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To the Honorable Commissior e attached original documents or copy thereof.

1. Name of Conveying party(ies):

Paul Chan
Allan Joseph Hilling Smith
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JUN 22 2001

6-22-01

Additional name(s) of conveying party(ies attached? __ Yes _x_ No

2. Name and address of receiving party(ies)

Name: William A. Cook Australia Pty. Ltd.

Street Address: 12 Electronics Street

Brisbane Technology Park, Eight Miles Plains

City: Queensland 4113, Australia

Name: Cook Incorporated

Street Address: 925 South Curry Pike

City: Bloomington State: IN ZIP: 47402

Additional name(s) & address(es) attached? __Yes _X_ No

3. Nature of conveyance:

_X_ Assignment ___ Merger

___ Security Agreement ___ Change of Name

___ Other _____

Execution Date: February 24, 2001

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: _____

A. Patent Application No. 09/819,407 B. Patent No.

Additional numbers attached? ___ Yes _X_ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Anton P. Ness

Street Address: P.O. Box 2269

City: Bloomington State: IN ZIP: 47902

6. Total number of applications and patents involved 1

7. Total fee (37 CFR 3.41) $40.00

___ Enclosed

_X_ Authorized to be charged to deposit account

8. Deposit account number: 13-2528

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9. Statement and signature

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Anton P. Ness
Name of Person Signing

Anton P. Ness
Signature

June 19, 2001
Date

Total number of pages including cover sheet, attachments, and document 16

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MEMORANDUM OF ASSIGNMENT

THIS MEMORANDUM OF ASSIGNMENT is made PAUL CHAN of 8 Oyster Point Esplanade, Scarborough, Queensland 4020 and ALLAN JOSEPH HILLING SMITH of 59 Delavan Street, Wishart, Queensland 4122 and DAVID MICHEL of 1 Gordon Road, Redland Bay, Queensland 4165 all of Australia (hereinafter called "the Assignor") of the one part and
WILLIAM A. COOK AUSTRALIA PTY LTD (A. C. N. 005 526 723) 12 Electronics Street, Brisbane Technology Park, Eight Mile Plains, Queensland 4113, Australia and COOK INCORPORATED of 925 South Curry Pike, Bloomington, Indiana 47402, United States of America (hereinafter called "the Assignee") of the other part;
NOW THIS ASSIGNMENT WITNESSETH THAT for the sum of ONE AUSTRALIAN DOLLAR ($AU1.00) and other good and valuable consideration (receipt of which is hereby acknowledged), the Assignor, does hereby assign and transfer and set over to the said Assignee, all of our right title and interest in and to an invention entitled PERFUSION INCUBATOR a description of which is attached hereto including the right to apply for Letters Patent throughout the World and all Patents that may be granted thereon, and the Assignee does accept from the Assignor all of their right title and interest in and to the said invention and the right to apply for Letters Patent throughout the World and all Patents that may be granted thereon, to hold absolutely.
AND, the Assignor hereby agrees to sign, confirm and authenticate any and all forms, declarations and papers properly required by any Patent Office in support of any application for Letters Patent or similar protection and will promptly deliver the same to the Assignee provided that the Assignee shall bear all costs, fees and expenses relating thereto;
IN WITNESS WHEREOF the parties hereto have hereunto affixed their hands and seals on the day and year hereinafter written.

SIGNED, SEALED AND DELIVERED)
by the said PAUL CHAN)
was hereunto affixed)
this 24th day of FEBRUARY 2000)
in the presence of -)

PAUL CHAN

Witness

SIGNED, SEALED AND DELIVERED)
by the said ALLAN JOSEPH HILLING)
SMITH was hereunto affixed)
this 24th day of FEBRUARY 2000)
in the presence of -)

ALLAN JOSEPH HILLING SMITH

Witness

SIGNED, SEALED AND DELIVERED)
by the said DAVID MICHEL)
was hereunto affixed)
this 24th day of FEBRUARY 2000)
in the presence of -)

DAVID MICHEL

Witness

THE COMMON SEAL OF)
WILLIAM A. COOK AUSTRALIA PTY)
LTD was hereunto affixed)
this 25th day of FEBRUARY 2000)
in the presence of -)

Signature of authorised person

ACCOUNTS CO-ORDINATOR
Office held

JOY DAVIDSON
Name of authorised person

Signature of authorised person

MANAGING DIRECTOR
Office held

GUS TADDEO
Name of authorised person

THE COMMON SEAL OF
COOK INCORPORATED
was hereunto affixed
this 21st day of March 2000
in the presence of:-

Signature of authorised person

Senior Vice President
Office held Business Development

Brian L. Bates
Name of authorised person

Signature of authorised person

Senior Vice President
Office held Product Development + Administration

John A. DeFord
Name of authorised person

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P/00/009
Regulation 3.2

AUSTRALIA
Patents Act 1990

ORIGINAL

PROVISIONAL SPECIFICATION FOR AN INVENTION ENTITLED

Invention Title: **PERFUSION INCUBATOR**

Name of Applicant: **WILLIAM A COOK AUSTRALIA PTY LTD** and **COOK INCORPORATED**

Address for Service: **COLLISON & CO.** 117 King William Street, Adelaide, S.A. 5000

The invention is described in the following statement :

This invention relates to incubators and more particularly incubators for cell culturing, in particular culturing embryos of mammalian species.

