[0005] Therefore, there is a need to provide a device and method for efficiently capturing and retrieving cells, particularly rare cells, that overcome, or at least ameliorate, one or more of the disadvantages described above. There is a need to optimize the efficiency of the retrieval of isolated rare cells using methods, materials and/or device configurations in such a way that the rare cells do not adhere to the components of the device and filters, so that the rare cells can be easily and efficiently retrieved for downstream procedures.

[0006] There is a need to provide less invasive screening test methods for the early detection of cancer.

SUMMARY

[0007] In a first aspect, there is provided an apparatus for capturing and retrieving a cell from a sample, comprising at least one column, the column comprising:

(i) an inner wall defining an inner chamber, the inner chamber having an inlet opening at a first end of the column for receiving the sample, and an outlet opening at a second end of the column;

(ii) a perforated plug disposed within the inner chamber adjacent to the second end of the column;

(iii) a sleeve insert having an opening at a first end and an opening at a second end, the sleeve insert comprising a channel tapered at the second end and disposed within the inner chamber with its second end adjacent to the perforated plug; and

(iv) a filtering means housed within the sleeve insert, the filtering means comprising a sieve sandwiched between two sealing means.

[0008] In a second aspect, there is provided a method of capturing and retrieving a cell from a sample, comprising the steps of:

(a) introducing the sample to the inlet opening of the apparatus as described herein to allow the sample to flow through the sleeve insert and filtering means of the apparatus; and

(b) collecting the residue retained on the surface of the sieve in the filtering means of the apparatus.

[0009] In a third aspect, there is provided an isolated cell population having the following characteristics:

(i) being endothelial cells derived from a tumor and isolated from blood;

Claims:

1. An isolated cell population having the following characteristics:
   (i) being endothelial cells derived from a tumor and isolated from blood;
   (ii) each cell having at least two clearly distinct nuclei;
   (iii) each cell having a major axis of greater than about 10 μm;
   (iv) expression of endothelial cell genes or proteins;
   (v) non-expression of leukocyte-specific genes or proteins; and
   (vi) non-expression of megakaryocyte or platelets-specific genes or proteins.

2. The isolated cell population of claim 1, wherein the endothelial cell genes are selected from the group consisting of *PECAM1, VWF* and *CDH5*; wherein optionally

the leukocyte and megakaryocytic or platelet-specific genes are selected from the group consisting of *PTPRC, ITGA2B* and *GP1BA*.

3. A method for detecting the isolated cell population of claim 1 or 2 in a sample of a subject, the method comprising:

(a) contacting cells from the sample with at least one antibody coupled to a detectable label to allow binding of the antibody to one or more target biomarkers expressed on the cells;

(b) removing unbound antibody from the sample; and

(c) detecting and analyzing the detectable label bound to the antibody to detect the isolated population of cells.

4. A method for detecting the isolated cell population of claim 1 or 2 in a sample of a subject, the method comprising:

(a) lysing the cells present in the sample;

(b) contacting the lysed cell sample from step (a) with a reverse primer from a first primer pair, the reverse primer from the first primer pair being directed to a target RNA region, and a reverse transcriptase to effect reverse transcription of the RNA into cDNA;

(c) subsequently contacting the sample from step (b) with:

(i) a forward primer from the first primer pair, the forward primer from the first primer pair being directed to a target cDNA region,

(ii) a reverse primer and a forward primer from a second primer pair, the reverse primer and forward primer from the second primer pair being directed to a target DNA region, and

(iii) a DNA polymerase

to simultaneously amplify the target cDNA region and the target DNA region in a pre-amplification step; and

(d) analyzing the amplified target cDNA region and/or the amplified target DNA region.

5. The method of claim 4, further comprising: subjecting the sample from step (c) to a semi-nested PCR using the reverse primer in step (b) or the forward primer in step (c)(i), and a nested primer that binds within the amplified target cDNA region, or subjecting the sample from step (c) to a nested PCR using a nested primer pair that binds within the amplified target DNA region; wherein optionally steps (b) and (c) are conducted in the same reaction mixture; wherein optionally the analysis in step (d) comprises analyzing the amplified target cDNA for gene expression; and wherein optionally the analysis in step (d) comprises analyzing the amplified target cDNA for mutations.

6. A method of diagnosing a cancer in a subject, comprising analyzing a sample from the subject for presence of the isolated population of cells of claim 1 or 2, wherein presence of the isolated population of cells indicates that the subject has cancer.

