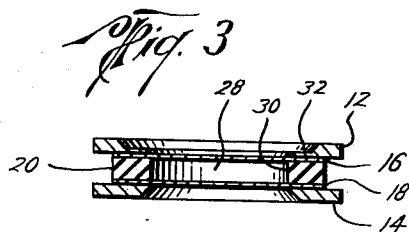
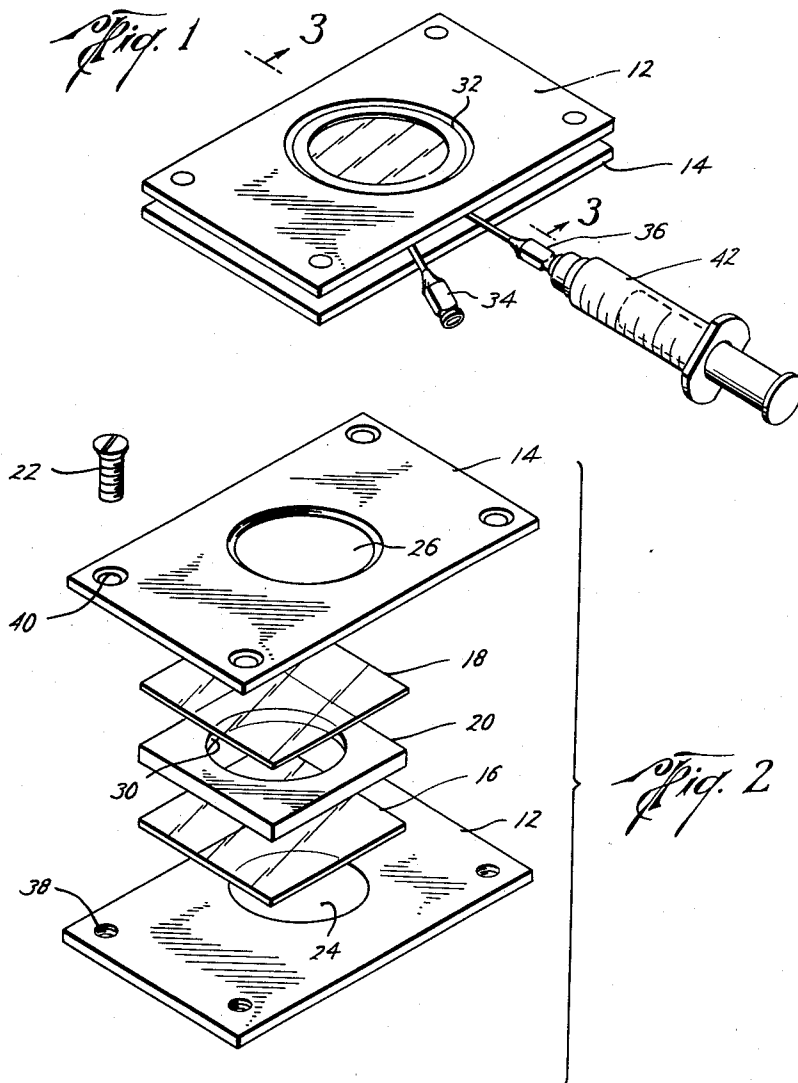


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TISSUE CULTURE DEVICE
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TISSUE CULTURE DEVICE

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2 Claims. (Cl. 88—40)

This invention relates to a tissue culture device and more particularly to one which may be used intact with microscopes and especially phase contrast microscopes.

Most tissue culture chambers are not as suitable as desired for microscopic examination of the contents of the chambers while the contents are still in the chambers and most microscopic slides have not been easily adapted to good tissue culture chambers. Several devices attempt to combine both the attributes of a good tissue culture chamber and a good microscopic slide but none of these devices fully accomplish this aim.

It is a general object of the present invention to provide a tissue culture device including a chamber which device after its usefulness for a particular culture may be dismantled and the cells fixed, stained and the slide mounted for a permanent record; which permits microscopic examination of any magnitude including oil magnitude at any desired time without altering the device or environment of the cells; which can be perfused with known or unknown fluids and its nutrient exchanged at will; which possesses good aseptic technical qualities; which contains adequate surface for extended culturing; which uses relatively small amount of nutrient; which does not need plasma clots; which permits vital staining (staining of living tissue) without disrupting the cellular growth or altering the structure of the device; and which provides a system of cultivation conducive to cultural survival over long periods of time.

