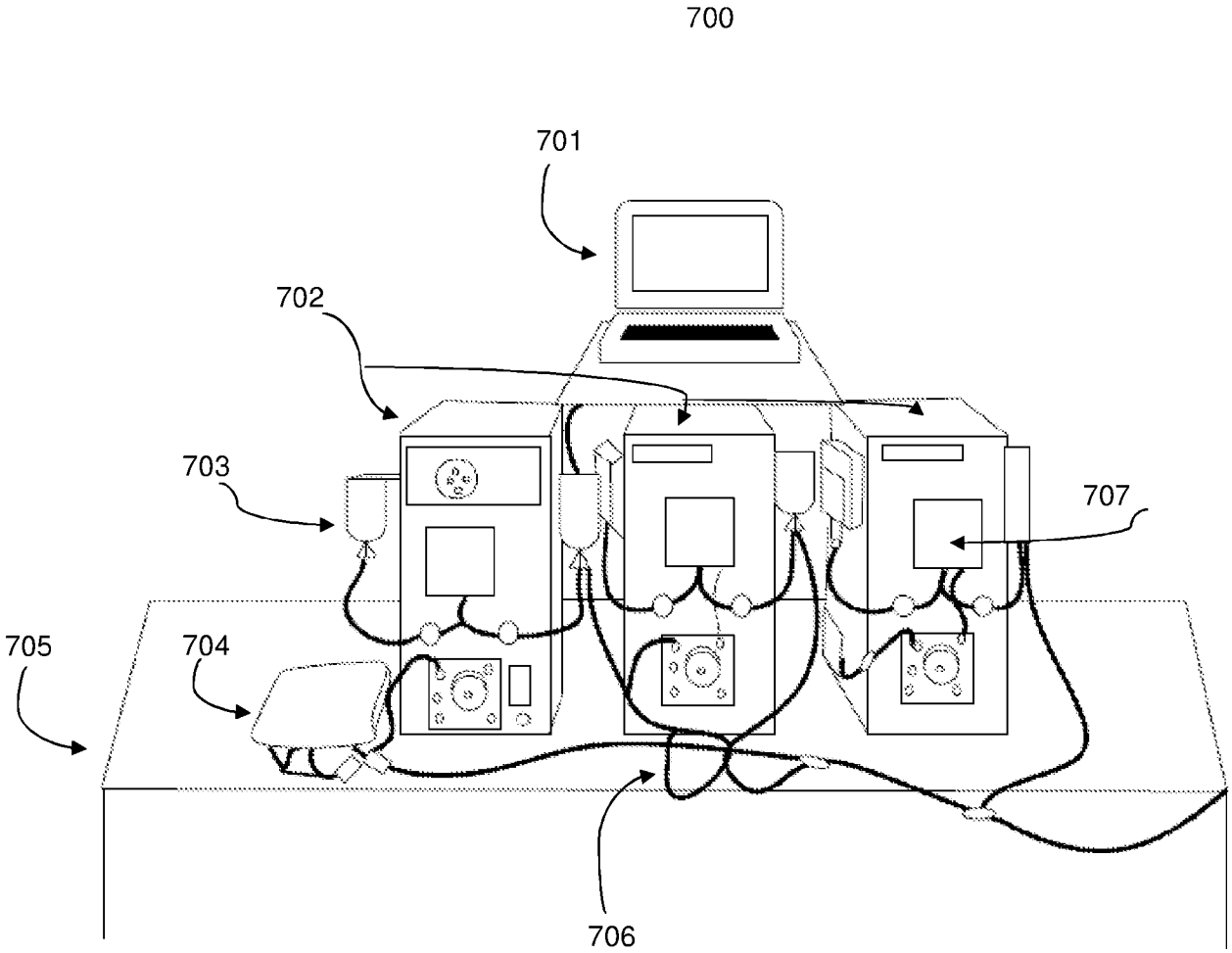


FIG. 7

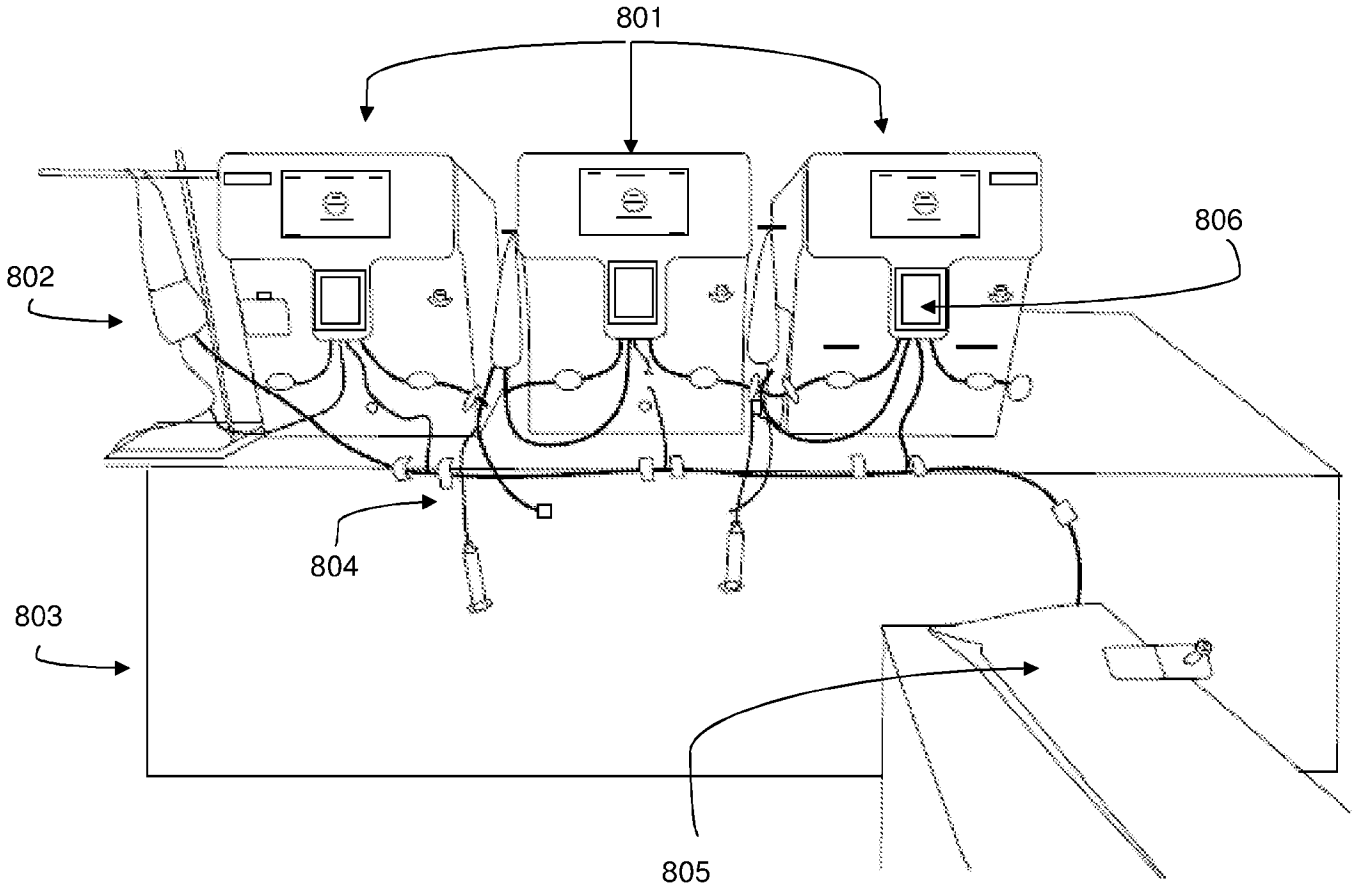


FIG. 8

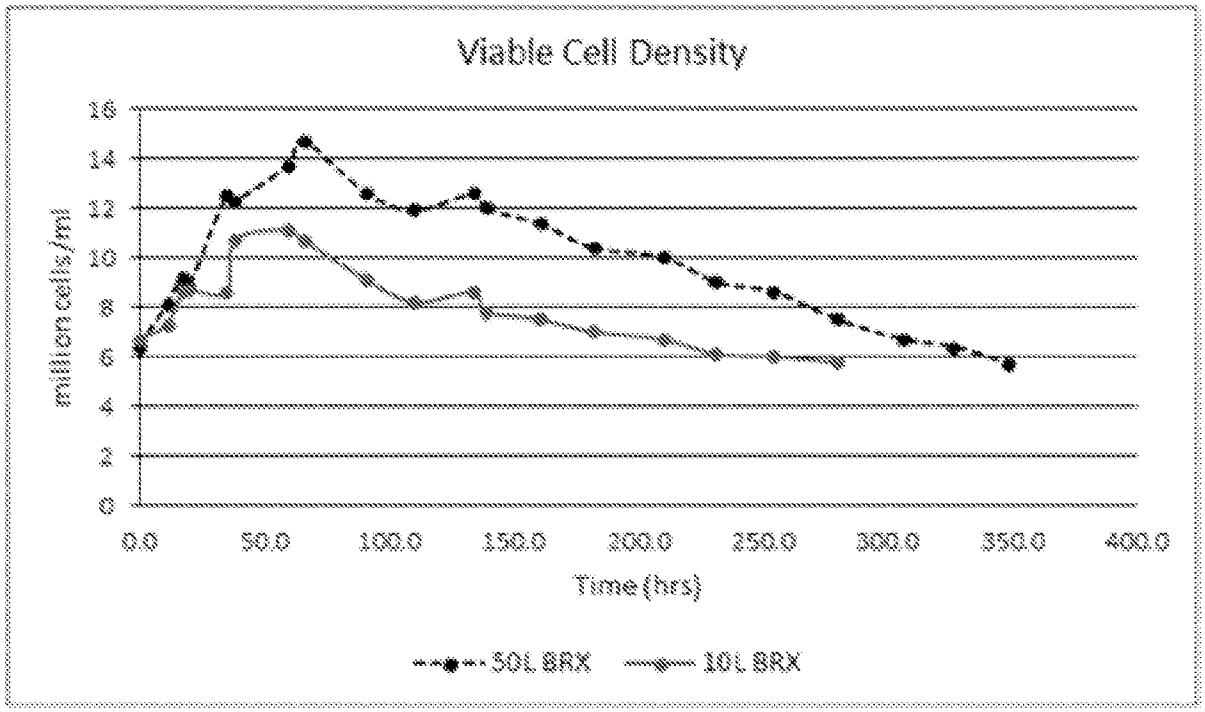

Viable Cell Density
million cells/ml
16
14
12
10
8
6
4
2
0
0.0
50.0
100.0
150.0
200.0
