preferably fully automated mammalian cell separation, based upon distinct expression levels of a given transgene expression product.

While the MACS device sold by Miltenyi Biotec® allows the removal of dead cells from cultures of mammalian cell lines using magnetic beads in combination with a magnetic material column operated under a strong permanent magnet, MACS does not allow for selective sorting of magnetic beads, and it does not allow for a sorting of high and low producer cells to preferably identify and select high producer cells. Alternative methods and apparatuses that rely on the labeling of high-producer cells with antibodies have been disclosed. The fast isolation of high producer cells may involve the use of fluorescence cameras that image cell colonies growing in soft agar and are combined with the robotic picking of highly fluorescent colonies. Examples are TAP's CellCelector™ for stem cell picking (Caron et al., 2009). Alternatively, Genetix's ClonePix™ relies on the formation of immuno-precipitates from the secreted proteins in semi-solid culture media, similarly coupled to cameras and a cell-picking arm. In these approaches the cells are not grown as free suspension but as clumps and are picked early during cloning, in particular, before stable expression may have established. The equipment involved has a relatively low throughput in that it is unable to analyze 100,000 transfected cells and more, which, however, is generally needed to find the most productive clones. In addition, the approaches are relatively slow, requiring days to be performed. The microfluidic-based approach, of the present invention, is designed to mitigate and/or address drawbacks of the prior art.

SUMMARY

In one embodiment, the present disclosure is directed at a sorting method for cells that display a protein of interest and, in certain embodiments, produce a transgene of interest, such as a therapeutic protein, preferably at a high level and optionally from a complex polyclonal population.

In certain embodiments, the present disclosure can identify high-producer cell lines using magnetic beads in an easy-to-use microfluidic system in a relatively short amount of time (e.g., less than 36 or 24 hours). In other embodiments, viable cells (e.g., high-producer cells) are sorted using a single use (disposable) cartridges in a consistently sterile environment, as required to achieve GMP compatible cell sorting.

The disclosure also concerns method for selecting cells depending on their level of expression of a protein of interest from a population of heterogeneously expressing cells using magnetic beads.

The disclosure is also directed at a method for identifying and, preferably selecting, cells displaying a protein of interest on their surface comprising:

(a) providing a sample comprising said cells;
(b) providing functionalized magnetic beads comprising one or more affinity groups, and optionally carrier beads, wherein said affinity group(s) is adapted to bind cells displaying the protein on their surface;
(c) mixing the cells with said functionalized magnetic beads and optionally with said carrier beads,

wherein said affinity group of the beads binds cells displaying said protein on their surface to produce magnetically-labeled cells (MLCs) having a magnetic label,

(d) separating, e.g. in at least one washing step, non-magnetically labelled cells from said MLCs, and
(e) identifying and, preferably selecting cells displaying the protein on their surface.

In one aspect, there is provided a method for identifying and selecting, cells displaying a protein of interest on their surface comprising:

(a) providing a sample comprising said cells;
(b) providing capture beads in the form of functionalized superparamagnetic beads comprising one or more affinity groups, wherein said affinity group(s) is/are adapted to bind cells displaying the protein on their surface, and carrier beads in the form of non-functionalized ferromagnetic beads;
(c) mixing the cells with said capture beads and carrier beads in a reaction chamber,

wherein said affinity group of the capture beads binds cells displaying said protein at their surface to produce magnetically-labeled cells (MLCs) having a magnetic label, and applying an external magnetic field having an amplitude and a polarity to said reaction chamber, wherein, in said external magnetic field, mixing of the capture beads and the cells displaying the protein is promoted by said carrier beads;

(d) separating, e.g. in at least one washing step, not magnetically-labelled cells from said MLCs; and

identifying and selecting, cells displaying the protein on their surface.

The protein of interest may be a marker protein or a transgene expression product (TEP).

The cells may be recombinant cells and the sample may comprise the recombinant cells that were transfected with a transgene, wherein the protein of interest may be a transgene expression product (TEP); and wherein the MLCs may lose their magnetic label over a time interval after binding to the affinity group and wherein the MLCs may be identified, and preferably selected, based on the time interval.

The recombinant cells secreting the TEP may be separated from recombinant cells displaying, but not secreting, the TEP based on said time interval.