Another object of the present invention is to provide such a tissue culture device permitting the use of hypodermic needles for the extraction and insertion of nutrients and other matter.

And still a further object of the present invention is to provide such a tissue culture device which is reliable, durable, and easy to handle.

Other objects and advantages will be more apparent from the following description of a preferred example of the invention, given for the purpose of disclosure, and taken in conjunction with the accompanying drawings, where like character references refer to like parts throughout the several views and where

Fig. 1 is a perspective view of the tissue culture device of the present invention showing a hypodermic syringe and needle for insertion or withdrawal of matter from the chamber and a hypodermic needle for maintaining constant pressure in the chamber,

Fig. 2 is an exploded view of the tissue culture device inverted from the position shown in Fig. 1, and

Fig. 3 is a view along line 3—3 of Fig. 1.

Referring now to the drawings, and particularly to Fig. 2, the tissue culture device as a whole includes first and second retaining plates 12 and 14 respectively each having a transparent cover slip 16 and 18 adjacent its interior side and a gasket 20 between the cover slips 16 and 18, all held together in a sandwich by bolts 22. Apertures 24 and 26 are formed in the first and second retaining plates 12 and 14 respectively and are in mutual alignment with one another so that when the tissue culture

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device is assembled as shown in Figs. 1 and 3 there is a chamber 28 formed by an aperture 30 in the gasket 20 and the cover slips 16 and 18 with the contents of the chamber 28 being observable through the apertures 24 and 26 and the cover slips 16 and 18.

Because of the harmful effects of bowing of the cover slips, as will be made more apparent later, and the desire to have a maximum diameter to the chamber 28 for extended culture growth without increasing the dimensions of the cover plates to such a size that they are not convenient for handling these cover plates 12 and 14 need to be made of a rigid material. Stainless steel plates measuring 2" x 3" x 1/8" have been found very satisfactory. Stainless steel has the desired qualities of rigidity and is readily available. The 2" x 3" length and width is convenient to handle and allows a comparatively large aperture 24 or 26 to be formed while using retaining plates 12 and 14 of only 1/8" in thickness.

With the particular dimensions of the retaining plates 12 and 14 previously given an aperture of 1 1/16" in diameter centered in each plate 12 and 14 may be used which is of ample size for most extended culture growth and yet will leave sufficient material in the retaining plates 12 and 14 to prevent harmful bowing.

As the lower end of the objectives of some microscopes, and especially of phase contrast microscopes, needs to be placed in close proximity to the specimen, and as some of the tissue to be examined by the microscope may be at the periphery of the apertures, the aperture 24 in the first retaining plate 12, which is the one to be placed adjacent the objective of the microscope, is counter-sunk as at 32 (see Fig. 3) so that the objective may be placed close to the cover slip 16 at the periphery of aperture 24.

The cover slips 16 and 18 are normally identical and are preferably standard optical cover slips of No. 1 thickness which is from 0.13 to 0.17 millimeter in thickness. Such cover slips are thin enough to permit the objective of a microscope to be in close proximity to a tissue culture specimen lying on the underneath side of the cover slip 16 or 18 adjacent the microscope. These standard optical cover slips 16 and 18 of No. 1 thickness are marketed in various sizes and one standard size is 50 x 43 millimeters which is approximately the same size as the gasket 20 and therefore such size cover slip is desirable.

The gasket 20 makes a sealing engagement with the cover slips 16 and 18 to form the chamber 28 and it also permits introduction and withdrawal at will of needles such as the hypodermic needles 34 and 36 best seen in Fig. 1. This gasket 20 must be non-toxic to the particular cultures in the chamber 28, needle penetratable, and self-sealing so that when hypodermic needles are withdrawn there is no passage of fluid to or from the chamber 28. Further, this gasket 20 must be of material that when placed in the sandwich will form a fluid tight seal with the cover slips 16 and 18. Pure gum latex has been found to have all these properties and is highly satisfactory. However, for some reason certain pure gum latex gaskets are toxic to varying extents upon many tissue cultures and care must be used in the choice of the gasket material. Pure gum latex (floating stock) sold by Hamilton Rubber and Manufacturing Corporation, P.O. Box, 361, Trenton, New Jersey, as stock No. 107 has been found to be highly satisfactory in that it is non-toxic to all tissue cultures tested and has all the other necessary properties mentioned before.

