A fluidic apparatus and/or method for differentiating viable cells

Field of the invention

The present invention relates to the detection of microorganisms, for example, in environmental sampling, food and water safety, pathogen detection and disease control. The invention in particular relates to the detection and/or differentiation of viable and dead cells.

Background of the invention

The differentiation between viable and dead cells presents an important challenge in many applications, for example microbial diagnostics. In the case of pathogenic microorganisms, it is important to identify viable cells as these cells are metabolically active and/or reproductive with the potential to infect and cause diseases.

Methods for differentiating viable and dead cells include cell culture followed by identification (e.g. microscopic identification), animal infectivity models or flow cytometry. These methods can be time consuming. Another method is the use of flurogenic dyes such as 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) and propidium iodide together with microscopy which is also time consuming as well as labour intensive.

More recently, molecular biology techniques such as DNA diagnostics have been developed which can detect the presence of microorganisms rapidly. However, DNA persists in a sample after the death of a cell and can still be detected by such techniques. Techniques exploiting the ability of compounds to enter and bind to DNA of membrane-compromised cells have been used to increase the sensitivity of differentiating viable intact cells in conjunction with DNA diagnostics.

It is desirable to improve on the available technology.

Summary of the invention

In general terms, the invention provides a fluidic apparatus for a reaction for differentiating viable and dead or membrane compromised cells in a sample and a method for performing the same in a fluidic apparatus.

According to a first aspect, the present invention provides a fluidic apparatus for a reaction for differentiating viable cells, comprising a cartridge comprising:

(a) a substantially optically transparent reaction chamber configured to expose to a light source a sample comprising cells and a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule and covalently binding to the nucleic acid molecule on exposure to a light source;

(b) at least one inlet in fluid communication with the reaction chamber; and

(c) at least one outlet in fluid communication with the reaction chamber;

and at least one light source, the reaction chamber being configured such that in use it will receive light transmitted from the at least one light source.

According to another aspect, the present invention provides a method for differentiating viable cells comprising one of:

(a) (i) introducing a sample comprising cells through an inlet into a reaction chamber of a fluidic cartridge; and

(ii) introducing into the reaction chamber a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at

least one nucleic acid molecule and covalently binding to at least one nucleic acid molecule on exposure to a light source; allowing the sample and phenanthridium compound to combine to form a reaction sample;

or

(b) (i) allowing a sample comprising cells to combine with a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule and covalently binding to at least one nucleic acid molecule on exposure to a light source to form a reaction sample; and

(ii) introducing the reaction sample through an inlet into a reaction chamber of a fluidic cartridge; followed by

(iii) exposing the reaction sample in the reaction chamber to a light source configured to transmit light to the reaction chamber to covalently bind the phenanthridium compound to the nucleic acid molecule(s) from the dead or membrane-compromised cells to form a reacted sample; and

(iv) allowing the reacted sample to exit from the reaction chamber through an outlet.

Either step a(i) may be performed before step a(ii) or step a(ii) may be performed before step a(i).

The details of one or more non-limiting embodiments of the invention are set forth in the accompanying figures and further description. Other features and advantages of the drawings may be better and more completely understood from the description, drawings and claims.

Brief description of the figures

Figure 1 shows an exemplary embodiment of the fluidic apparatus.

Figure 2 shows an exemplary embodiment of a cartridge.

Figure 3 shows an exemplary embodiment of a cartridge with a straight channel first loaded with the phenanthridium compound. Figure 3(a) illustrates the top view and Figure 3(b) illustrates the side view of the cartridge.

Figure 4 shows an exemplary embodiment of a cartridge and method wherein the phenanthridium compound and the sample may be mixed to form a reaction sample and introduced together into the cartridge. Figure 4(a) illustrates the top view and (b) illustrates the side view of the cartridge.

Figure 5 shows an exemplary embodiment of the cartridge with a straight channel and a first inlet for the sample and a second inlet for the phenanthridium compound.

Definitions

Reaction sample refers to a sample to undergo or undergoing a transformation or reaction.

Reacted sample refers to a sample which has undergone a substantially completed transformation or reaction.

Detailed description of the invention

The fluidic apparatus of the present invention may be a continuous flow apparatus which may be automated. For example, the fluidic apparatus may further comprise at least one processor for controlling the light source and/or fluid flow. The processor may be configured to control at least one of: the light source and fluid flow through the cartridge.

In addition to the design of the channels, fluid flow through the fluidic apparatus may be controlled by at least one valve. The opening and closing of the valves may be controlled by a processor.

The cartridge of the fluidic apparatus comprises:

(a) a substantially optically transparent reaction chamber configured to expose to a light source a sample comprising cells and a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule and covalently binding to the nucleic acid molecule on exposure to a light source;

(b) at least one inlet in fluid communication with the reaction chamber; and

(c) at least one outlet leading outlet in fluid communication with the reaction chamber.

