

## CLAIMS

1. A method of monitoring one or more cell aggregates, the method comprising:
  - moving a plurality of cell aggregates in a channel along a flow path, wherein the flow path defines a closed recirculating flow of the plurality of cell aggregates in a loop configured for dynamic monitoring of the plurality of cell aggregates, the plurality of cell aggregates in the channel being in a medium, the flow path being configured to pass through a collective sensing zone of a set of electrodes;
  - obtaining impedance-related signals from the collective sensing zone, the impedance-related signals corresponding to each of the medium and one of the plurality of cell aggregates in the medium;
  - determining one or more electrical signatures for each of the plurality of cell aggregates, wherein the one or more electrical signatures are based on the impedance-related signals obtained from the set of electrodes; and
  - determining a state or a change in the state of each of the plurality of cell aggregates based on the one or more electrical signatures, wherein the state includes a physiological and/or morphological state of each of the plurality of cell aggregates.
2. The method according to claim 1, wherein the one or more electrical signatures are determined at single-particle resolution.
3. The method according to claim 1 or claim 2, wherein the flow path is configured such that the plurality of cell aggregates are in a continuous flow mode in the collective sensing zone concurrently with the obtaining of the impedance-related signals.
4. The method according to any one of claims 1 to 3, wherein the impedance-related signals are obtained when the plurality of cell aggregates are in motion relative to the set of electrodes.
5. The method according to any one of claims 1 to claim 4, further comprising: returning the plurality of cell aggregates to a reservoir after the plurality of cell aggregates have passed through the collective sensing zone.

6. The method according to any one of claims 1 to 5, wherein the one or more electrical signatures is one or more of an opacity of one or more of the plurality of cell aggregates and an electrical size of the one or more of the plurality of cell aggregates.
7. The method according to claim 6, wherein the opacity is a ratio between a first impedance and a second impedance, and wherein the first impedance is obtained at a higher frequency than the second impedance.
8. The method according to claim 7, wherein the first impedance and the second impedance are obtained at a beta dispersion range of frequencies.
9. The method according to claim 7, wherein a second frequency is selected from a range of frequencies corresponding to impedance-related signals of the medium such that the second frequency corresponds to a minimum electrical double layer (EDL) effect.
10. The method according to claim 6, wherein the electrical size is determined based on the second impedance.
11. The method according to any one of claims 1 to 10, wherein the one or more electrical signatures are EIS-based (electrical impedance spectroscopy-based) electrical signatures.
12. The method according to claim 11, wherein the one or more EIS-based electrical signatures are obtained from differential values between impedance-related signals measured at a first electrode and a second electrode, wherein the first electrode and the second electrode are selected from the set of electrodes.
13. The method according to claim 12, wherein the cell aggregate is in one of a first sensing zone and a second sensing zone, the first sensing zone being provided by the first electrode and a source electrode, the second sensing zone being provided by the second electrode and the source electrode, and wherein the first sensing zone and the second sensing zone form the collective sensing zone.

14. The method according to any one of claims 6 to 13, further comprising:  
determining a change in an optical size of the one or more of the plurality of cell aggregates based on the one or more electrical signatures.
15. The method according to any one of claims 6 to 13, further comprising:  
determining a change in a cell count of the one or more of the plurality of cell aggregates based on the one or more electrical signatures.
16. The method according to any one of claims 6 to 13, further comprising:  
determining a cell aggregate growth of the one or more of the plurality of cell aggregates based on the one or more electrical signatures.
17. The method according to any one of claims 6 to 13, further comprising:  
determining a change in cell viability of the one or more of the plurality of cell aggregates based on the one or more electrical signatures.
18. The method according to any one of claims 6 to 13, further comprising:  
determining a change in health of the one or more of the plurality of cell aggregates based on the one or more electrical signatures.
19. The method according to any one of claims 6 to 13, further comprising:  
determining a cytotoxicity response of the one or more of the plurality of cell aggregates based on the one or more electrical signatures.
20. The method according to any one of claims 6 to 13, further comprising:  
determining a drug efficacy with respect to the one or more of the plurality of cell aggregates based on the one or more electrical signatures.
21. The method according to any one of claims 6 to 20, the method comprising:  
determining if the one of the plurality of cell aggregates has increased in its optical size based on whether there is an increase in the electrical size and whether there is concurrently a relatively small change or no change in the opacity.

22. The method according to any one of claims 6 to 20, the method comprising:  
determining if cell proliferation has occurred in the one of the plurality of cell aggregates based on whether there is a decrease in the opacity and whether there is concurrently an increase in the electrical size.
23. The method according to any one of claims 6 to 20, the method comprising:  
determining a decrease in cell viability based on whether there is an increase in the opacity and whether concurrently there is a decrease or no change in the electrical size, wherein the cell viability corresponds to one or more of the following: a decrease in a proportion of living cells, an increase in a proportion of dead cells, and a deterioration in a health of the one of the plurality of cell aggregates.
24. The method according to any one of claims 6 to 20, the method comprising:  
determining drug susceptibility with respect to the one of the plurality of cell aggregates based on whether there is a decrease in electrical size concurrent with a relatively small decrease or no change in the opacity.
25. The method according to any one of the preceding claims, wherein any one of the plurality of cell aggregates is a spheroid, an encapsulated microcarrier, or a cell-adhered microcarrier.
26. A system comprising:  
at least one reservoir configured to culture a plurality of cell aggregates;  
at least one monitoring device having a set of electrodes configured to define a collective sensing zone; and  
a flow path defining a closed recirculating flow of the plurality of cell aggregates in a channel between one of the at least one reservoir and the collective sensing zone of a corresponding one of the at least one monitoring device, wherein the at least one monitoring device is configured to monitor the plurality of cell aggregates in the channel according to the method of any one of the preceding claims.

27. A microfluidic chip comprising:

a channel;

a source electrode excitable at a frequency; and

a first electrode spaced apart from the source electrode, the source electrode and the first electrode being disposed in the channel and configured to provide at least part of a collective sensing zone, wherein the channel is configured to enable a plurality of cell aggregates to move through the collective sensing zone in turn to be monitored according to the method as claimed in any one of claims 1 to 25.

28. The microfluidic chip of claim 27, further comprising:

a second electrode disposed in the channel and spaced apart from the source electrode, wherein in operation, the first electrode and the second electrode are spaced apart along the channel to provide a differential value based on impedance-related signals obtainable at the first electrode and the second electrode.

29. A method making the microfluidic chip of claim 27, the method comprising:

detachably sealing one end of each of a plurality of slots defined in an electrode layer with an aluminum foil under an electrode layer;

filling each slot with a flowable metal to form a set of electrodes in the electrode layer;

separating the aluminum foil from the electrode layer after solidification of the flowable metal; and

assembling the electrode layer with a channel layer to form a channel such that the set of electrodes are disposed in the channel.