In one form, although this may not necessarily be the only form, the invention is said to reside in a perfusion incubator, including a media supply, a media conditioning unit, at least one well assembly and a well assembly heating unit, a peristaltic pump and a media collection unit, the well assembly including a plurality of wells, each having a media inlet and a media outlet, the media inlet connected to the media supply and the media outlet connected to the media collection unit via the peristaltic pump. Whenever on the placing of a cell to be cultured in each well and flowing media through the well culturing of the cell can occur.

Preferably there is provided an illumination device so that the cell being cultured in the cell assembly can be observed by means of a microscope. Preferably therefore there is a microscope mount associated with the perfusion incubator.

In a further embodiment each well includes means to provide a flow path from the media inlet to the media outlet within the well so that media flow is not directly around the embryo in the well.

In a preferred embodiment of the well each media inlet is positioned so as to allow a tangential entry of media to the cell at a mid point in the well and the media outlet being positioned above the media inlet with the cell or embryo to be cultured in a lower portion of the cell. The flow of media in the cell is formed by this construction into a vortex which will tend to draw media from around the cell without direct flow over the cell.

Preferably each well has a stepped side well defining an upper chamber and a smaller lower chamber. Preferably each well has a lid which in use is adapted to extend partially into the upper chamber. Preferably the lid is of a substantially transparent material so as to allow for viewing of the embryo in the lower chamber.

The entire well assembly may be transparent so that the cell can be illuminated from below.

In a further form the invention is said to reside in a perfusion incubator unit having at least one well, the or each well having a stepped side well defining an upper

chamber and a smaller lower chamber and a lid, a media inlet to the or each well and media outlet to the or each well, the media inlet being positioned so as to allow tangential entry of media to the well at a lower portion of the upper chamber and the media outlet being positioned above the media inlet.

Preferably the lid is adapted to extend partially into the upper chamber and may include O-ring sealing means.

Preferably the lid is of a substantially transparent material so as to allow viewing of an embryo in the lower chamber.

In a preferred embodiment of the invention the perfusion incubator cell is substantially transparent so that with illumination from below the embryo being cultured can be achieved.

It can be seen, therefore, that by this invention the perfusion incubator is a system which based on the principle of perfusion of specifically designed culture media provides a suitable environment for the production, development and storage of pre-implantation embryos from mammalian species. The system maintains purpose built culture wells at a pre-set temperature while perfusing carbon dioxide enriched culture media over the cells.

This very generally describes the invention but to assist with understanding, reference will now be made to the accompanying drawings which show a preferred embodiment of the invention.

In the drawings.

Figure 1 shows a schematic view of a perfusion incubator according to the present invention,

Figure 2 shows a isometric view of a perfusion incubator,

Figure 3 shows a cross sectional view of a culture chamber for the perfusion incubator,

Figure 4 shows an isometric view of a well assembly; and

Figure 5 shows an isometric view of the lid for the culture chamber.

Now looking at the drawings in detail we see that the perfusion incubator includes a media heater unit 1 which has a gas inlet 2, the peristaltic pump 4 draws media from test tube 5 in the media heater unit through media inlet line 6, through the chamber 7 (as will be discussed in detail below) through the media outlet line 9 and after the peristaltic pump 4 travels through line 10 into media collection unit 11.

The media flow lines 6, 9 & 10 are not shown in figure. 2.

Below the chamber 7 is an illumination source 13. Above the chambers is a chamber heater unit 14 and above this is a microscope lens 15. The microscope lens is held in microscope holder 16 as can be seen in Figure 2. In Figure 2 the chamber heater unit 14 has been raised so that the chambers below it can be seen.

As can be seen in the side view in Figure 3 the chamber 7 has a first well generally shown as 20 and a second well generally shown as 21. Each well has a stepped cross section with a largest upper section 23 into which the lid fits, a middle section 24 and a smallest lower section 25. The media inlet to each cell 26 is at the bottom of the middle section 24 and the media outlet 27 is at the top of the middle section 24. In use a cell to be cultured is placed in the lower section 25. The lid 30, as can be seen in Figures 4 and 5, has an upper portion 31 to enable placement and removal of the lid and is of a size which can be gripped by the fingers. A central portion of the lid 32 is adapted to fit into the upper sections 23 of the well with an O-ring in the slot 33 providing sealing of the lid. The lower portion of the lid 35 is adapted in use to fit into the middle section 24 of the well but allows media to flow around it towards the media outlet 27. The lower surface 36 of the lid 30 and the upper surface 37 are both finely polished so that viewing through the lid enables viewing of the cell being cultured in the lower section of the well.