250.0
300.0
350.0
400.0
Time (hrs)
50L BRX
10L BRX

FIG. 9

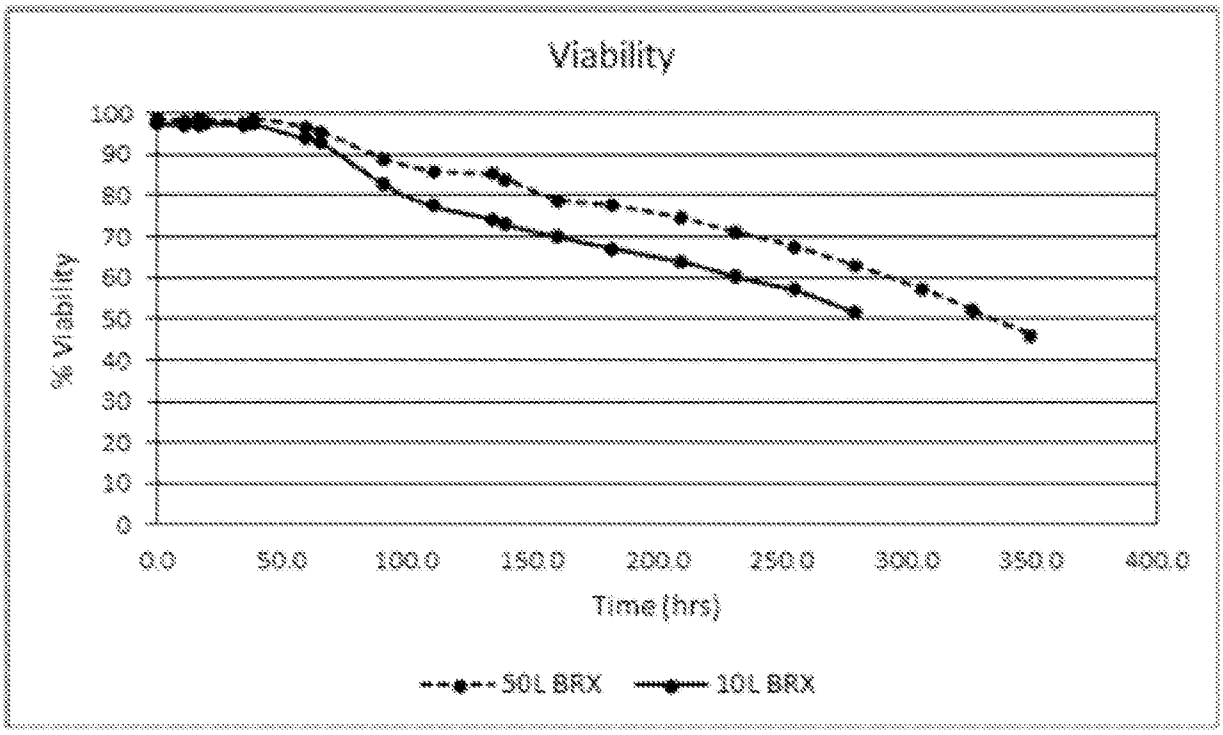

Viability
% Viability
100
90
80
70
60
50
40
30
20
10
0
0.0
50.0
100.0
150.0
200.0
250.0
300.0
350.0
400.0
Time (hrs)
50L BRX
10L BRX