The MLCs that lose the magnetic label in less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 hour(s) after binding, in less than 24, in less

The selected cells may have a level of protein expression, display or secretion that is at least 10% higher than the cells present in the original population. selected cells have a level of protein expression, display or secretion may preferably be 20%, 40%, 60%, 80%, or more preferably over 90% higher than the cells present in the original population. Cell may also be selected on the basis of their lower protein expression and the selected cells may have a level of protein expression is at least 10% lower than the cells present in the original population. The selected cells may have a level of protein expression that is preferably 20%, 40%, 60%, 80%, or more preferably over 90% lower than the cells present in the original population.

The capture beads may be superparamagnetic beads and the carrier beads may be ferromagnetic beads.

The ratio of capture beads to said carrier beads may be between 2:1 and 50:1, 5:1 and 25:1, preferably between 8:1 and 12:1 or around 10:1.

The amplitude and/or the polarity may be changed to define successive operation modes, wherein said mixing in (c) may be performed in a mixing mode and said separating in (d) may be performed in a bead separation mode.

The cells may be recombinant cells and the protein expressed on the surface may be a TEP and the identifying in (e) is performed by eluting the cells from the reaction chamber that lose their magnetic label (ergo separate from the magnetic bead) in less than 48 hrs, preferably less than 36 or 24 hrs after binding .

In the mixing and bead separation mode, the magnetic device(s) may operate in a circular or alternating mode at 1 Hz- 1000Hz and 0.1 to 10000 mA , preferably 40 to 500 Hz and at 200-500 mA.

The mixing mode and/or bead separation mode may each last less than 60 seconds.

The disclosure is also directed at a cartridge for selecting cells based on their level of display, and preferably secretion (release from a surface of a cell; shedding), of a

protein, such as a TEP, from a population of cells comprising the cells displaying, preferably secreting said protein, comprising:

a. microfluidic channels,
b. a reaction chamber for mixing magnetic beads in suspension, wherein the reaction chamber has at least one inlet and at least one outlet channel for introducing and removing a fluid into and from, respectively, said reaction chamber,
c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,
d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,
e. a waste container in fluid communication with the reaction chamber through the outlet channel,

wherein, each container of c. to d. is further in communication through one of the microfluidic channels to a venting pore comprising an air filtering element.

In another aspect, there is provided a cartridge for selecting cells based on their level of display of, and secretion of, a protein from a population of cells comprising the cells displaying, and secreting, said protein, according to the method described herein comprising:

a. microfluidic channels,
b. a reaction chamber for mixing magnetic beads in suspension, wherein the reaction chamber has at least one inlet and at least one outlet channel for introducing and removing a fluid into and from said reaction chamber,
c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,
d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,
e. a waste container in fluid communication with the reaction chamber through the outlet channel,

wherein, each container of c- d is further in communication through one of the microfluidic channels to a venting pore comprising an air filtering element.

The disclosure is also directed to an integrated system for selecting cells, e.g. recombinant cells, based on their level of display, and preferably secretion (release from a surface of a cell; shedding), of a protein, e.g., a TEP, expressed on the surface of the cells, from a population of cells comprising cells displaying, preferably secreting, said protein, wherein the system comprises a cartridge comprising:

a. microfluidic channels,
b. a reaction chamber for mixing magnetic beads in suspension; wherein the reaction chamber has at least a first inlet and at least a second outlet channel for introducing and removing a fluid into and from said reaction chamber,
c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,
d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,
e. a waste container in fluid communication with the reaction chamber through the outlet channel, wherein each container of c. to d. is further in communication through one of the microfluidic channels to an venting pore comprising an air filtering element;

f. one or more devices that create a controllable magnetic field (magnetic field devices= MFDs), in particular one or more electromagnets, arranged around or at the reaction chamber;

g. data processing equipment (e.g. a computer) configured to adjust a magnetic field created by the MTD(s) within the reaction chamber via frequency and/ or amplitude adjustments, wherein each frequency and/ or amplitude adjustment defines an operation mode within the reaction chamber.

In another aspect, there is provided an integrated system for selecting cells based on their level of display of, and secretion of, a protein from a population of cells comprising the cells displaying, and secreting, said protein, comprising:

a. microfluidic channels,

b. a reaction chamber for mixing magnetic beads in suspension; wherein the reaction chamber has at least a first inlet and at least a second outlet channel for introducing and removing a fluid into and from said reaction chamber,

c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,

d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,

e. a waste container in fluid communication with the reaction chamber through the outlet channel, wherein each container of c-d is further in communication through one of the microfluidic channels to an venting pore comprising an air filtering element;

f. one or more devices that create a controllable magnetic field (magnetic field devices= MFDs), arranged around or at the reaction chamber;

g. data processing equipment configured to adjust the magnetic field created by the MFDs within the reaction chamber via frequency and/ or amplitude adjustments, wherein each frequency and/ or amplitude adjustment defines an operation mode within the reaction chamber,

wherein the data processing equipment is configured to set a succession of said operation modes comprising a mixing mode, a capture mode, an immobilization mode, a bead separation mode and a recovery mode, wherein

the data processing equipment is adapted to set the MFDs to operate:

