

A further method dealing with cell-cell interactions is disclosed in WO 2016/046346. The method involves determining the number of cells that are in contact with a further cell irrespective of the number of cells that a cell is in contact with.

In view of the foregoing, there is a need for means and methods that allow high-throughput and cost-efficient determination of cell-cell interactions in biological samples, in particular samples comprising PBMCs or bone-marrow cells.

Thus, the technical problem underlying the present invention is the provision of improved means and methods for determining the propensity of cells to interact.

The technical problem is solved by provision of the embodiments characterized in the claims.

Summary

In one aspect, the present disclosure refers to a method for determining the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells comprising at least two distinguishable subpopulations of cells, wherein the method comprises the following steps: (a) determination of the number of total interactions between cells of a-the first distinguishable subpopulation and cells of a-the second distinguishable subpopulation; (b) determination of the number of total interactions in the population of cells by randomly assigning cells comprised in the population of cells to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells, respectively, are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a); and (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number.

In another aspect, the present disclosure refers to a method for diagnosing a disease or predisposition to a disease of a cell donor, wherein the method comprises the determination of the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells obtained from the donor, wherein the population of cells comprises at least two distinguishable

subpopulations of cells and wherein the method comprises the following steps: (a) determination of the number of total interactions between cells of ~~a~~-the first distinguishable subpopulation and cells of ~~a~~-the second distinguishable subpopulation; (b) determination of the number of total interactions in the population of cells by randomly assigning cells comprised in the population of cells to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells, respectively, are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a); (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number; and (d) determination whether the cell donor suffers from a disease or is predisposed to suffer from a disease based on the propensity of cells to interact.

In yet another aspect, the present disclosure refers to a method for determining whether a subject suffering from or predisposed to a disease will respond or is responsive to treatment with a therapeutic agent by determining the alteration of the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells obtained from the subject, wherein the population of cells comprises at least two distinguishable subpopulations of cells and wherein the method comprises the following steps: (a) determination of the number of total interactions between cells of ~~a~~-the first distinguishable subpopulation and cells of ~~a~~-the second distinguishable subpopulation; (b) determination of the number of total interactions in the cell sample by randomly assigning cells comprised in the population of cells to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a); (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number; and (d) determination of the alteration of the propensity of cells to interact by comparing the propensity prior to addition of a therapeutic agent and subsequent to addition of a therapeutic agent and thereby determining whether the subject will respond or is responsive to treatment with the therapeutic agent.

In yet another aspect, the present disclosure refers to a method for screening of a therapeutic agent by determining the alteration of the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells due to addition of one or more test substance(s), wherein the population of cells comprises at least two distinguishable subpopulations of cells and wherein the method comprises the following steps: (a)

determination of the number of total interactions between cells of ~~a~~the first distinguishable subpopulation and cells of ~~a~~the second distinguishable subpopulation; (b) determination of the number of total interactions in the cell sample by randomly assigning cells comprised in the cell sample to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells, respectively, are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a); (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number; and (d) determination of the alteration of the propensity of cells to interact by comparing the propensity prior to addition of one or more test substance(s) and subsequent to addition of one or more test substance(s) and thereby determining whether the one or more test substance(s) qualify as therapeutic agent.

Accordingly, the present invention relates to methods for determining the propensity of cells to interact in a population of cells comprising at least two distinguishable subpopulations of cells, wherein the method comprises the following steps (a) determination of the number of interactions between cells of a first distinguishable subpopulation and cells of a second distinguishable subpopulation; (b) determination of the number of interactions in the population of cells by randomly assigning cells comprised in the population of cells to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells, respectively, are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a); and (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number. In a preferred embodiment, step (b) is repeated

and the determined number of interactions is averaged, preferably wherein step (b) is repeated more than at least 1000 times, at least 2000 times or at least 3000 times.