FIG. 10

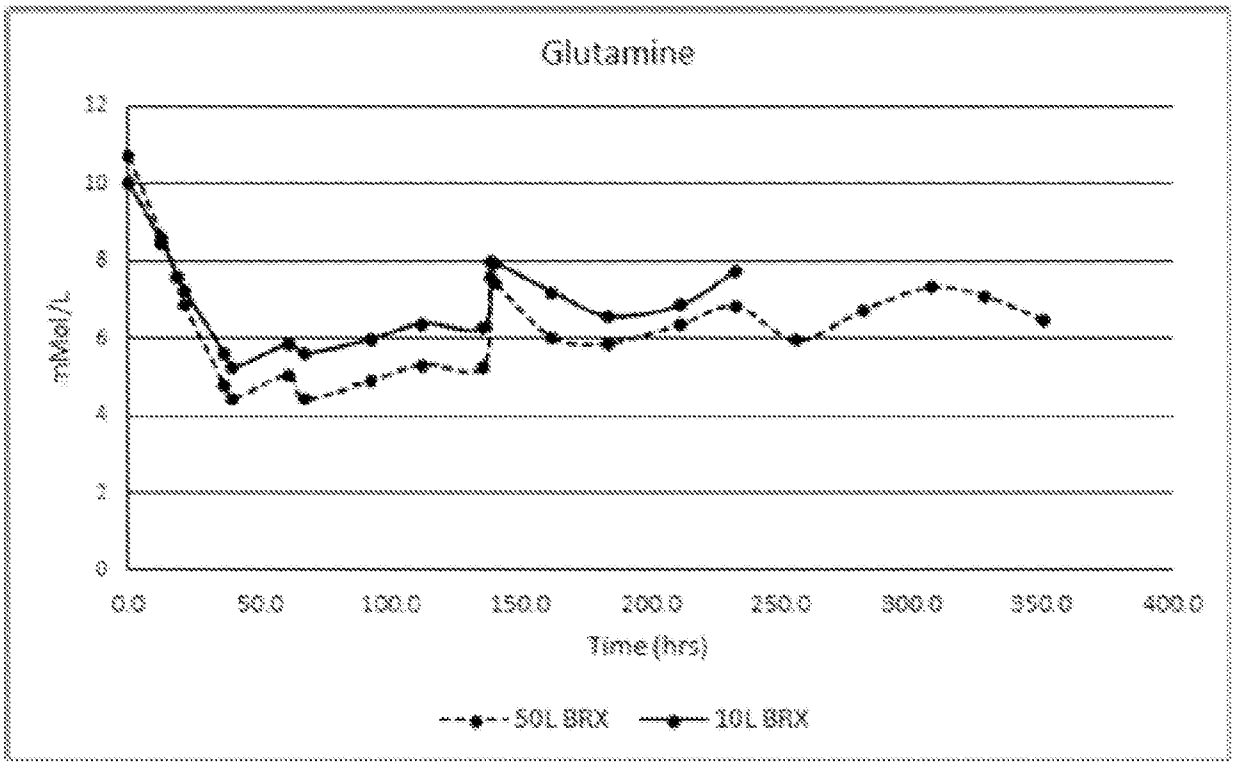

Glutamine
12
10
8
6
4
2
0
mMol/L
0.0
50.0
100.0
150.0
200.0
250.0
300.0
350.0
400.0
Time (hrs)
50L BRX
10L BRX