- in a circular or alternating mode at 1-1000 Hz, and at 0.1 to 10,000 mA, during the mixing and bead separation mode;
- in circular or alternating mode at a frequency and amplitude lower than in the mixing mode, such as at 0.5 to 40 Hz and at 300 to 600 mA, during the capture mode;
- at 0 Hz and at an amplitude ,such as at 300 to 600 mA, during the immobilization mode; and
- at an, relative to the immobilization mode, increased frequency, such as between 40 Hz-500Hz and at a lowered amplitude, such as at 30-300 mA during the recovery mode.

The data processing equipment may be configured to set a succession of said operation modes comprising a mixing mode, a capture mode, an immobilization mode, a bead separation mode and/or a recovery mode.

The data processing equipment may be adapted to set the MFDs to operate:

- in a circular or alternating mode at 1-1000 Hz, preferably 40 Hz- 500Hz and at 0.1 to 10,000 mA, preferably 200-500 mA during the mixing and bead separation mode, wherein, e.g., the circular mode may switch between clockwise and counterclockwise;
- in circular or alternating mode at a frequency and amplitude lower than in the mixing mode, such as at 0.5 to 40 Hz and at 300 to 600 mA, during the capture mode;
- at 0 Hz and at an amplitude, such as at 300 to 600 mA, during the immobilization mode; and
- at an, relative to the immobilization mode, increased frequency, such as between 40 Hz-500Hz and at a lowered amplitude, such as at 30-300 mA during the recovery mode.

The reaction chamber of the system or cartridge may comprise a mixture of carrier and capture beads.

The cartridge may further include a recovery container for receiving magnetically labelled cells, preferably magnetically labelled recombinant cells from the reaction chamber.

The cartridge or system may further comprise at least one second inlet and at least one second outlet channel in fluid communication with said reaction chamber, wherein the second inlet channel diverges off the at least one first outlet channel and the second outlet channel diverges off the at least one first inlet channel, wherein the recovery container is in fluid communication with the reaction chamber through the second inlet channel and the second outlet channel is connected to a further venting pore comprising an air filtering element.

The air venting pore of the recovery container may be connected to a pump for recovering the magnetically labelled cells within the reaction chamber by pumping air through the venting pore of the recovery container so that the reaction chamber content is flushed into the recovery container through an inlet channel.

The reaction chamber volume may be between 10 µl and 500 µl.

The cartridge may be self-contained and/or disposable.

The disclosure is also directed at a kit comprising, in one container, a cartridge as described herein, wherein the reaction chamber may comprise capture beads and carrier beads (which may alternatively be contained in a further container), and, in a separate container, instructions of how to use the capture beads and carrier beads in the cartridge.

In another aspect, there is provided a kit comprising, in one container, a cartridge comprising:

a. microfluidic channels,

b. a reaction chamber for mixing magnetic beads in suspension, wherein the reaction chamber has at least one inlet and at least one outlet channel for introducing and removing a fluid into and from said reaction chamber,

c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,

d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,

e. a waste container in fluid communication with the reaction chamber through the outlet channel,

wherein, each container of c-d is further in communication through one of the microfluidic channels to a venting pore comprising an air filtering element;

wherein capture beads in the form of functionalized superparamagnetic beads and carrier beads in the form of ferromagnetic beads are contained in the reaction chamber or are provided in a further container, and, in a separate container, instructions of how to use the capture beads and carrier beads in the cartridge.

The capture beads may be superparamagnetic beads and the carrier beads may be ferromagnetic beads, wherein the ratio of superparamagnetic beads to ferromagnetic beads is between 2:1 and 50:1.

The disclosure is also directed at cells identified and preferably selected via the methods, systems and/or cartridges described herein.

In another aspect, there is provided cells identified and selected via a method described herein.

The disclosure is also directed at an isolated population of cells comprising, preferably recombinant cells secreting a transgene expression product, at a level of more than 20, 40, 60, 80 pcd, wherein the isolated population of claims does not contain more 40% of a original cell population from which the isolated cell population was isolated.