In a preferred embodiment the perfusion incubator according to the invention is constructed and operated as discussed below.

The tube heater will contain at least six tubes containing culture media which may or may not be the same as that in other tubes. The media is held at the users specified temperature and equilibrated with specific gas mixtures capable of maintaining the required pH in the culture media.

Each pair of culture wells is connected by non-toxic, non-permeable tubing to a tube of media in the front and to a peristaltic pump in the rear of the culture wells. From the peristaltic pump the tubing continues to the media waste receiver tubes where used media is collected as waste or for further analysis. The peristaltic pump draws solution from the heated reservoirs through the culture wells to the waste containers. The whole process of temperature, light and fluid flow control may be maintained by a microprocessor.

In a referred embodiment the culture wells themselves may be made of plastic materials such a polycarbonate and they have highly polished surfaces. Underneath each culture well is a high output light emitting diode. A set of 5 pairs of culture wells may be contained in a heating block. In operation mode the chamber in the heater unit which is contained in the lid is placed down over the culture wells. In the lid is a clear glass strip. Above the heater block is a microscope holder. With a microscope in place and the light emitting diode beneath the culture well turned on, an operator is able to observe the embryos directly 'in situ'.

It is useful in growing a healthy embryo to observe the growth pattern. A motor driven system to position the microscope over each culture well may be used.

The motor driven system may be provided to position the microscope over each consecutive culture well, for instance, at one minute intervals which would make it possible to accumulate digital time lapse photography of growth patterns of individual embryos in each culture well. The illumination beneath each culture well may only be turned on as required to capture an image. Light output from an LED is specifically in the orange-yellow band to be of low energy but provide high contrast. In addition to this, it is preferable the light emitting source contains no ultra-violet radiation as this may damage the embryos.

Embryos for culture are placed in the bottom of the culture well where they stick in the margin between the horizontal and vertical axis of the lower section. The embryos are not dislodged by the action of culture media flowing across them. Culture media is introduced above the lower section as a tangential flow to the inner surface of the culture well and removed from the top of the well. This creates an upward moving vortex which displaces media in the lower section of the culture well. Without this vortex action media exchange in the bottom section of the well is by diffusion only, which results in embryo death or at best, embryos with very

poor morphology which would not be suitable for implantation. A particular feature of the culture wells is the vortex action to exchange media in the bottom section. This action can be observed by the injection of small amounts of dye and following the fluid path.

The reason that the embryos are not placed directly in the fluid path is that exogenous and growth factors excreted by the embryos would be flushed away immediately. By placing in a lower section where the media exchange rate is less than the true flow rate, then the exogenous and growth factors remain in the proximity of the embryos for at least a short time.

In operation mode, with the lids in place, there is a negative pressure of approximately 1kPa within the culture well as media is being pulled through by the peristaltic pump. This slight negative pressure may cause outgassing of the dissolved gasses which maintain the pH of the culture media. Henry's Law states that the mass of gas dissolved by a given volume of solvent at a constant temperature is proportional to the pressure of the gas in equilibrium with the solvent. Small bubbles which form in the culture well may isolate embryos from culture media with resultant death of the embryos. These minute bubbles cannot be dislodged at the flow rates used in this device. The flow rate used per well can vary between 40 microlitres per hour up to 4000 microlitres per hour when in flush mode.

To overcome this bubble problem media at 0.5°C above the operating temperature of the culture chamber is equilibrated with gas mixture. As gases dissolve with the liberation of heat the Le Chatelier principle dictates that a rise in temperature will cause a decrease in gas solubility. Equilibrating the culture media at 0. 5°C above the chamber temperature with the gas mixture prior to the media's entry into the culture well, ensures that the increase in solubility because of the 0. 5°C temperature drop and decrease in solubility because of the 1kPa pressure drop are both compensated for and no bubbles form in the culture well. Without the pre-equilibration of the media at the higher temperature, the culture well may not perform effectively.

Once the embryos are ready for implantation they can be perfused with cryo-protectant and the whole block can be frozen in liquid nitrogen.

Throughout this specification various indications have been given as to the scope

of this invention but the invention is not limited to any one of these but may reside in two or more of these combined together. The examples are given for illustration only and not for limitation.

Throughout this specification and the claims that follow unless the context requires otherwise, the words 'comprise' and 'include' and variations such as 'comprising' and 'including' will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Dated this day of 2000

WILLIAM A. COOK AUSTRALIA PTY LTD and COOK INCORPORATED
By their Patent Attorneys,
COLLISON & CO.