159159

METHOD AND APPARATUS FOR CELL DISRUPTION

Field of the Invention

The invention relates to assay methods for extracting material from a biological sample. In particular, the invention relates to cell disruption methods and devices.

Background

In the extraction of biologically interesting material from cells, it is often the case that only very small samples sizes are available, and consequently, a very small quantity of material is expected to be extracted. To this end, to test this biological material, an assay system must extract the material; as efficiently and effectively as possible.

One such class of assay systems is the cell disruption device, which is used to homogenize tissue samples and disrupt cells, for the purpose of extracting biological material from a sample. A sample may comprise a range of materials including human and animal cells, bacteria (eg E-coli), yeast etc. The biological material to be extracted will therefore depend on the application to which the device is applied.

For human and animal cells, such as for the extraction of material to conduct DNA tests, may involve small sample quantities which must be extracted as efficiently and quickly

G00002

as possible. Further, given the need for commercial laboratories to undertake hundreds or thousands of samples, issues such as cross contamination must also be addressed.

Summary of Invention

In a first aspect the invention provides a cell disruption device for extracting biological material from a biological sample, the device comprising: a chamber for receiving the biological sample; a piezo electric disc within said chamber and supported about a peripheral edge so as to vibrate on receiving an excitation input; wherein said disc is partially covered by an abrasive patch.

In a second aspect the invention provides a cell disruption device for extracting biological material from a biological sample, the device comprising: a chamber for receiving the biological sample; a piezo electric disc within said chamber and supported about a peripheral edge so as to vibrate on receiving an excitation input, and; a quantity of loose particulate matter within said chamber; wherein said loose particulate matter is arranged to interact with the biological sample in said chamber on vibration of said disc.

In a third aspect the invention provides a method for extracting biological material from cells of a biological sample, the method comprising the steps of: placing the biological sample in a chamber, said chamber including a piezo electric disc supported about a peripheral edge; placing a quantity of loose particulate matter in said chamber; exciting said piezo electric disc, and consequently impacting said biological matter with said

particulate matter so as to disrupt said cells, and; extracting the biological material from said disrupted cells.

The cell disruption device according to the present invention therefore operates by disrupting samples through a mechanical shearing action generated from the high-speed vibration of the piezoelectric disc in the cartridge. Mechanical action may include a combination of fluid pressurization, abrasion, shear and sonication occurring within the cartridge when the piezo disc vibrates.

Fluid pressurization arises from the space contraction when the disc deflects into the confined space. On the other hand, abrasion and shearing are attributed to the abrasive patch on the piezo disc. Sound energy is generated from the high frequency disc vibration thus resulting in the sonication effect.

The abrasive material forming the patch may include material such as aluminium oxide or silica. The patch acts as a stiffening layer on the portion of the disc to which it is applied. Hence it restrains the deflection and vibration of the disc. Pressurization and sonication declines consequently. However the abrasive patch enhances the homogenization process as it causes shearing. Thus the area of the abrasive patch must be optimized so as to obtain effective sample disruption.

In one embodiment, the abrasive patch is concentrically placed on the disc. In a further embodiment, the chamber may be a selectively removable cartridge.

In a further embodiment, the diameter of the abrasive patch may be in the range 0 to 10 mm. Alternatively, the diameter of the abrasive patch is in the range 3 mm to 10 mm. In a still further embodiment, the diameter may be in the range 6 to 10 mm.

In a further embodiment, the percentage area of said abrasive patch is in the range 0 to 25% of the disc. Alternatively, the percentage area may be in the range 2 to 25% of the disc. In a still further embodiment, the percentage area may be in the range 9 to 25% of the disc.

In one embodiment, a quantity of loose particulate matter may be introduced into the chamber, to assist in the disruption of the biological material. Further, the loose particulate matter may in the form of a friable tablet of said matter and arranged to dissociate on operation of said disruption device. Such an arrangement may assist in fast and repeatable use of the device, particularly by unskilled or semi-skilled operators. The matter may be silica and/or aluminium, oxide. A quantity that may yield effective results may be in the range 25 to 45mg, or specifically 35mg.

