[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 example, 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 example, 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 example, there is provided 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.

[0010] In a fourth example, there is provided a method for detecting the isolated cell population as described herein in a sample of a subject, the method comprising:

(a) capturing and retrieving the cells from the sample using the apparatus as described herein or the method as described herein.

[0011] In another example, the method of the fourth example further comprises 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.

[0012] In another example, the method of the fourth example further comprises 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.

[0013] In another example, the method of the fourth example further comprises: 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.

[0014] In yet another example, the method of the fourth example further comprises: subjecting the sample from step (d) to a nested PCR using a nested primer pair that binds within the amplified target DNA region.

[0015] In a fifth example, there is provided a method for detecting the isolated cell population of the third example 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.

[0015a] In another example, there is provided a method for detecting an isolated cell population having the following characteristics:

(i) being endothelial cells derived from blood vessel of 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,

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.

[0016] In a sixth example, there is provided a method for detecting the isolated cell population of the third example 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.

[0016a] In another example, there is provided a method for detecting an isolated cell population having the following characteristics:

(i) being endothelial cells derived from blood vessel of 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,

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.

[0017] In another example, the method of the sixth example further comprises: 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.

[0018] In yet another example, the method of the sixth example further comprises: subjecting the sample from step (c) to a nested PCR using a nested primer pair that binds within the amplified target DNA region.

[0019] In a seventh example, there is provided a method of diagnosing a cancer in a subject, comprising analyzing a sample from the subject for presence of the isolated population of cells as described herein, wherein presence of the isolated population of cells indicates that the subject has cancer.

[0019a] In one aspect, the present disclosure refers to a method of diagnosing a colorectal cancer in a subject, comprising analyzing a sample from the subject for presence of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;
(ii) each cell cluster having at least two clearly distinct nuclei;
(iii) each cell cluster having a major axis of greater than about 10 μm;
(iv) expression of endothelial cell genes or proteins, comprising *FOLH1*;
(v) non-expression of leukocyte-specific genes or proteins;

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and
(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,
wherein presence of the isolated population of cell clusters indicates that the subject has cancer.

[0020] In an eighth example, there is provided 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 as described herein, 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.

[0020a] In another aspect, the present disclosure refers to a method for monitoring and/or predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the patient after treatment for determining the number of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;
(ii) each cell cluster having at least two clearly distinct nuclei;
(iii) each cell cluster having a major axis of greater than about 10 μm;
(iv) expression of endothelial cell genes or proteins comprising *FOLH1*;
(v) non-expression of leukocyte-specific genes or proteins;
(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and
(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,

wherein a reduction in the number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a baseline sample obtained from the patient prior to treatment indicates that the patient is responding positively to the treatment.

[0021] In a ninth example, there is provided 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 as described herein, 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.

[0021a] In another aspect, the present disclosure refers to a method for predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the cancer patient before treatment for determining the number of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;

(ii) each cell cluster having at least two clearly distinct nuclei;

(iii) each cell cluster having a major axis of greater than about 10 μm;

(iv) expression of endothelial cell genes or proteins comprising *FOLH1*;

(v) non-expression of leukocyte-specific genes or proteins;

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and

(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,

wherein an equal or higher number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond positively to the treatment, and wherein a lower number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond negatively to the treatment.

[0022] In a tenth example, there is provided 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 as described herein, 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.

[0022a] In another aspect, the present disclosure refers to a method for analyzing blood vessel characteristics of a colorectal tumor in a subject, the method comprising analyzing a sample from the subject for determining the number of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;

(ii) each cell cluster having at least two clearly distinct nuclei;

(iii) each cell cluster having a major axis of greater than about 10 μm;

(iv) expression of endothelial cell genes or proteins comprising *FOLH1*;

(v) non-expression of leukocyte-specific genes or proteins;

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and

(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,

wherein an increased number of the isolated population of cell clusters compared to a baseline sample indicates that the colorectal tumor has larger blood vessels compared to the baseline sample, and wherein a reduced number of the isolated population of cell clusters compared to a baseline sample indicates that the colorectal tumor has smaller blood vessels compared to the baseline sample.

[0023] In an eleventh example, there is provided a kit for use in the method of the second, the fourth, the seventh, the eighth, the ninth or the tenth examples, the kit comprising:

(a) the apparatus as described herein.

