

CLAIMS

1. An *in vitro* method for determining and/or monitoring at least one condition of/in a three dimensional cell culture system (2) comprising at least one growth section (3),
5 wherein the at least one condition comprises a physiological condition and further comprises a condition selected from the group consisting of
 - (i) vitality, and
 - (ii) metabolism status,wherein the physiological condition is determined using erythrocytes as detectors for
10 said condition by determining the flow rate, physiological osmolality, and/or oxygen consumption in the three dimensional cell culture system (2), the vitality is determined and/or monitored by measuring living cell fluorescent dyes, and the metabolism status is determined and/or monitored by measuring the autofluorescence of NADH and/or FAD, and/or by determining the NADH/NAD⁺ and/or NADH/FAD ratio.
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2. The method of claim 1, wherein the method for determining the flow rate in the three dimensional cell culture system (2) using erythrocytes as detectors comprises the steps of:
 - (i) providing a perfusable three dimensional cell culture system (2) comprising at
20 least one growth section (3),
 - (ii) taking pictures of erythrocytes in said cell culture system at least at two different time points,
 - (iii) selecting at least one erythrocyte and determining the position of the at least one erythrocyte on the at least two pictures,
 - 25 (iv) assigning a motion vector to the at least one erythrocyte representing the positional change of said at least one erythrocyte on the at least two pictures, and
 - (v) determining the flow rate in said cell culture system on the basis of the motion vector.
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3. The method of claims 1 to 2, wherein the method for determining the physiological osmolality in the three dimensional cell culture system (2) using erythrocytes as detectors comprises the steps of:

- (i) providing the three dimensional cell culture system (2) comprising at least one growth section (3), and
- (ii) determining the shape of at least one erythrocyte in said cell culture system at a time point during culturing of said cell culture system.

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4. The method of claim 3, wherein

- (i) a spherical shape of the at least one erythrocyte indicates that the physiological osmolality is decreased (hypotonic condition), or
- (ii) a crenate shape of the at least one erythrocyte indicates that the physiological osmolality is increased (hypertonic condition), or
- (iii) a biconcave shape of the at least one erythrocyte indicates that the physiological osmolality is stable (isotonic condition).

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5. The method of claims 1 to 4, wherein the method for determining the oxygen consumption in the three dimensional cell culture system (2) using erythrocytes as detectors comprises the steps of:

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- (i) providing the three dimensional cell culture system (2) comprising at least one growth section (3),
- (ii) determining an average haemoglobin saturation of erythrocytes at a first position and at a second position in said cell culture system, and
- (iii) calculating the oxygen consumption in said cell culture system on the basis of the average haemoglobin saturation at the different positions.

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6. The method of claims 3 to 5, wherein the three dimensional cell culture system (2) is perfusable.

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7. The method of claim 1, wherein the method for determining and/or monitoring the vitality of the three dimensional cell culture system (2) by measuring living cell fluorescent dyes comprises the steps of:

- (i) providing the three dimensional cell culture system (2) comprising at least one growth section (3) which is loaded with living cell fluorescent dyes, and
- (iia) measuring the average fluorescence intensity in at least a part of the at least one growth section (3) of said cell culture system at a first time point and at a second time point during culturing of said cell culture system, and comparing

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- the average fluorescence intensity at the second time point with the average fluorescence intensity at the first time point, and/or
- (iib) measuring the average fluorescence intensity in at least a part of the at least one growth section (3) of said cell culture system at a time point during culturing of said cell culture system, and comparing the average fluorescence intensity at said time point with the average fluorescence intensity of at least one control cell culture system.
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8. The method of claim 7, wherein
- 10 (i) a decrease of the average fluorescence intensity at the second time point when compared to the average fluorescence intensity at the first time point indicates that the vitality of said cell culture system (2) is decreased, or
- (ii) a retention of the average fluorescence intensity at the second time point when compared to the average fluorescence intensity at the first time point indicates that the vitality of said cell culture system (2) is maintained.
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9. The method of claim 7 or 8, wherein
- (i) an increase of the average fluorescence intensity at said time point compared to the average fluorescence intensity of the control cell culture system indicates that said cell culture system (2) is vital, or
- 20 (ii) the comparability of the average fluorescence intensity at said time point with the average fluorescence intensity of the control cell culture system indicates that said cell culture system (2) is not vital.
- 25 10. The method of claims 1 to 9, wherein the at least one growth section (3) comprises a multilayer cell assembly, a tissue, an organoid, or an organ.