Brief Description of Drawings

It will be convenient to further describe the present invention with respect to the accompanying drawings that illustrate possible arrangements of the invention. Other arrangements of the invention are possible and consequently, the particularity of the accompanying drawings is not to be understood as superseding the generality of the preceding description of the invention.

Figure 1A is a cross-sectional view of a cartridge according to one embodiment of the present invention;

Figure 1B is a plan view of a piezo-electric disc according to one embodiment of the present invention;

Figure 2 is a cross-sectional view of a cartridge according to one embodiment of the present invention;

Figure 3 is a graphical representation showing material extracted against patch diameter;

Figure 4 is a graphical presentation showing material extracted using three methods.

Figure 5 is a graphical presentation showing material extracted against quantity of abrasive particles.

Detailed Description

Figure 1A is an elevation view of a cell disruption device 5 according to one embodiment of the present invention. The cell disruption device 5 includes a piezo-electric disc 10 supported around its peripheral edge and housed within a cartridge 15. The cartridge 15 is arranged to fit within a housing (not shown) which provides containment as well as electrical input to operate the piezo-electric disc 10.

Figure 1B shows a plan view of the piezo-electric disc 10. A concentric abrasive layer or patch 30 is placed on the disc 10 so as to leave a peripheral portion 25 of the disc uncovered. On exciting the disc 10, various harmonic wave shapes 35, 40, may be

experienced. The benefit provided by the present invention is two-fold. The amplitude of the vibrating disc pressurizes the chamber 20 in which the sample material has been placed. This pressurization acts to impact on the cell membrane so as to cause perforations in the cell wall and consequently free the biological material from the sample. A second action, whereby shear and abrasion is applied to the cells within the chamber through contact with the abrasive patch 30, is also achieved. Accordingly, the vibration of the disc provides a repeated impact against the cell membrane, acting as a fatigue type loading, whilst contact with the abrasive patch 30 abrades the cell membrane. As can be seen from Figure 3, however, the synergistic effect of the two actions deteriorates beyond an acceptable yield of biological material once the abrasive patch exceeds 10 mm in diameter or 50% of the diameter of the disc 10. A more complete explanation of Figure 3 will be provided later.

It follows that there is a balance between allowing a large amplitude of the disc and the stiffening effect by the abrasive patch, limiting this amplitude, which then provides the beneficial abrading benefit.

Figure 2 shows a second aspect of the present invention. In this arrangement, the cell disruption device uses a similar chamber 47 in which is placed a piezo-electric disc 50. In this case, there is no abrasive patch and therefore the amplitude achieved on exciting the disc to various harmonic wave forms 60, 65, is unimpeded. This has the effect of dramatically increasing the kinetic energy transferred to the sample 55. However, added to the sample is particulate matter 57 being abrasive material such as silica or aluminum oxide. The high kinetic energy imparted to the cell is also imparted to the

particulate matter creating impact events within the chamber between the sample cells and the particulate matter. By providing an un-stiffened disc the added kinetic energy leads to a substantial yield of biological material.

Experimental Data

Fresh avian tissue was purchased and used in the study. 30 mg of tissue was excised using a scalpel and checked via a mass balance. The tissue was placed in the cartridge of the cell disruption device and 400 µl of lysis buffer was pipetted in. The lysis buffer comprises of ATL buffer with 10% proteinase K (both from Qiagen, Germany). The cartridge comprises of a piezo disc enclosed within a plastic chamber. In the first part of the study, an abrasive patch on the disc was created via the application of epoxy glue and aluminium oxide (average particle size of 256 µm). The diameter (3, 6, 10 and 15 mm) of the abrasive patch was controlled via a mask. The cartridge was subsequently sealed with the ABI PCR film (Carlsbad CA) and connected to the cell disruption device.

The sample was homogenized till a uniform lysate was obtained. The homogenization period was observed to be 3 – 10 minutes. The suspension was pipette out and DNA was extracted via the Qiagen DNeasy kit. The extraction procedure was in accordance to the kit protocol. DNA was quantified using Nanodrop 2000 (Wilmington, DE). The sample size was 4 per group.