[0024] In another example, the kit of the eleventh embodiment further comprises 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 the fourth embodiment,

ii. the forward primer of step (d)(i) of the method of the fourth embodiment,

iii. the primer pair of step (d)(ii) of the method of the fourth embodiment, and

iv. the nested primer and nested primer pair of the method of the fourth embodiment;

(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 the fourth embodiment,

ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (d) of the method of the fourth embodiment or the semi-nested or nested PCR of the method of the fourth embodiment, 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.

[0025] In a twelfth example, there is provided a kit for use in the method of the fifth, the sixth, the seventh, the eighth, the ninth or the tenth examples, the kit comprising one or 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 the fifth example,

ii. the forward primer of step (c)(i) of the method of the fifth example,

iii. the primer pair of step (c)(ii) of the method of the fifth example, and

iv. the nested primer and nested primer pair of the method of the fifth example;

(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 the fifth example,

ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (c) of the method of the fifth example or the semi-nested or nested PCR of the method of the fifth example, 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 as described herein; and optionally means for detecting the detectable label.

[0025a] In another example, there is provided a kit for use in the method of any one of the aspects described herein, 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 paragraph [0016a],

ii. the forward primer of step (c)(i) of the method of paragraph [0016a],

iii. the primer pair of step (c)(ii) of the method of paragraph [0016a], and

iv. the nested primer and nested primer pair of the method of paragraph [0016a];

(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 paragraph [0016a],

ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (d) of the method of paragraph [0016a]or the semi-nested or nested PCR of the method of paragraph [0016a], 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.

[0026] In another example, the kit of the eleventh or the twelfth example further comprises instructions for performing the methods as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

Claims:

1. A method of diagnosing a colorectal cancer in a subject, comprising analyzing a sample from the subject for presence of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;

(ii) each cell cluster having at least two clearly distinct nuclei;

(iii) each cell cluster having a major axis of greater than about 10 μm;

(iv) expression of endothelial cell genes or proteins, comprising *FOLH1*;

(v) non-expression of leukocyte-specific genes or proteins;

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and

(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,

wherein presence of the isolated population of cell clusters indicates that the subject has colorectal cancer.

2. The method of claim 1, wherein the endothelial cell genes are further 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. The method of claim 1 or 2, 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.

4. A method for monitoring and/or predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the patient after treatment for determining the number of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;

(ii) each cell cluster having at least two clearly distinct nuclei;

(iii) each cell cluster having a major axis of greater than about 10 μm;

(iv) expression of endothelial cell genes or proteins comprising *FOLH1*;

(v) non-expression of leukocyte-specific genes or proteins;

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and

(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,

wherein a reduction in the number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a baseline sample obtained from the patient prior to treatment indicates that the patient is responding positively to the treatment.

5. A method for predicting the response to treatment of a colorectal cancer patient, the method comprising analyzing a sample obtained from the cancer patient before treatment for determining the number of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;

(ii) each cell cluster having at least two clearly distinct nuclei;

(iii) each cell cluster having a major axis of greater than about 10 μm;

(iv) expression of endothelial cell genes or proteins comprising *FOLH1*;

(v) non-expression of leukocyte-specific genes or proteins;

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and

(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,

wherein an equal or higher number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond positively to the treatment, and wherein a lower number of the isolated population of cell clusters compared to the number of the isolated population of cell clusters in a sample obtained before treatment from a patient or a group of patients that have responded positively to the treatment indicates that the colorectal cancer patient will respond negatively to the treatment .

6. A method for analyzing blood vessel characteristics of a colorectal tumor in a subject, the method comprising analyzing a sample from the subject for determining the number of an isolated population of cell clusters, each cell cluster comprising at least two cells and having the following characteristics:

(i) being endothelial cell clusters derived from blood vessel of a colorectal tumor and isolated from blood;

(ii) each cell cluster having at least two clearly distinct nuclei;

(iii) each cell cluster having a major axis of greater than about 10 μm;

(iv) expression of endothelial cell genes or proteins comprising *FOLH1*;

(v) non-expression of leukocyte-specific genes or proteins;

(vi) non-expression of megakaryocyte or platelets-specific genes or proteins; and

(vii) being cytomorphologically consistent with malignancy but not having primary tumor mutations or chromosomal abnormalities,

wherein an increased number of the isolated population of cell clusters compared to a baseline sample indicates that the colorectal tumor has larger blood vessels compared to the baseline sample, and wherein a reduced number of the isolated population of cell clusters

compared to a baseline sample indicates that the colorectal tumor has smaller blood vessels compared to the baseline sample.

7. The method of any one of claims 4 to 6, wherein the endothelial cell genes are further 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*.