The disclosure also includes the use of mammalian cells disclosed herein as therapeutic cells, including, but not limited to gene therapy or regenerative medicine use.

The transgene secreted may be a therapeutic protein.

The time interval between the mixing the cells with said functionalized magnetic beads and optionally with said carrier beads, and the identifying and, preferably selecting cells displaying the protein on their surface may be less than 1 hour, less than 30 minutes, less than 20 minutes, less than 15 minutes or less than 10 minutes.

The subject matter of the claims and all claimed combinations is incorporated by reference in this description and remains part of the disclosure event if claims are abandoned.

BRIEF DESCRIPTION OF THE DRAWINGS

The objects and features of the present invention are set forth with particularity in the appended claims. The present invention, both as to its organization and manner of operation, together with further objects and advantages, may best be understood by reference to the following description, taken in connection with the accompanying drawings, wherein

Figure 1 is a schematic presentation showing the generation of cells with various immunoglobulin production levels. CHO-M cells were co-transfected with expression vectors for immunoglobulin gamma (IgG) and an antibiotic selection marker, as well as a plasmid encoding eBFP2. Polyclonal populations stably expressing various levels of IgG were sorted by FACS on the basis of BFP and surface IgG display. IgG secretion of selected cell clones were validated by ELISA.

Figure 2 shows diagrams of GFP - or BFP - labeled reference cells mediating various IgG display and secretion levels: CHO-M-derived cell clones displaying various levels of cell surface IgG, but with variables levels of IgG secretion were selected by FACS as reference cell populations. BFP-labeled median displayer BS2 cells, high displayer BLC cells and very high displayer BHB cells are compared to the GFP-labeled F206 very high producer cell clone. The IgG displayed at the cell surface was labeled with APC-conjugated anti-IgG antibodies, prior to flow cytometry analysis (A). The IgG titter produced by parallel cultures of the indicated cell clones (B), or their specific productivity

WHAT WE CLAIM IS:

1. A method for identifying and selecting, cells displaying a protein of interest on their surface comprising:

(a) providing a sample comprising said cells;

(b) providing capture beads in the form of functionalized superparamagnetic beads comprising one or more affinity groups, wherein said affinity group(s) is/are adapted to bind cells displaying the protein on their surface, and carrier beads in the form of non-functionalized ferromagnetic beads;

(c) mixing the cells with said capture beads and carrier beads in a reaction chamber,

wherein said affinity group of the capture beads binds cells displaying said protein at their surface to produce magnetically-labeled cells (MLCs) having a magnetic label, and applying an external magnetic field having an amplitude and a polarity to said reaction chamber, wherein, in said external magnetic field, mixing of the capture beads and the cells displaying the protein is promoted by said carrier beads;

(d) separating, e.g. in at least one washing step, not magnetically-labelled cells from said MLCs; and

(e) identifying and selecting, cells displaying the protein on their surface.

2. The method of claim 1, wherein the cells are recombinant cells and the sample comprises the recombinant cells that were transfected with a transgene, wherein the protein of interest is a transgene expression product (TEP); and wherein the MLCs lose their magnetic label over a time interval after binding to the affinity group(s) and wherein the MLCs are identified, and selected, based on the time interval.

3. The method of claim 2, wherein, based on the time interval, recombinant cells secreting the TEP are separated from recombinant cells displaying, but not secreting, the TEP.

4. The method of any of claims 2 to 3, wherein the MLCs that lose the magnetic label in less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 hour(s) after binding, in less than 24, in less than 36, in less

than 48, in less than 60, in less than 72, in less than 84 or in less than 96 hours after binding are selected.

5. The method of any one of the preceding claims, wherein the ratio of capture beads to said carrier beads is between 2:1 and 50:1, 5:1 and 25:1, preferably between 8:1 and 12:1 or around 10:1.

6. The method of any one of the preceding claims, further comprising changing the amplitude and/or the polarity to define successive operation modes, wherein said mixing in (c) is performed in a mixing mode, in which the external magnetic field is a circulating magnetic field with a certain field strength and frequency, and said separating in (d) is performed in a bead separation mode, in which the external magnetic field is a circulating magnetic field with a field strength that is higher than in the mixing mode, and a frequency that is lower than the frequency in the mixing mode.

7. The method of claim 6, wherein the cells are recombinant cells and wherein the protein expressed on the surface is a TEP and wherein the identifying in (e) is performed by eluting the cells from the reaction chamber that lose their magnetic beads within less than 48 hrs after binding.