As shown in the appended Examples, the methods of the present invention, inter alia, provide a more robust, more accurate and, thus, improved measure of cell-cell interactions in biological settings. Methods of the prior art, such as those described in

Claims

1. Method for determining the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells comprising at least two distinguishable subpopulations of cells, wherein the method comprises the following steps:
 - (a) determination of the number of total interactions between cells of a—the first distinguishable subpopulation and cells of a—the second distinguishable subpopulation;
 - (b) determination of the number of total interactions in the population of cells by randomly assigning cells comprised in the population of cells to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells, respectively, are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a); and
 - (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number.
2. A method for diagnosing a disease or predisposition to a disease of a cell donor, wherein the method comprises the determination of the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells obtained from the donor, wherein the population of cells comprises at least two distinguishable subpopulations of cells and wherein the method comprises the following steps:
 - (a) determination of the number of total interactions between cells of a—the first distinguishable subpopulation and cells of a—the second distinguishable subpopulation;
 - (b) determination of the number of total interactions in the population of cells by randomly assigning cells comprised in the population of cells to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable

subpopulation of cells, respectively, are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a);

- (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number; and
- (d) determination whether the cell donor suffers from a disease or is predisposed to suffer from a disease based on the propensity of cells to interact.

3. A method for determining whether a subject suffering from or predisposed to a disease will respond or is responsive to treatment with a therapeutic agent by determining the alteration of the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells obtained from the subject, wherein the population of cells comprises at least two distinguishable subpopulations of cells and wherein the method comprises the following steps:

- (a) determination of the number of total interactions between cells of a—the first distinguishable subpopulation and cells of a—the second distinguishable subpopulation;
- (b) determination of the number of total interactions in the cell sample by randomly assigning cells comprised in the population of cells to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a);
- (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number; and
- (d) determination of the alteration of the propensity of cells to interact by comparing the propensity prior to addition of a therapeutic agent and subsequent to addition of a therapeutic agent and thereby determining

whether the subject will respond or is responsive to treatment with the therapeutic agent.

4. A method for screening of a therapeutic agent by determining the alteration of the propensity of cells in a first distinguishable subpopulation to interact with cells of a second distinguishable subpopulation in a population of cells due to addition of one or more test substance(s), wherein the population of cells comprises at least two distinguishable subpopulations of cells and wherein the method comprises the following steps:
 - (a) determination of the number of total interactions between cells of a—the first distinguishable subpopulation and cells of a—the second distinguishable subpopulation;
 - (b) determination of the number of total interactions in the cell sample by randomly assigning cells comprised in the cell sample to first and second distinguishable subpopulation of cells, wherein the absolute number of cells in the first and second distinguishable subpopulation of cells, respectively, are identical to the number of cells in the first and second distinguishable subpopulation of cells in (a);
 - (c) determination of the propensity of cells to interact by dividing (a) through (b), wherein propensity increases with the resulting number; and
 - (d) determination of the alteration of the propensity of cells to interact by comparing the propensity prior to addition of one or more test substance(s) and subsequent to addition of one or more test substance(s) and thereby determining whether the one or more test substance(s) qualify as therapeutic agent.
5. The method of any one of claims 1 to 4, wherein the first and second distinguishable subpopulations are of the same cell type but can be distinguished by differing expression profiles of cell markers in each subpopulation.

6. The method of any one of claims 1 to 5, wherein step (b) is repeated and the determined number of interactions is averaged, preferably wherein step (b) is repeated more than at least 1000 times.
7. The method of any one of claims 1 to 6, wherein the cells are (a) peripheral blood mononuclear cells (PBMCs) or (b) bone marrow cells.
8. The method of ~~any one of~~ claims ~~1-2 to 7~~ or 3, wherein the disease is a myeloproliferative disorder, inflammatory disorder, latent virus infection, cellular growth disorder, cellular chemotaxis disorder, metabolic disorder or autoimmune disorder.
9. The method of claim 8, wherein the disease is leukemia or lymphoma.
10. The method of any one of claims 1 to 9, wherein the cells are present in form of a monolayer.