For example, the light source may be a light emitting diode (LED). The light source may emit blue light to enable the reaction to occur.

As illustrated in the exemplary embodiment shown in Figure 1, the sample may be introduced into the reaction chamber 5 of cartridge 4 using a syringe 1. Alternatively, the fluidic apparatus of the present invention may be incorporated as a component of a larger fluidic apparatus wherein sample collection, sample processing, the viable cell differentiation reaction and analysis of organisms are automated.

The reaction chamber may comprise a channel with a flow through geometry. In an exemplary embodiment, the reaction chamber may comprise a straight channel (Figures 3-5). The fluid flow through the reaction chamber may be controlled by valves so that the reaction may be substantially completed as the fluid flows through the reaction chamber. In an alternative embodiment, the

reaction chamber may comprise a channel comprising at least one bend. The bend(s) may slow the flow of fluid through the reaction chamber. In particular, the reaction chamber 5 may comprise a serpentine-shaped channel or be shaped to approximate a "W" (Figures 1-2). Slowing the flow of fluid through the reaction chamber may thus enable the reaction to be substantially completed as the fluid flows through the reaction chamber while exposed to the light source. In addition, the flow of fluid through the reaction chamber may also be controlled by valves of the fluidic apparatus. Accordingly, the flow of fluid through the reaction chamber may be manipulated/controlled by either the channel design and/or valves. For example, the reaction sample comprising the sample and phenanthridium compound may be allowed to flow through the reaction chamber for ten minutes before exposure to the light source and this step may further ensure thorough mixing. The reaction sample may then flow through the portion of the reaction chamber exposed to the light source for a further ten minutes for the reaction to occur.

The sample and the phenanthridium compound may be introduced separately, sequentially or together into the reaction chamber 5 through one inlet. Either the sample may be introduced first followed by the phenanthridium compound or vice versa.

In an exemplary embodiment (Figure 3), the reaction chamber 5 may comprise one inlet 2. The inlet 2 may be for introducing the sample 10 into the reaction chamber 5 first loaded with the phenanthridium compound 9 (Figure 3(b)(i)). The phenanthridium compound 9 may be in the form of a solid or liquid. As the sample 10 flows through the reaction chamber 5, it mixes with the phenanthridium compound 9 and flows through to a region 15 of the reaction chamber and is exposed to light from LED 6 (Figure 3(b)(ii)). The reaction is initiated and substantially completed and the reacted sample eventually exits through the outlet 3.

Alternatively, the sample may be mixed with the phenanthridium compound to form a reaction sample 11 before introducing the reaction sample 11 via inlet 2 into the reaction chamber 5 (Figure 4a), where the reaction occurs on exposure to light from LED 6.

In an alternative embodiment, the cartridge may comprise two inlets. The first inlet 2 may be for introducing the sample into the reaction chamber. The second inlet 12 may be for introducing the phenanthridium compound 9 into the reaction chamber. The phenanthridium compound 9 and the sample 10 may optionally be mixed in a mixer 13 (Figure 5(a) and (b)).

In another alternative embodiment as shown in Figures 5(c) and (d), instead of having a mixer, the mixing of the phenanthridium compound 9 and the sample 10 may be performed by controlling the flow of the reaction sample 11 forwards and backwards (indicated by the arrow) in the region 15 via valve(s) 14.

The phenanthridium compound may be any phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells. After penetrating the dead or membrane-compromised cells of the sample as it flows through the fluidic apparatus, the phenanthridium compound intercalates with at least one nucleic acid molecule and covalently binds with the nucleic acid molecule on exposure to a light source. In a particular example, the phenantrhidium compound comprises propidium monoazide.

The reaction chamber 5 may be substantially optically transparent and configured for exposing the fluid within the reaction chamber to a light source for reaction to occur (Figure 2). In an exemplary embodiment, the reaction chamber 5 may be substantially optically transparent to blue light. Refering to Figure 1, the reaction chamber 5 may be positioned with respect to a light source such that light from the light source is transmitted to the reaction chamber 5. The light source may be in the form of a light emitting diode 6

(LED). The LED 6 may be configured to transmit light to the reaction chamber for a predetermined time. This predetermined time should be sufficient to allow for a substantially complete reaction as the fluid flows through the reaction chamber. In particular, the LED emits blue light. The fluidic apparatus may further comprise a heat sink 7 for the LED 6. The heat sink may include a fan 8.