7. The method of claim 6, wherein the cancer is selected from the group consisting of carcinoma, sarcoma, lymphoma, germ cell tumor, blastoma, colon cancer, rectal cancer, breast cancer, prostate cancer, renal cell cancer, transitional cell carcinoma, lung cancer, cholangiocarcinoma, brain cancer, non-small cell lung cancer, pancreatic cancer, gastric carcinoma, bladder cancer, esophageal cancer, mesothelioma, melanoma, thyroid cancer, head and neck cancer, osteosarcoma, hepatocellular carcinoma, carcinoma of unknown primary, ovarian carcinoma, endometrial carcinoma, glioblastoma, neuroblastoma, Hodgkin lymphoma and non-Hodgkin lymphomas; wherein optionally

the cancer is an invasive and/or metastatic cancer; or

a stage I cancer, stage II cancer, stage III cancer or stage IV cancer; or

an early stage cancer.

8. A method for monitoring and/or predicting the response to treatment of a cancer patient, the method comprising analyzing a sample obtained from the patient after treatment for determining the number of the isolated population of cells of claim 1 or 2, wherein a reduction in the number of the isolated population of cells compared to the number of the isolated population of cells in a baseline sample obtained from the patient prior to treatment indicates that the patient is responding positively to the treatment.

9. A method for predicting the response to treatment of a cancer patient, the method comprising analyzing a sample obtained from the cancer patient before treatment for determining the number of the isolated population of cells of claim 1 or 2, wherein an equal or higher number of the isolated population of cells compared to the number of the isolated population of cells in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the cancer patient will respond positively to the treatment, and wherein a lower number of the isolated population of cells compared to the number of the isolated population of cells in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the cancer patient will respond negatively to the treatment .

10. A method for analyzing blood vessel characteristics of a tumor in a subject, the method comprising analyzing a sample from the subject for determining the number of the isolated population of cells of claim 1 or 2, wherein an increased number of the isolated population of cells compared to a baseline sample indicates that the tumor has larger blood vessels compared to the baseline sample, and wherein a reduced number of the isolated population of cells compared to a baseline sample indicates that the tumor has smaller blood vessels compared to the baseline sample.

11. An apparatus for capturing and retrieving a cell from a sample, comprising at least one column, the column comprising:

(i) an inner wall defining an inner chamber, the inner chamber having an inlet opening at a first end of the column for receiving the sample, and an outlet opening at a second end of the column;

(ii) a perforated plug disposed within the inner chamber adjacent to the second end of the column;

(iii) a sleeve insert having an opening at a first end and an opening at a second end, the sleeve insert comprising a channel tapered at the second end and disposed within the inner chamber with its second end adjacent to the perforated plug; and

(iv) a filtering means housed within the sleeve insert, the filtering means comprising a sieve sandwiched between two sealing means.

12. The apparatus of claim 11, comprising one column, or

two or more columns; wherein optionally

the second end of the column is adapted for connection to one or more pumps for controlling flow-rate of the sample through the column; wherein optionally

the pump is adapted for passing the sample at a flow-rate selected from the group consisting of at least about 0.05 mL/min, at least about 0.10 mL/min, at least about 0.15 mL/min, at least about 0.20 mL/min, at least about 0.25 mL/min, at least about 0.30 mL/min, at least about 0.40 mL/min, and at least about 0.50 mL/min; wherein optionally

the sieve comprises non cell-adhesive material; wherein optionally

the non cell-adhesive material is selected from the group consisting of silicon, silicon dioxide, silicon nitride, epoxy-based negative photoresist and ceramic; wherein optionally

the epoxy-based negative photoresist comprises SU-8; and wherein optionally

the sieve comprises a plurality of pores having a diameter selected from the group consisting of at least about 6 μm, at least about 7 μm, at least about 8 μm, at least about 9 μm, at least about 10 μm, at least about 12 μm, at least about 14 μm, at least about 16 μm, at least about 18 μm and at least about 20 μm.

13. A method of capturing and retrieving a cell from a sample, comprising the steps of:

(a) introducing the sample to the inlet opening of the apparatus of claim 11 or 12 to allow the sample to flow through the sleeve insert and filtering means of the apparatus; and

(b) collecting the residue retained on the surface of the sieve in the filtering means of the apparatus.

14. The method of claim 13, wherein the sample comprises a biological fluid optionally

selected from the group consisting of whole blood, blood serum, blood plasma, cerebrospinal fluid, lymph fluid, cystic fluid, sputum, stool, pleural effusion, mucus, ascitic fluid and urine; wherein optionally

the sample comprises a single cell optionally selected from the group consisting of a suspected cancer cell, a suspected tumor-derived cell, a suspected cell derived from an embryo or a foetus, and a cell from a pathogenic organism; or

a plurality of cells wherein at least some of the plurality of cells are optionally selected from the group consisting of: suspected cancer cells, suspected tumor-derived cells, suspected cells derived from an embryo or a foetus, and cells from a pathogenic organism; wherein optionally

two or more of the plurality of cells form a cell cluster; wherein optionally

the single cell or at least some of the plurality of cells is multinucleated; wherein optionally

the sample is a blood sample, and the cell captured and retrieved therefrom comprises at least two clearly distinct nuclei; wherein optionally

the major axis of the cell is at least 10 μm; wherein optionally

the cell expresses a gene selected from the group consisting of *PECAM1, VWF* and *CDH5*; wherein optionally

the cell does not express a gene selected from the group consisting of *PTPRC, ITGA2B* and *GP1BA*; and wherein optionally

collecting the residue in step (b) comprises using a pipette to retrieve the residue.