FIG. 11

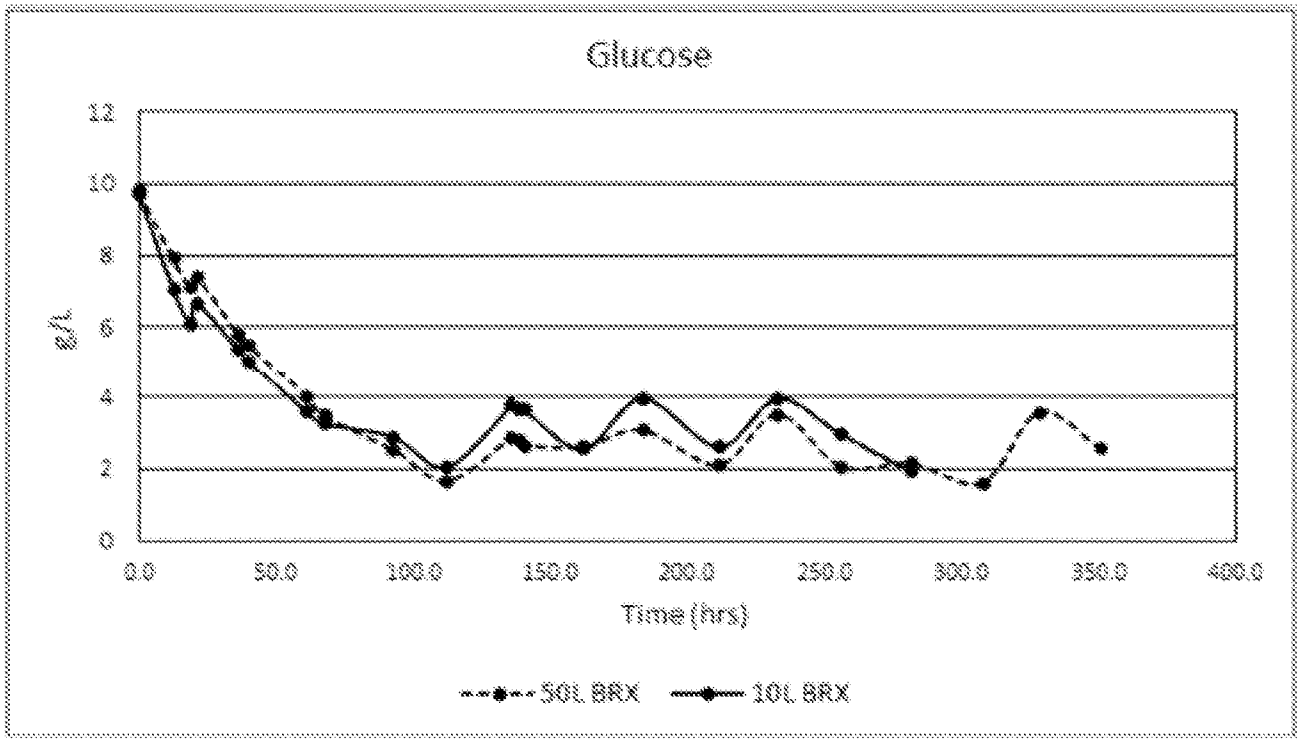

Glucose
12
10
8
6
4
2
0
g/L
0.0
50.0
100.0
150.0
200.0
250.0
300.0
350.0
400.0
Time (hrs)
50L BRX
10L BRX

FIG. 12

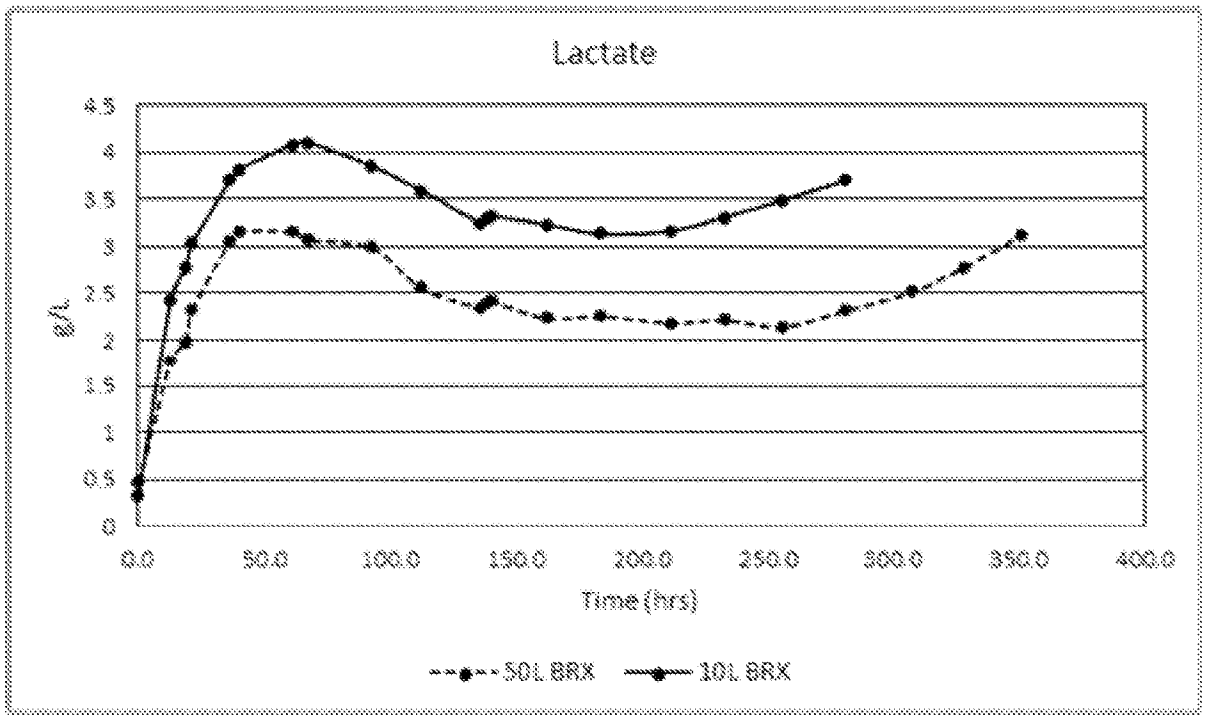

Lactate
g/L
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.0
50.0
100.0
150.0
200.0
250.0
300.0
350.0
400.0
Time (hrs)
50L BRX
10L BRX

FIG. 13

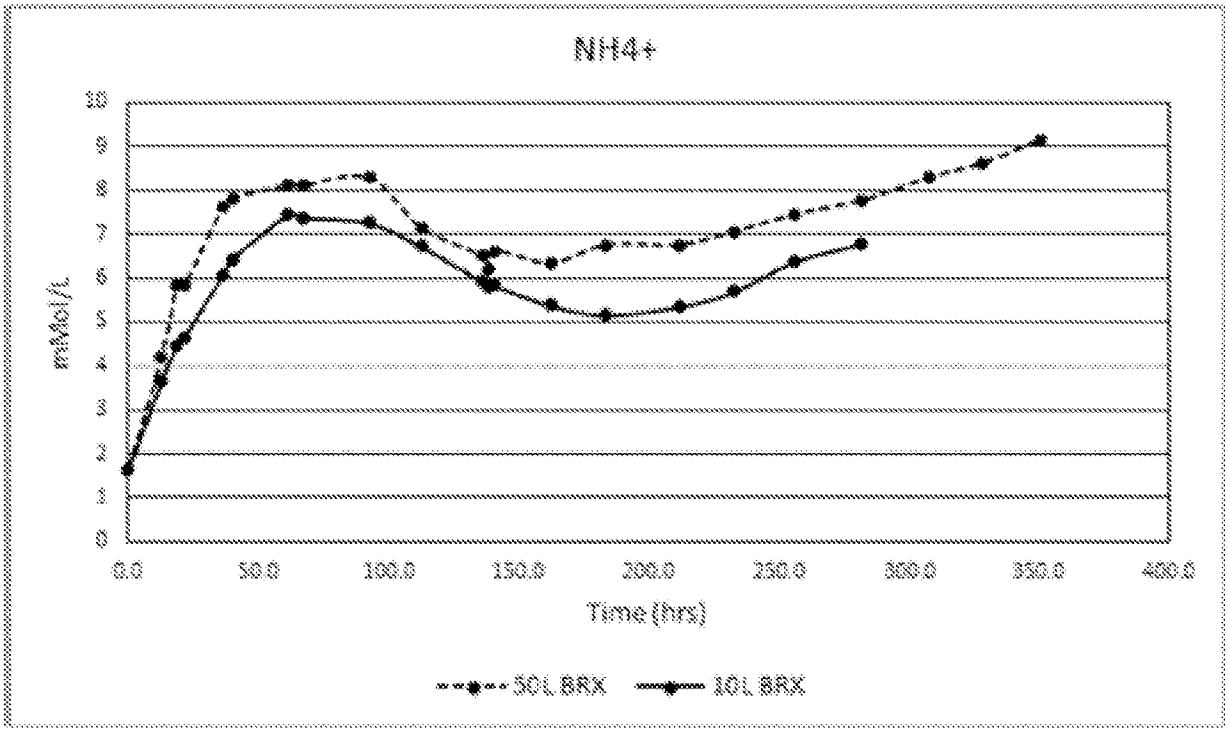

NH4+
mMol/L
Time (hrs)
0.0
50.0
100.0
150.0
200.0
250.0
300.0
350.0
400.0
50L BRX
10L BRX

