physical limitations of column separation (see for example US10196631B2, JP2018138913A, EP2734538A2, WO2015166049A1, US2014315297A1 and US2017299585A1).

3) Centrifugal and density separation: this is the most widely used standard mode of cell separation based on the physical properties of cells such as size and density. These methods use centrifuges and density gradients to capture the cells of interest. The main drawback of these methods is that cells are purified solely based on their physical properties and not their biological properties, such as surface antigens. Cell therapy requires the isolation of specific cell populations based on their surface antigens (called CD antigens) and cannot be differentiated solely on the physical properties of the cells. Extended centrifugation and the use of density reagents can also negatively influence cell behaviour and viability (see for example JP2012143256A and WO2018194061A1).

As a result of these and other drawbacks in cell separation methods and systems in the art, there is a need for a method, apparatus and system that would provide an efficient separation of target cells based on their surface antigens without using magnetic particles and without using non-magnetic beads coated with antibody receptors that can be directly used in therapy.

3. Summary

In order to address this need, the invention provides a method, a cartridge and an apparatus that results in an efficient and specific separation of viable target cells based on molecules, for example antigens, that are present on the surface of said target cells without using magnetic particles or beads coated with antibody receptors. The present invention results in a high degree of purity and high degree of cell viability of the isolated target cells in a buffer or medium of choice.

These objects are solved by means of a method for separating viable target cells from a sample; a cartridge configured to be used in such a method; a kit-of-parts, and/or an apparatus configured to be used in the aforementioned method according to the claims. Distinct embodiments are derivable from the dependent claims and description.

Accordingly, in one aspect, the invention relates to a method for separating viable target cells from a sample comprising the steps of:

a. contacting a sample comprising a suspension of viable target cells that display a molecule on the cell surface with non-porous microparticles that have a density of about 1.45 g/cm^3 or greater, a diameter of about 10µm to 200µm, and a capture ligand covalently immobilized to the microparticle surface that is capable of specifically binding to said molecule;
b. incubating said sample without substantial agitation to form a target cell/microparticle complex;

magnetic particles that are eluted and present in the final cell suspension in other systems. The method, cartridge and apparatus according to the invention allow for a rapid and controlled cell separation procedure that can last less than one hour, thereby reducing cell death and changes to cell metabolism and unwanted activation of cells. Furthermore, the method, cartridge and apparatus according to the invention can provide a closed, sterile environment and can be adapted for low volume (for example 10 ml of sample) or high volume (several liters of sample) applications. The method, cartridge and apparatus according to the invention result in separated target cells that are eluted in a medium or physiological buffer of choice with no unwanted additives or impurities, with limited processing time and high cell viability and health.

Therefore, in a further aspect, the invention also relates to a medicament comprising a target cell suspension obtainable according to the method of the invention, a target cell suspension obtainable according to the method of the invention for use as a medicament and a method of treating a subject in need of a target cell suspension obtainable according to the method of the invention for the treatment of a disease.

In another aspect, provided herein is a closed housing cartridge configured to be used in the separation of viable target cells from a sample according to the method as described herein.

In another aspect, provided herein is a kit-of-parts comprising the closed housing cartridge as described herein and a container comprising a suspension of non-porous microparticles that have a density of about 1.45 g/cm^3 or greater, a diameter of about 10µm to 200µm, and a capture ligand covalently immobilized to the microparticle surface that is capable of specifically binding to a molecule on the surface of a cell.

In another aspect, provided herein is an apparatus configured to separate viable target cells from a sample according to the method as described herein comprising:
- a means for securely mounting the closed housing cartridge as described herein such that the valves of the cartridge can be mechanically operated by an electronic control system,
- a means for connecting the cartridge to one or more containers with one or more solutions and/or suspensions,
- a means for moving solutions and/or suspensions, and
- a control system configured to set, measure, and/or adjust the flow of solutions and/or suspensions via the valves of the cartridge while the method as described herein is performed.

In another aspect, provided herein is a medicament comprising a viable target cell suspension obtainable according to the method as described herein, wherein the eluted viable target cell suspension is essentially free from capture ligand.

In another aspect, provided herein is a viable target cell suspension obtainable according to the method as described herein for use as a medicament, wherein the eluted viable target cell suspension is essentially free from capture ligand.

In another aspect, provided herein is the use of a viable target cell suspension obtainable according to the method as described herein in the manufacture of a medicament for prevention and/or treatment of disease in a subject, wherein said viable target cell suspension that is essentially free from capture ligand is to be administered to said subject.

4. Brief Description of the Drawings

FIG. 1 Results of Bradford supernatant analysis, calculated as the percentage of proteins that is left in the supernatant solution, from the starting protein concentration.

FIG. 2: Calculated surface saturation of 40 µm non-porous silica microparticles (Glantreo Ltd., Ireland) covered with anti-CD90 antibody (BIOTEM, France), based on experimental results acquired with the Bradford spectrophotometric method of supernatants.

FIG. 3: Depicted are the parts, i.e. filter, incubation tube and syringes connected in one system, that can be used for the manual cell separation method of target cells according to the invention. The system is sterile and is meant for one-time use. The syringe 1 is used for the incubation of the viable target cells and non-porous microparticles and for the elution buffer. The filter 3 is used to separate viable target cells in the sample from the non-porous microparticles. Long tube 4 and short tube 5 are used for easier connection with the filter 3 as well as operation of the first syringe 1 and second syringe 2. The second syringe 2 is used as a receiving syringe for the collection of viable target cells. The first syringe 1 and the second syringe 2 can be replaced with clean syringes when needed for preforming the method.

FIG. 4a: Microscope image of non-porous microparticles after performing step a and b of the cell separation method according to the present invention using positive control cells. Silica non-porous microparticles having a diameter of 40 µm (purchased from Glantreo Ltd., Cork, Ireland) and having immobilized anti-CD90 antibody on their surface bind CD90 positive cells (Jurkat cells), forming target cell/microparticle complexes. CD90 positive cells are distinguished as the small round glowing structures positioned on the surface of larger spherical non-porous microparticles.