15. A method for detecting the isolated cell population of claim 1 or 2 in a sample of a subject, the method comprising:

(a) capturing and retrieving the cells from the sample using the apparatus of claim 11 or 12, or the method of claim 13 or 14.

16. The method of claim 15, further comprising the steps of:

(b) contacting the cells from step (a) with at least one antibody coupled to a detectable label to allow binding of the antibody to one or more target biomarkers expressed on the cells;

(c) removing unbound antibody from the sample; and

(d) detecting and analyzing the detectable label bound to the antibody to detect the isolated population of cells.

17. The method of claim 3 or 16, wherein the antibody is capable of specific binding to a target biomarker selected from the group consisting of PAI-1, Vimentin, FOXC1, keratin-8, keratin-18, keratin-19, Ep-CAM, CD45, VWF, PECAM-1, CD146, CD41, CD34, PSMA, CD105, CD309, CD144, CD202B and Angiopoietin 2; wherein optionally

the detectable label is selected from the group consisting of a fluorescent group, a radioisotope, a stable isotope, an enzymatic group, a chemiluminescent group and a biotinyl group.

18. The method of claim 15, further comprising the steps of:

(b) lysing the cells from step (a);

(c) contacting the lysed cell sample from step (b) with a reverse primer from a first primer pair, the reverse primer from the first primer pair being directed to a target RNA region, and a reverse transcriptase to effect reverse transcription of the RNA into cDNA;

(d) subsequently contacting the sample from step (c) with:

(i) a forward primer from the first primer pair, the forward primer from the first primer pair being directed to a target cDNA region,

(ii) a reverse primer and a forward primer from a second primer pair, the reverse primer and forward primer from the second primer pair being directed to a target DNA region, and

(iii) a DNA polymerase

to simultaneously amplify the target cDNA region and the target DNA region in a pre-amplification step; and

(e) analyzing the amplified target cDNA region and/or the amplified target DNA region.

19. The method of claim 18, further comprising: subjecting the sample from step (d) to a semi-nested PCR using the reverse primer in step (c) or the forward primer in step (d)(i), and a nested primer that binds within the amplified target cDNA region, or subjecting the sample from step (d) to a nested PCR using a nested primer pair that binds within the amplified target DNA region; wherein optionally steps (c) and (d) are conducted in the same reaction mixture; wherein optionally the analysis in step (e) comprises analyzing the amplified target cDNA for

gene expression; and wherein optionally the analysis in step (e) comprises analyzing the amplified target cDNA for mutations.

20. The method of any one of claims 4-5, and 18-19, wherein the first or the second primer pair is selected from the group consisting of: (i) SEQ ID NO: 1 and SEQ ID NO: 2; (ii) SEQ ID NO: 3 and SEQ ID NO: 4; (iii) SEQ ID NO: 5 and SEQ ID NO: 6; (iv) SEQ ID NO: 7 and SEQ ID NO: 8; (v) SEQ ID NO: 9 and SEQ ID NO: 10; (vi) SEQ ID NO: 11 and SEQ ID NO: 12; (vii) SEQ ID NO: 13 and SEQ ID NO: 14; (viii) SEQ ID NO: 15 and SEQ ID NO: 16; (ix) SEQ ID NO: 17 and SEQ ID NO: 18; (x) SEQ ID NO: 19 and SEQ ID NO: 20; (xi) SEQ ID NO: 21 and SEQ ID NO: 22; (xii) SEQ ID NO: 23 and SEQ ID NO: 24; (xiii) SEQ ID NO: 25 and SEQ ID NO: 26; (xiv) SEQ ID NO: 27 and SEQ ID NO: 28; (xv) SEQ ID NO: 29 and SEQ ID NO: 30; (xvi) SEQ ID NO: 31 and SEQ ID NO: 32; (xvii) SEQ ID NO: 33 and SEQ ID NO: 34; (xviii) SEQ ID NO: 52 and 53; and (xix) SEQ ID NO: 54 and 55; wherein optionally