FIG. 14

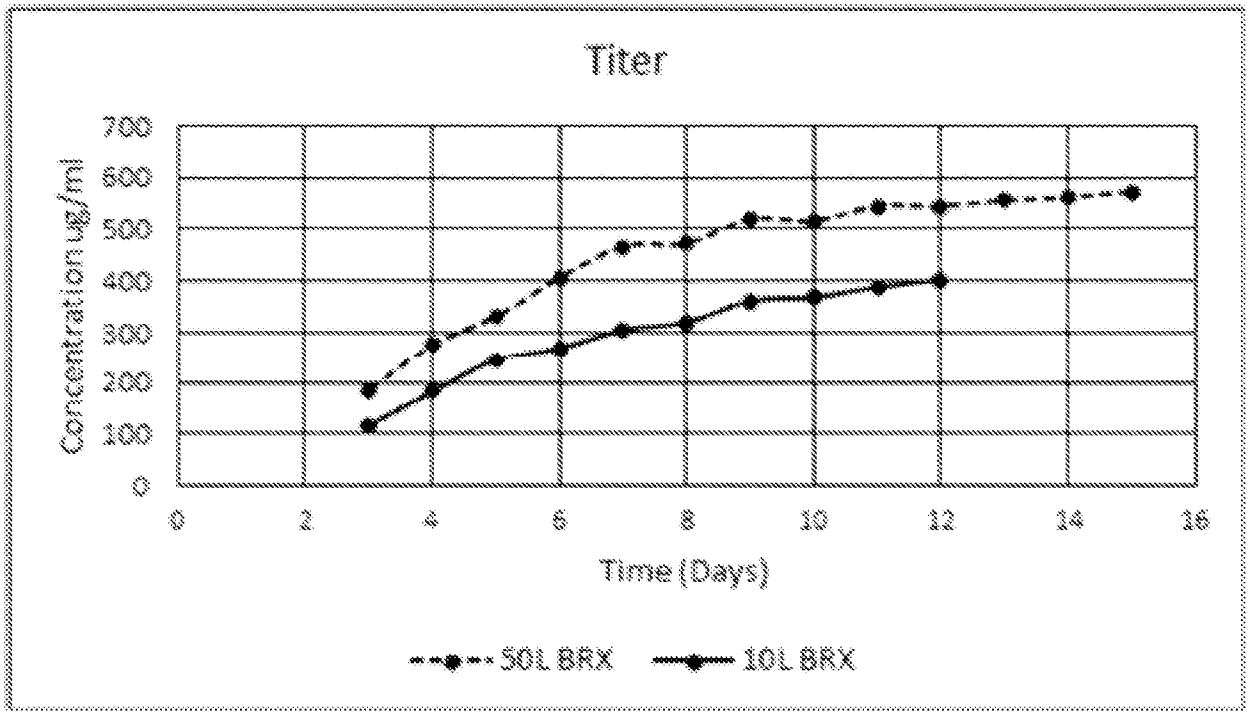
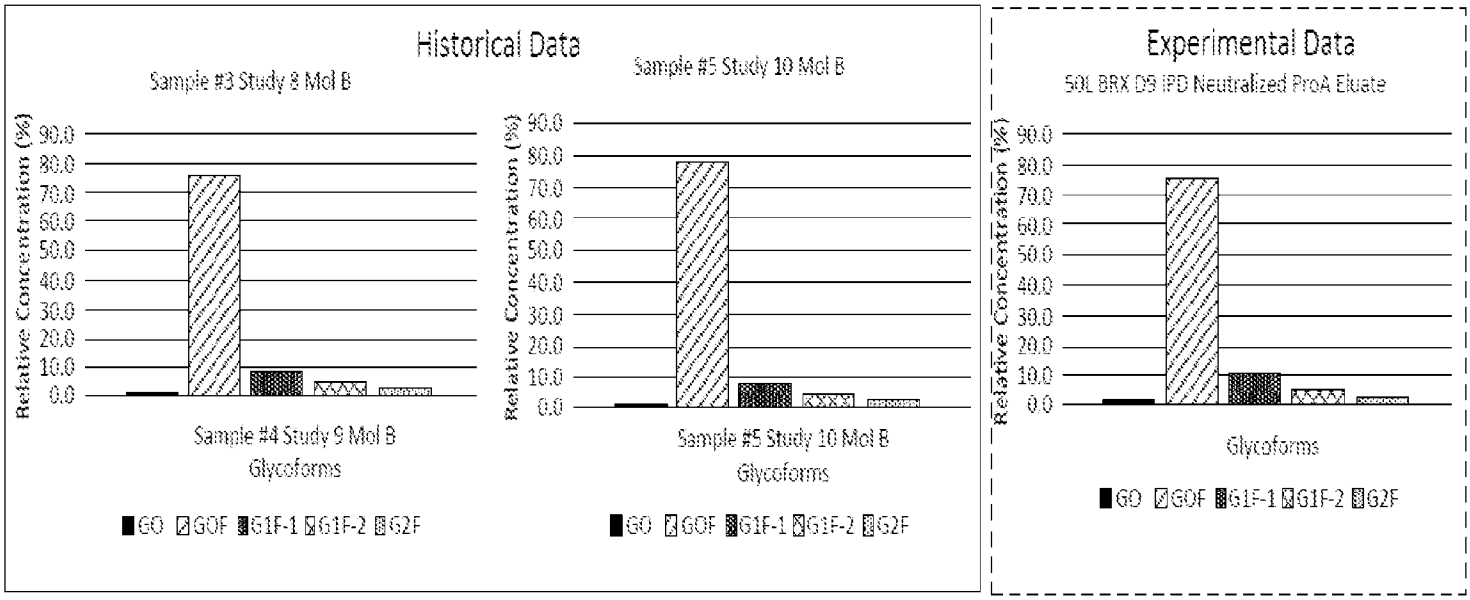