The diameter of the disc is 20 mm. The abrasive patch has to be smaller than the disc, hence the maximum diameter was set at 15 mm. A skilled person will appreciate that nucleic acid extraction is performed with a tissue mass of 5 – 30 mg, in view of the confined space within the cartridge. To this end, this arrangement formed the basis of the experimental approach.

The area of the abrasive patch is dependent of the diameter of the patch. Abrasive patches measuring 3, 6, 10 and 15 mm in diameter were tested with the homogenization of avian gizzard (30mg). The absence of the patch was also evaluated (diameter of 0 mm). DNA was extracted from the lysate and quantified. The efficacy of the tissue dissociation process was gauged from the amount of DNA extracted using the cartridges of differing patch diameters and is shown in Figure 3 and Table #1.

dia of sand patch (mm)	Amount of DNA per unit tissue mass (ng/mg)	
	Ave	Std
0	2354	409
3	1617	403
6	2935	259
10	1377	234
15	853	163

Table #1: Experimental Results

As shown in Figure 3, it was observed that there was a decline in the amount of DNA extracted, per unit mass of tissue, from the avian gizzard when the diameter of the abrasive patch increases from 0 to 3 mm. This was because at 0 mm, tissue disruption occurs predominately through pressurization. However the inclusion of the stiff abrasive

patch limits disc deflection. The abrasive effect was minimal between 0 to 3 mm, hence it did not contribute significantly to the homogenization process. Consequently, tissue disruption declines when the sand patch diameter increases from 0 to 3 mm.

Abrasion and shearing becomes significant when the patch diameter was raised from 3 to 6 mm. This was sufficient to compensate the pressurization drop as the disc becomes stiffer with a larger abrasive patch. Effective tissue homogenization occurs with a 6 mm diameter patch. However performance diminishes beyond 6 mm as the disc stiffens with an enlarged abrasive patch. The shearing effect could not offset the drop in pressurization.

850 ng/mg of DNA was adopted as the lowest acceptable limit, indicative of effective homogenization. This could only be achieved when the sand patch measures 0 – 15 mm in diameter, with an area of 0 – 78.5 mm^2. The 6 mm diameter patch was effective, being 30% of the disc diameter, effective in supporting tissue dissociation.

Further performance enhancement was desired but a different design had to be adopted to achieve this.

In testing a further aspect of the present invention loose abrasive particles were included in the cartridge in substitution of the abrasive patch.

It was discovered that the amount of the loose abrasive also affects homogenization. The loose abrasive in the chamber space constitutes a load on the piezodisk and this

reduces the deflection and vibration of the disk. Hence pressurization and sonication declines. However the collision between the abrasive particles and tissue enhances the homogenization process as it causes sample shearing. Thus similar to the abrasive patch, the amount of loose abrasive used must also be optimized so as to obtain effective sample disruption.

A comparative study was done using the 6 mm diameter abrasive patch and 35 mg of abrasive particles. These results were compared against that of the rotor stator control. Both the abrasive patch and loose abrasive cartridge designs were superior to that of the control in terms of the amount of DNA extracted per unit tissue mass as shown in Figure 4. Though the outcomes from the loose and patch abrasives were not significantly different, the former was better.

30 mg of gizzard tissue was homogenized in cartridges with 35 ± 2.5 mg loose abrasive particles with the results shown in Figure 4. DNA extracted from gizzard tissue homogenized with different cartridge designs. * indicates significant difference against control ($P < 0.050$).

Further, loose abrasive measuring 5, 15, 25, 35 and 45 mg were tested with the homogenization of avian gizzard (30mg). The absence of the abrasive was also evaluated (0 mg). DNA was extracted from the lysate and quantified. The efficacy of the tissue dissociation process was gauged from the amount of DNA extracted per unit tissue mass using the cartridges containing the different abrasive masses (Figure 5).