the primer pair for semi-nested or nested PCR is selected from the group consisting of: (i) SEQ ID NO: 35 and SEQ ID NO: 2; (ii) SEQ ID NO: 3 and SEQ ID NO: 36; (iii) SEQ ID NO: 5 and SEQ ID NO: 37; (iv) SEQ ID NO: 38 and SEQ ID NO: 8; (v) SEQ ID NO: 39 and SEQ ID NO: 10; (vi) SEQ ID NO: 40 and SEQ ID NO: 12; (vii) SEQ ID NO: 41 and SEQ ID NO: 14; (viii) SEQ ID NO: 42 and SEQ ID NO: 16; (ix) SEQ ID NO: 17 and SEQ ID NO: 43; (x) SEQ ID NO: 44 and SEQ ID NO: 20; (xi) SEQ ID NO: 45 and SEQ ID NO: 22; (xii) SEQ ID NO: 46 and SEQ ID NO: 24; (xiii) SEQ ID NO: 25 and SEQ ID NO: 47; (xiv) SEQ ID NO: 27 and SEQ ID NO: 48; (xv) SEQ ID NO: 49 and SEQ ID NO: 30; (xvi) SEQ ID NO: 31 and SEQ ID NO: 50; (xvii) SEQ ID NO: 51 and SEQ ID NO: 34; (xviii) SEQ ID NO: 56 and SEQ ID NO: 57; and (xix) SEQ ID NO: 58 and SEQ ID NO: 55.

21. A kit for use in the method of any one of claims 6-10, and 13-20, the kit comprising:

(a) the apparatus of claim 11 or 12.

22. The kit of claim 21, further comprising one or more of the following:

(b) one or more cell lysis buffers;

(c) a primer selected from the group consisting of:

i. the reverse primer of step (c) of the method of any one of claims 18-20

ii. the forward primer of step (d)(i) of the method of any one of claims 18-20

iii. the primer pair of step (d)(ii) of the method of any one of claims 18-20

iv. the nested primer and nested primer pair of the method of any one of claims 18-20

(d) one or more reagents, selected from the group consisting of:

i. a reverse transcriptase and one or more suitable reaction buffers for the reverse transcription in step (c) of the method of any one of claims 18-20

ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (d) of the method of any one of claims 18-20 or the semi-nested or nested PCR of the method of claim 19 or 20, and

iii. one or more labelled or unlabelled deoxyribonucleotides selected from the group consisting of dATP, dCTP, dGTP, and dTTP or dUTP; and

(e) an antibody capable of specific binding to a protein selected from the group consisting of PAI-1, Vimentin, FOXC1, keratin-8, keratin-18, keratin-19, Ep-CAM, CD45, VWF, PECAM-1, CD146, CD41, CD34, PSMA, CD105, CD309, CD144, CD202B and Angiopoietin 2, wherein the antibody is coupled to a detectable label; and optionally means for detecting the detectable label.

23. A kit for use in the method of any one of claims 3-10, 17 and 20, the kit comprising one of more of the following:

(a) one or more cell lysis buffers;

(b) a primer selected from the group consisting of:

i. the reverse primer of step (b) of the method of any one of claims 4-5, and 20,

ii. the forward primer of step (c)(i) of the method of any one of claims 4-5, and 20,

iii. the primer pair of step (c)(ii) of the method of any one of claims 4-5, and 20, and

iv. the nested primer and nested primer pair of the method of any one of claims 4-5, and 20;

(c) one or more reagents, selected from the group consisting of:

i. a reverse transcriptase and one or more suitable reaction buffers for the reverse transcription in step (b) of the method of any one of claims 4-5, and 20,

ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (d) of the method of any one of claims 4-5, and 20 or the semi-nested or nested PCR of the method of any one of claims 4-5, and 20, and

iii. one or more labelled or unlabelled deoxyribonucleotides selected from the group consisting of dATP, dCTP, dGTP, and dTTP or dUTP; and

(d) an antibody capable of specific binding to a protein selected from the group consisting of PAI-1, Vimentin, FOXC1, keratin-8, keratin-18, keratin-19, Ep-CAM, CD45, VWF, PECAM-1, CD146, CD41, CD34, PSMA, CD105, CD309, CD144, CD202B and Angiopoietin 2, wherein the antibody is coupled to a detectable label; and optionally means for detecting the detectable label.

24. The kit of claim 22 or 23, wherein the primers and/or reagents are pre-mixed in combinations suitable for the lysis, pre-amplification, and amplification steps according to any one of claims 4-5, and 18-20; wherein optionally

the primers are pre-mixed in combinations suitable for analysis of gene expression profiles or mutation signatures.

25. The kit of any one of claims 21-24, further comprising instructions for performing the method of any one of claims 3-10 and 15-20; wherein optionally

the kit comprises one or more containers comprising one or more reaction buffers for performing the methods of any one of claims 3-10 and 15-20.