Titer
Concentration µg/ml
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16
Time (Days)
50L BRX
10L BRX

FIG. 15

16(a)



16(b)

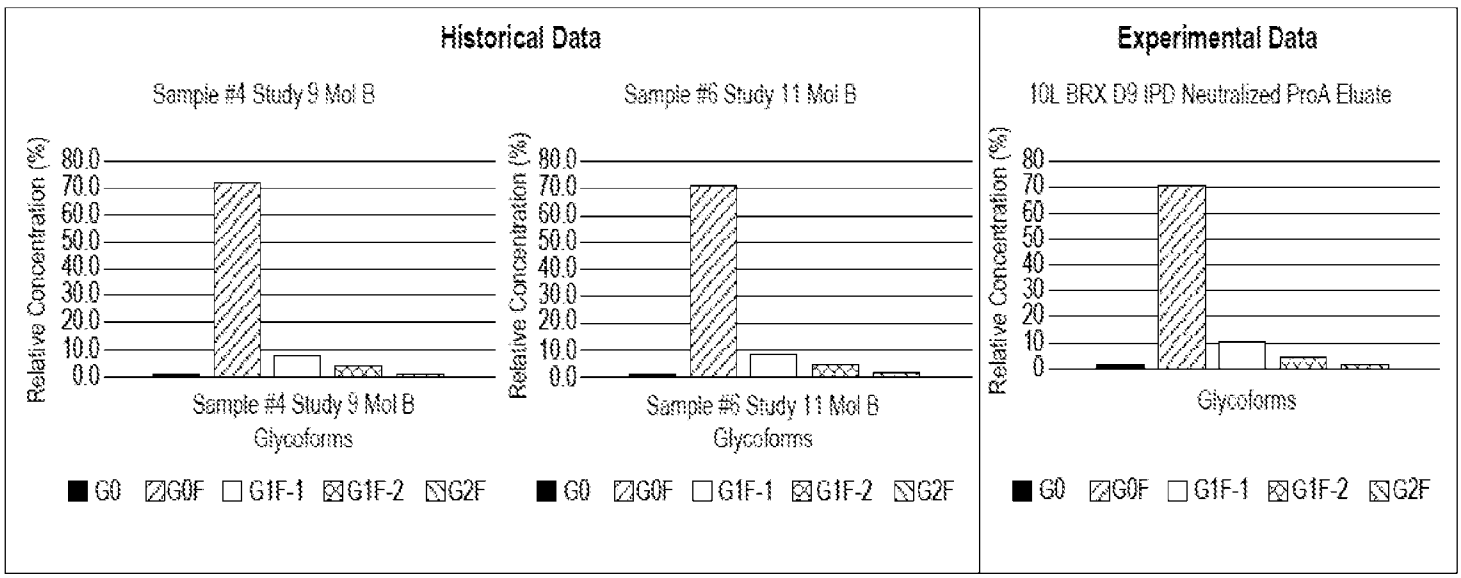


FIG. 16

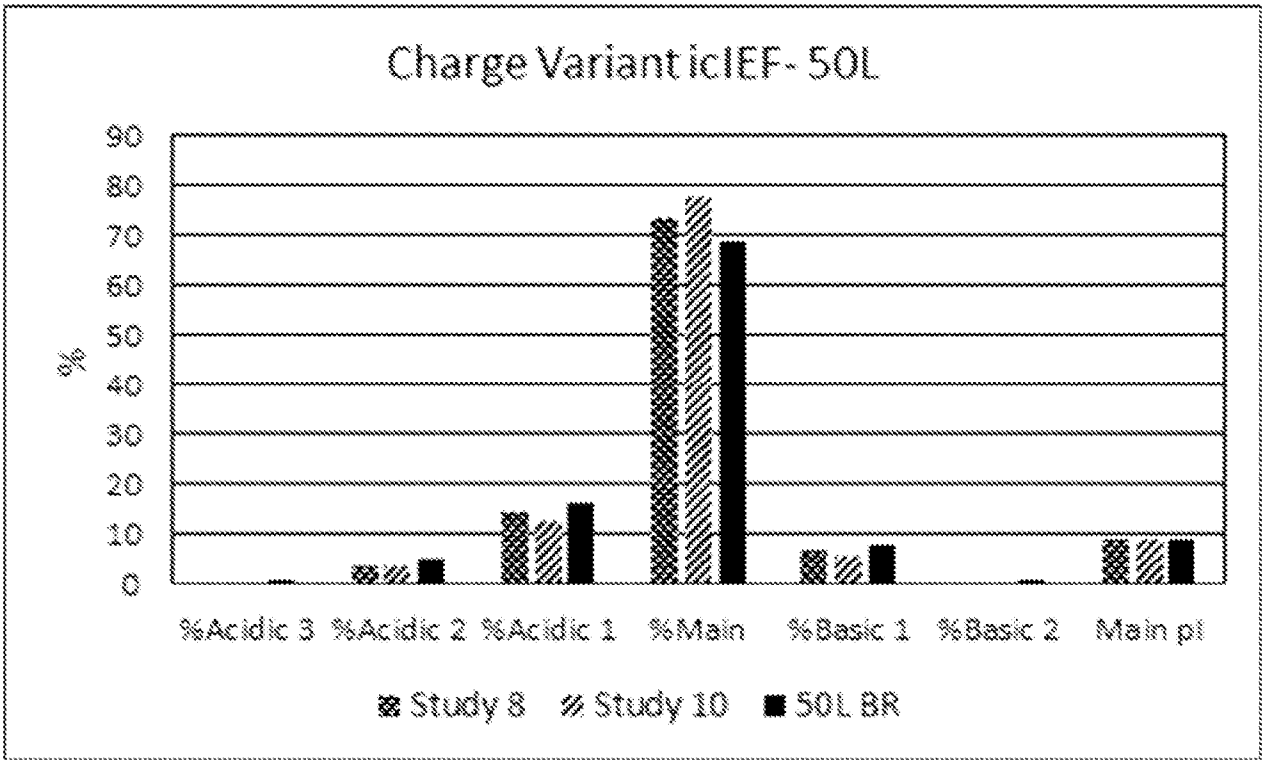

Charge Variant icIEF- 50L
90
80
70
60
50
40
30
20
10
0
%
%Acidic 3
%Acidic 2
%Acidic 1
%Main
%Basic 1
%Basic 2
Main pI
Study 8
Study 10
50L BR