The reacted sample eventually exits the reaction chamber 5, with the nucleic acid molecules of the dead or membrane-compromised cells of the sample covalently bound to the phenanthridium compound. The covalently bound nucleic acid molecules (e.g. DNA) of the dead or membrane compromised cells are unsuitable or unable to take part in further reactions, for example, PCR. On the other hand, nucleic acid molecules (e.g. DNA) from viable and/or substantially intact cells which are not covalently bound with the phenanthridium compound would be able to take part in such further reactions. On exiting the cartridge of the invention via the outlet, the reacted sample may be collected or allowed to flow to another apparatus or component for further manipulation, purification, analysing and/or detecting the presence of nucleic acid molecules (e.g. DNA) from viable and/or substantially intact cells.

The inlet(s) and outlet may also be used for washing the reaction chamber after each use. The cartridge may thus be regenerated for repeated use. The cartridge may be detachable from the fluidic apparatus. Washing of the cartridge may be performed within the fluidic apparatus for convenience or when the cartridge is detached. As the cartridge is detachable, this also enables the replacement of a used cartridge with a new cartridge if necessary.

The above illustrations and accompanying descriptions of the various embodiments of the present invention merely serve to aid in the understanding the principle of the invention. Accordingly, it should not be construed that the present invention described herein is limited to the illustrated embodiments of the apparatus and/or apparatus.

Claims

1. A fluidic apparatus for a reaction for differentiating viable cells, comprising a cartridge comprising:

(a) a substantially optically transparent reaction chamber configured to expose to a light source a sample comprising cells and a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule and covalently binding to the nucleic acid molecule on exposure to a light source,

(b) at least one inlet in fluid communication with the reaction chamber; and

(c) at least one outlet in fluid communication with the reaction chamber;

and at least one light source, the reaction chamber being configured such that in use it will receive light transmitted from the at least one light source.

2. The apparatus according to claim 1, further comprising at least one processor, configured to control at least one of: the light source and the fluid flow through the cartridge.

3. The apparatus according to claim 1 or 2, further comprising at least one valve for controlling fluid flow through the cartridge.

4. The apparatus according to any one of the preceding claims, wherein the at least one inlet comprises at least one inlet selected from the group consisting of: an inlet for introducing (i) the sample and phenanthridium compound separately, sequentially or together, and (ii) the sample into the reaction chamber first loaded with the phenanthridium compound.

5. The apparatus according to any one of claims 1 to 3 comprising a first inlet for introducing the phenanthridium compound and a second inlet for introducing the sample into the reaction chamber.

6. The apparatus according to any one of the preceding claims wherein the reaction chamber comprises a channel with a flow through geometry.

7. The apparatus according to any one of the preceding claims wherein the reaction chamber comprises a channel comprising at least one bend.

8. The apparatus according to any one of the preceding claims, wherein the reaction chamber comprises a serpentine-shaped channel.

9. The apparatus according to any one of the preceding claims wherein the light source is configured to transmit light to the reaction chamber for a predetermined time.

10. The apparatus according to any one of the preceding claims, wherein the light source emits blue light.

11. The apparatus according to any one of the preceding claims, wherein the phenanthridium compound comprises propidium monoazide .

12. The apparatus according to any one of the preceding claims, wherein the cartridge is detachable from the apparatus.

13. A method for differentiating viable cells comprising one of:

(a) (i) introducing a sample comprising cells through an inlet into a reaction chamber of a fluidic cartridge; and

(ii) introducing into the reaction chamber a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule and covalently binding to at least one nucleic acid molecule on exposure to a light source; allowing the sample and phenanthridium compound to combine to form a reaction sample;

or

(b) (i) allowing a sample comprising cells to combine with a phenanthridium compound capable of preferentially penetrating dead or membrane-compromised cells over viable and/or substantially intact cells to intercalate with at least one nucleic acid molecule and covalently binding to at least one nucleic acid molecule on exposure to a light source to form a reaction sample; and

(ii) introducing the reaction sample through an inlet into a reaction chamber of a fluidic cartridge; followed by

(iii) exposing the reaction sample in the reaction chamber to a light source configured to transmit light to the reaction chamber to covalently bind the phenanthridium compound to the nucleic acid molecule(s) from the dead or membrane-compromised cells to form a reacted sample; and

(iv) allowing the reacted sample to exit from the reaction chamber through an outlet.

14. The method according to claim 13, wherein either step a(i) is performed before step a(ii) or step a(ii) is performed before step a(i).

15. The method according to claim 13 or 14, further comprising controlling fluid flow through the cartridge.

16. The method according to any one of claims 13 to 15, further comprising controlling the light source to transmit light to the reaction sample in the reaction chamber for a predetermined time.

17. The method according to claim 16, wherein the predetermined time is the time for the sample to flow through the reaction chamber.

18. The method according to claim 16 or 17; wherein the predetermined time comprises ten minutes.

19. The method according to any one of claims 13 to 18, comprising using a processor configured to control at least one of: the fluid flow and the light source.

20. The method according to any one of claims 13 to 19 wherein the light source emits blue light.

21. The method according to any one of claims 13 to 20, wherein the phenanthridium compound comprises propidium monoazide.