	Amount of DNA per unit tissue mass (ng/mg)	
Abrasive (mg)	Ave	Std
0	2354	409
5	2286	486
15	1316	202
25	2236	191
35	3217	593
45	2666	720

Table #2: Experimental Results – Abrasive Particles

It was observed that the amount of DNA extracted from the avian gizzard was approximately constant when the loose abrasive ranges from 0 to 5 mg. It declined when the abrasive mass was raised to 15 mg. This occurred because tissue homogenization was predominant via pressurization and sonication at abrasive masses below 5 mg. But this effect is arrested by the drop in the deflection of the piezodisk due to the increase in abrasive mass beyond 5mg as that constituted additional loading. The abrasive effect on tissue homogenization is minimal in the 5 – 15 mg range. However the abrasive effect becomes significant when the abrasive increases from 15 to 35 mg. More importantly it was able to offset the decline in pressurization and sonication within the chamber. This improvement in tissue homogenization ceases when the abrasive mass was increased beyond 35 mg. The abrasive load on the piezodisk was critical enough to arrest disk deflection such that pressurization and sonication declines. Moreover fluid agitation was insufficient to promote particle collision within the chamber. Therefore the mass of loose abrasive in the cartridge is recommended to be 0 – 5 mg and 25 – 45mg so as to achieve effective sample homogenization.

Claims

1. A cell disruption device for extracting biological material from a biological sample, the device comprising:
   a chamber for receiving the biological sample;
   a piezo electric disc within said chamber and supported about a peripheral edge so as to vibrate on receiving an excitation input;
   wherein said disc is partially covered by an abrasive patch.

2. The cell disruption device according to claim 1, wherein said chamber is a selectively removable cartridge.

3. The cell disruption device according to claim 1 or 2, wherein the diameter of said abrasive patch is in the range 0 to 10 mm.

4. The cell disruption device according to claim 1 or 2, wherein the diameter of said abrasive patch is in the range 3 mm to 10 mm.

5. The cell disruption device according to claim 1 or 2, wherein the diameter of said abrasive patch is in the range 6 to 10 mm.

6. The cell disruption device according to claim 1 or 2, wherein the diameter of said abrasive patch is 6 mm.

7. The cell disruption device according to claim 1 or 2, wherein the percentage area of said abrasive patch is in the range 0 to 25% of the disc.

8. The cell disruption device according to claim 1 or 2, wherein the percentage area of said abrasive patch is in the range 2 to 25% of the disc.

9. The cell disruption device according to claim 1 or 2, wherein the percentage area of said abrasive patch is in the range 9 to 25% of the disc.

10. The cell disruption device according to claim 1 or 2, wherein the percentage area of said abrasive patch is 9% of the disc.

11. The cell disruption device according to any one of claims 1 to 10, wherein the abrasive patch is placed concentrically on said disc.

12. The cell disruption device according to any one of claims 1 to 11, further including a quantity of loose particulate matter in said chamber.

13. The cell disruption device according to claim 12, wherein said quantity of loose particulate matter includes any one or a combination of: silica or aluminium, oxide.

14. The cell disruption device according to claim 12 or 13, wherein the quantity is in the range 25 to 45 mg.

15. The cell disruption device according to claim 12 or 13, wherein the loose particulate matter is in the form of a friable tablet of said matter and arranged to dissociate on operation of said disruption device.

16. A cell disruption device for extracting biological material from a biological sample, the device comprising:

a chamber for receiving the biological sample;

a piezo electric disc within said chamber and supported about a peripheral edge so as to vibrate on receiving an excitation input, and;

a quantity of loose particulate matter within said chamber;

wherein said loose particulate matter is arranged to interact with the biological sample in said chamber on vibration of said disc.

17. The cell disruption device according to claim 16, wherein said disc is partially covered by an abrasive patch.

18. A method for extracting biological material from cells of a biological sample, the method comprising the steps of:

placing the biological sample in a chamber, said chamber including a piezo electric disc supported about a peripheral edge;

placing a quantity of loose particulate matter in said chamber;

exciting said piezo electric disc, and consequently impacting said biological matter with said particulate matter so as to disrupt said cells, and;

extracting the biological material from said disrupted cells.

19. The method according to claim 18, wherein said disc is partially covered by an abrasive patch.