FIG. 17

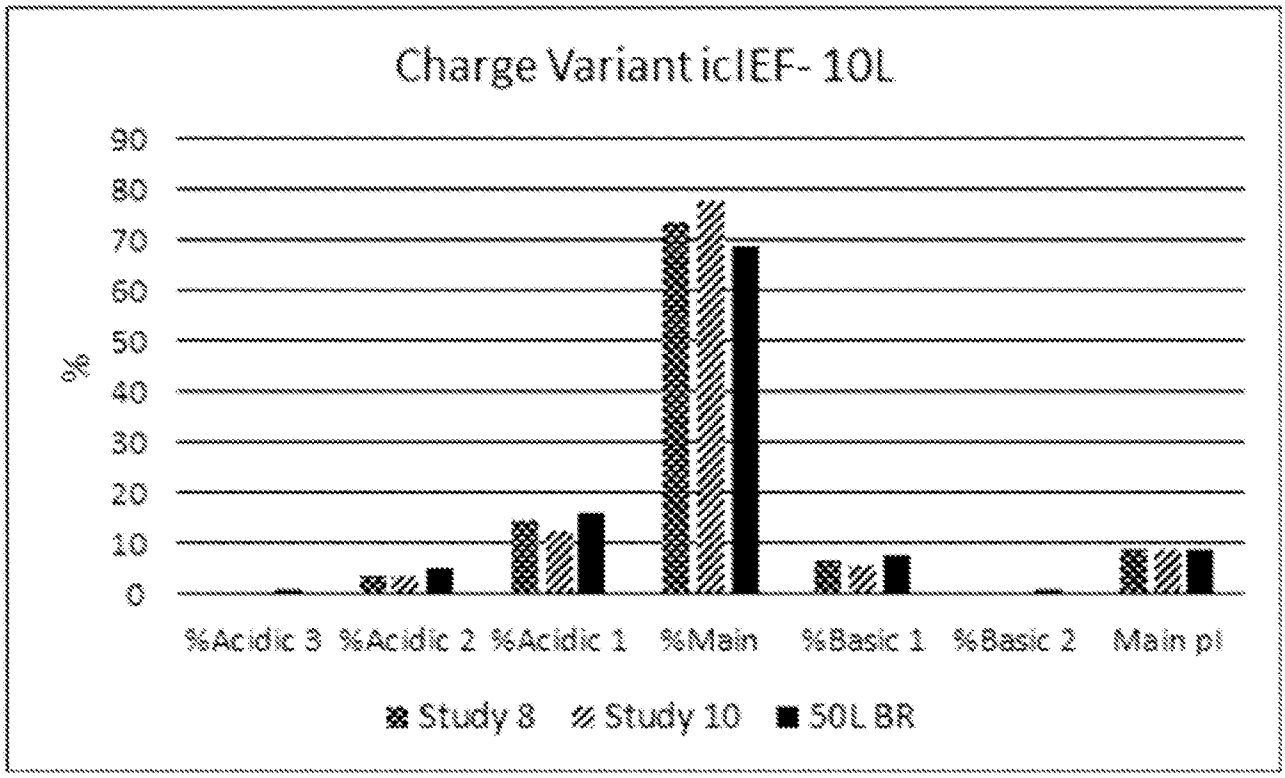

Charge Variant icIEF- 10L
90
80
70
60
50
40
30
20
10
0
%
%Acidic 3
%Acidic 2
%Acidic 1
%Main
%Basic 1
%Basic 2
Main pI
Study 8
Study 10
50L BR

FIG. 18

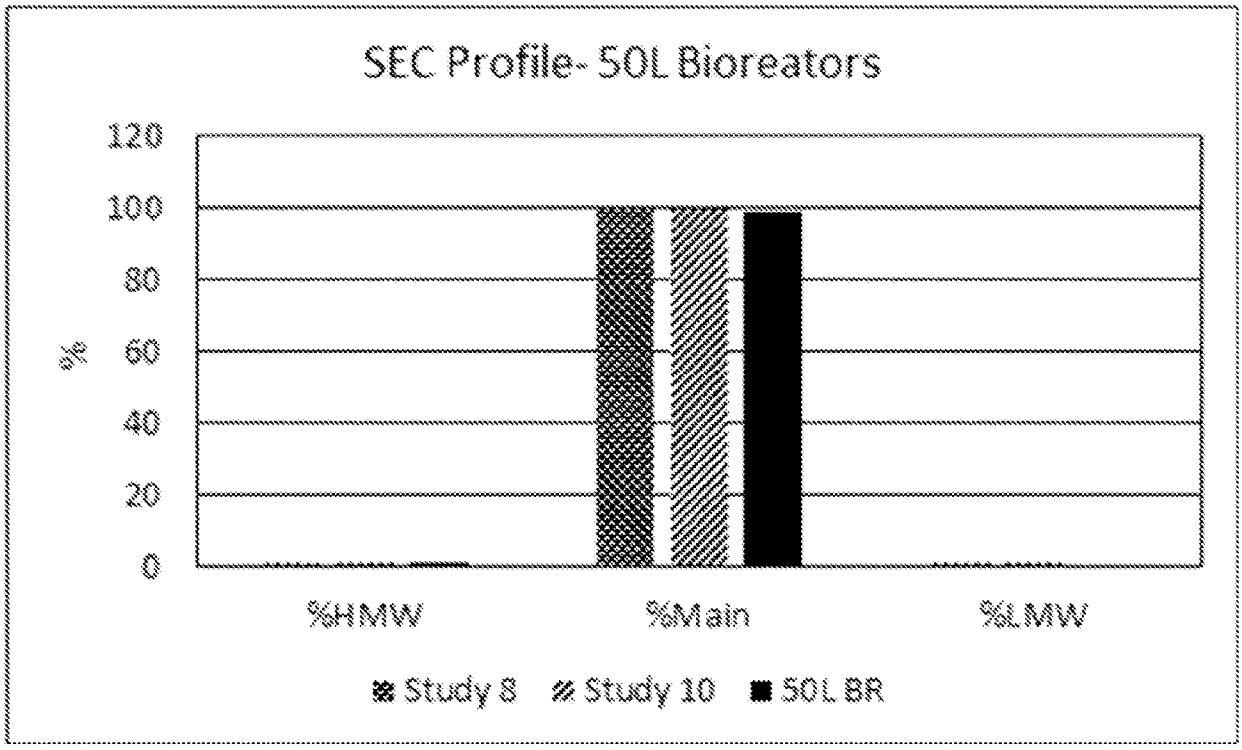

SEC Profile- 50L Bioreators
120
100
80
60
40
20
0
%
%HMW
%Main
%LMW
Study 8
Study 10
50L BR