8. The method of claim 7, wherein the identifying in (e) is performed by eluting the cells from the reaction chamber that lose their magnetic beads within less than 36 or 24 hours after binding.

9. The method of any one of claims 6 to 8, wherein
in the mixing and bead separation mode, the magnetic field is applied in a circular or alternating mode at 1 Hz- 1000Hz and 0.1 to 10000 mA.

10. The method of claim 9, wherein the magnetic magnetic field is applied in a circular or alternating mode at 40 to 500Hz and at 200 to 500 mA.

11. The method of any one of claims 6 to 10, wherein the mixing mode and/or bead separation mode each last less than 60 seconds.

12. A cartridge for selecting cells based on their level of display of, and secretion of, a protein from a population of cells comprising the cells displaying, and secreting, said protein, according to the method of any one of the preceding claims comprising:

a. microfluidic channels,

b. a reaction chamber for mixing magnetic beads in suspension, wherein the reaction chamber has at least one inlet and at least one outlet channel for introducing and removing a fluid into and from said reaction chamber,

c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,

d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,

e. a waste container in fluid communication with the reaction chamber through the outlet channel,

wherein, each container of c- d is further in communication through one of the microfluidic channels to a venting pore comprising an air filtering element.

13. An integrated system for selecting cells based on their level of display of, and secretion of, a protein from a population of cells comprising the cells displaying, and secreting, said protein, comprising:

a. microfluidic channels,

b. a reaction chamber for mixing magnetic beads in suspension; wherein the reaction chamber has at least a first inlet and at least a second outlet channel for introducing and removing a fluid into and from said reaction chamber,

c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,

d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,

e. a waste container in fluid communication with the reaction chamber through the outlet channel, wherein each container of c-d is further in communication through one of the microfluidic channels to an venting pore comprising an air filtering element;

f. one or more devices that create a controllable magnetic field (magnetic field devices= MFDs), arranged around or at the reaction chamber;

g. data processing equipment configured to adjust the magnetic field created by the MFDs within the reaction chamber via frequency and/ or amplitude adjustments, wherein each frequency and/ or amplitude adjustment defines an operation mode within the reaction chamber,

wherein the data processing equipment is configured to set a succession of said operation modes comprising a mixing mode, a capture mode, an immobilization mode, a bead separation mode and a recovery mode, wherein

the data processing equipment is adapted to set the MFDs to operate:
- in a circular or alternating mode at 1-1000 Hz, and at 0.1 to 10,000 mA, during the mixing and bead separation mode;
- in circular or alternating mode at a frequency and amplitude lower than in the mixing mode, such as at 0.5 to 40 Hz and at 300 to 600 mA, during the capture mode;
- at 0 Hz and at an amplitude ,such as at 300 to 600 mA, during the immobilization mode; and
- at an, relative to the immobilization mode, increased frequency, such as between 40 Hz- 500Hz and at a lowered amplitude, such as at 30-300 mA during the recovery mode.

14. The system of claim 13, wherein the one or more devices that create a controllable magnetic field is one or more electromagnets.

15. The system of claim 13, wherein the circular or alternating mode is set at 40 Hz-500Hz and at 200mA-500 mA during the mixing and bead separation mode.

16. The system of any one of claims 13 to 15, wherein the reaction chamber comprises a mixture of carrier beads in the form of ferromagnetic beads and capture beads in the form of functionalized superparamagnetic beads.

17. A kit comprising, in one container, a cartridge comprising:

a. microfluidic channels,

b. a reaction chamber for mixing magnetic beads in suspension, wherein the reaction chamber has at least one inlet and at least one outlet channel for introducing and removing a fluid into and from said reaction chamber,

c. a cell sample container in fluid communication with the reaction chamber through the inlet channel,

d. at least one washing reagent container in fluid communication with the reaction chamber through the inlet channel,

e. a waste container in fluid communication with the reaction chamber through the outlet channel,

wherein, each container of c-d is further in communication through one of the microfluidic channels to a venting pore comprising an air filtering element;

wherein capture beads in the form of functionalized superparamagnetic beads and carrier beads in the form of ferromagnetic beads are contained in the reaction chamber or are provided in a further container, and, in a separate container, instructions of how to use the capture beads and carrier beads in the cartridge.

18. The kit of claim 17, wherein the ratio of superparamagnetic beads to ferromagnetic beads is between 2:1 and 50:1.

19. The cells identified and selected via a method according to claims 1 to 11.