FIG. 19

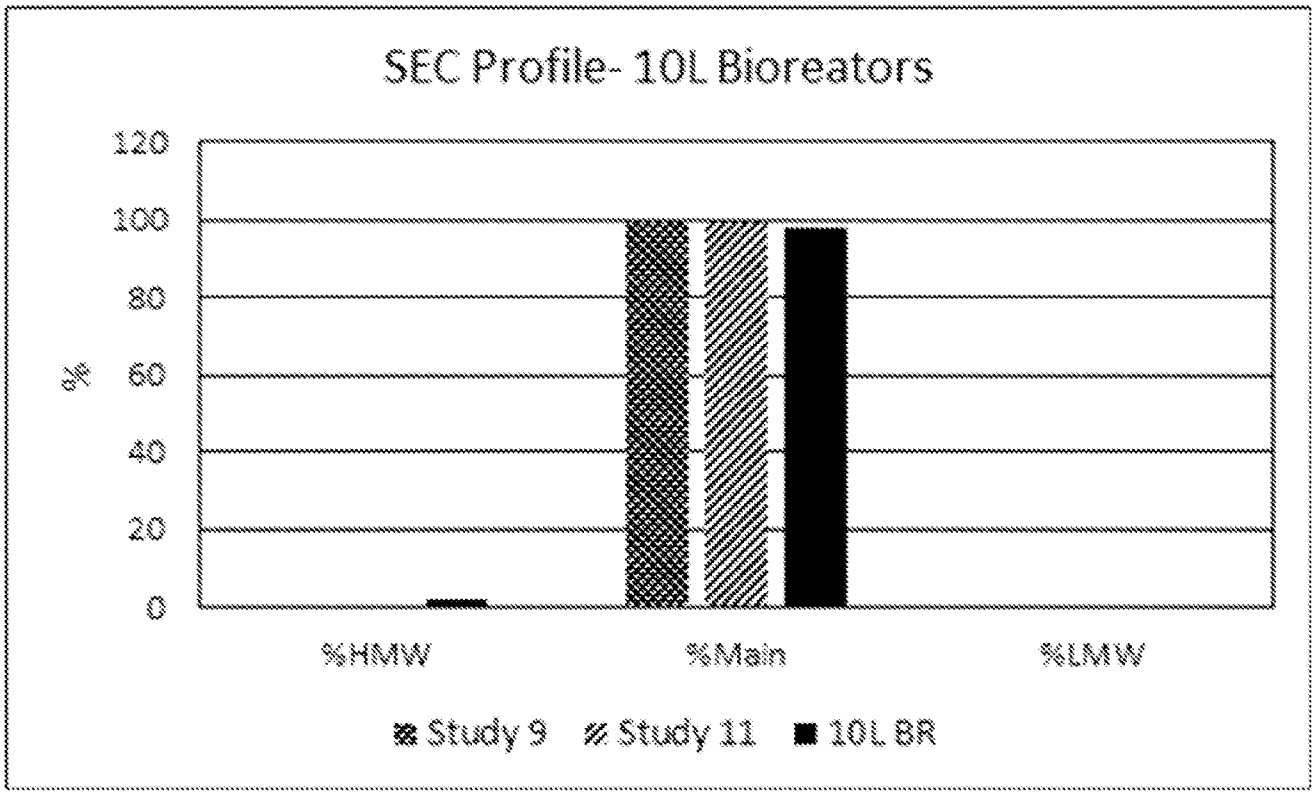

SEC Profile- 10L Bioreators
120
100
80
60
40
20
0
%
%HMW
%Main
%LMW
Study 9
Study 11
10L BR

FIG. 20

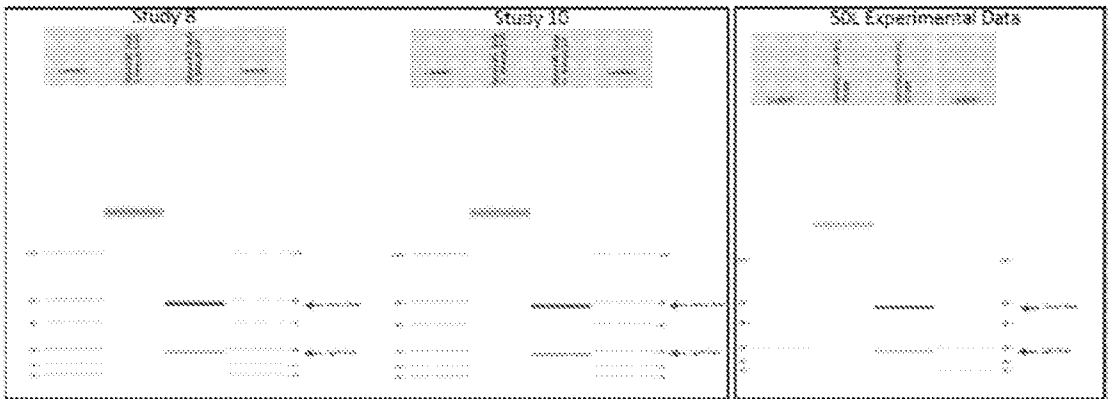


FIG. 21

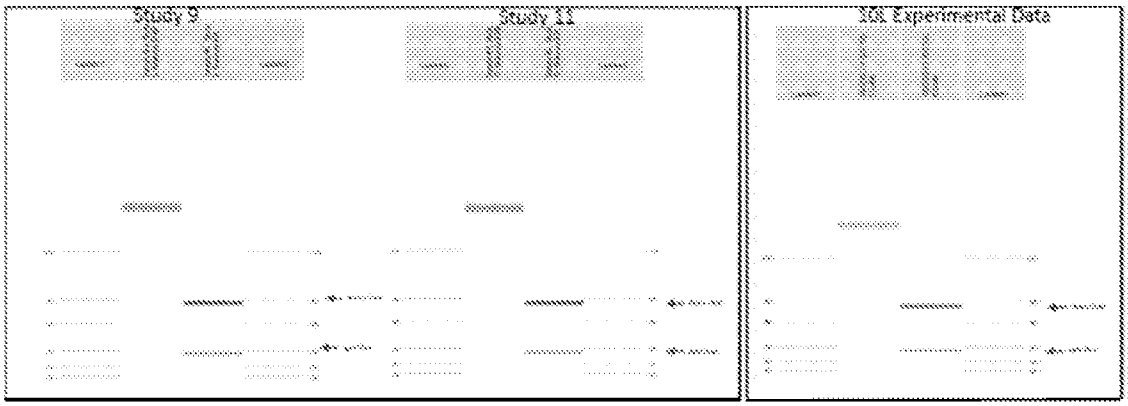


FIG. 22