To brace the underneath side of the cover slips 16 and 18 and thus to help prevent bowing and additionally to provide a maximum sealing surface between the gasket 20 and the cover slips 16 and 18, the aperture 30 in gasket 20 is preferably made the same diameter as the apertures 24 and 26 in retaining plates 12 and 14 respectively.

With the retaining plates 12 and 14 of the dimensions previously given a gasket 20 measuring 2" x 1 3/4" has been found to be very satisfactory as it has sufficient size to form a seal after a needle is withdrawn and has sufficient surface contacting the cover slips 16 and 18 to prevent slippage when the needle is inserted or withdrawn. Using cover slips of No. 1 thickness and retaining plates 1/8" thick, a gasket 20 approximately 1/8" thick is highly satisfactory in that the gasket is sufficiently thick to permit hypodermic needles to be easily inserted and withdrawn and yet the distance between the underneath side of the cover slip placed adjacent the objective of the microscope (the tissue culture specimen is usually located here) and the outer surface of the retaining plate nearest the condenser is no greater than the maximum working distance permitted by most phase contrast microscopes (having long working distance condensers) between the condenser of the microscope and the specimen to be examined, even in oil magnification study.

To hold the tissue culture device assembled in the position shown in Fig. 1, four corner holes 40 (see Fig. 2) are provided in one of the retaining plates such as the second retaining plates 14 and the holes 40 are countersunk to receive the Allen bolts 22 so that the heads of the Allen bolts 22 do not project above the surface of the retaining plate 14. The other retaining plate, 12 in this instance, has internally threaded holes 38 which are complementary with the holes 40 in the second retaining plate and in which the Allen bolts 22 may be threadedly engaged without extending beyond the outer surface of retaining plate 12.

The first and second retaining plates 12 and 14 need to be held together by means on opposite sides of the chamber 28 so that force on the cover slips 16 and 18 may be adjusted to prevent bowing of these cover slips and to adjust other stresses on them which affect their optical qualities. Additionally, the means to secure the retaining plates 12 and 14 to one another should not extend beyond the outermost surface of these retaining plates as any such projection would prevent use of the device with certain microscopes and would make it inconvenient in storage and in handling generally. These four Allen bolts 22 located as illustrated have been found to be satisfactory.

When the tissue culture device is being assembled the hypodermic needle 34, used as a vent, should be inserted through the gasket 20, as illustrated in Fig. 1, before a final tightening of the Allen bolts 22 takes place as pressure build-up in the chamber 28 during the final tightening sometimes ruptures cover slips 16 and 18. This vent needle 34 may be left in place with a cotton plug in it as convenient bacteriological filter or it may be withdrawn. Once the tissue culture device is assembled fluids may be introduced into the chamber 28 or withdrawn from it by using the hypodermic needle 36 and syringe 42 but whenever this is done the vent needle 34 should also be used to prevent pressure changes within the chamber 28.

With this particular device entries and withdrawals of the hypodermic needle may be made at will without the introduction of any contamination into the chamber, thus providing a device which may be perfused or have its nutrient changed at will and possessing good aseptic qualities. Further, because the nutrient may be changed at

will plasma clots, which are often objectionable in microscopic studies, are not necessary. Additionally, vital staining may take place without disrupting the cellular growth by introduction of the dyes directly into the chamber from the hypodermic needle 36 and syringe 42. Further, the dyes may be washed out of the culture and chamber by the same method. If staining of the culture is desired this can be done on the cover slips 16 and 18 with the cover slips being mounted as a permanent record.

The tissues to be cultured may be introduced into the chamber 28 in either of two ways. The tissue culture device may be assembled as illustrated in Fig. 1 and the tissue introduced by the hypodermic needle 36 and syringe 42, or, if the fragments are too large or plasma clots are desired, then the tissue is placed within the chamber 28 before the top cover slip is positioned and thereafter the device is assembled as illustrated in Fig. 1.

All parts of the tissue culture chamber are re-usable except one of the cover slips whenever staining is desired or for some reason it is desired to keep the culture on that particular cover slip. Therefore maintenance cost is low.

Although dimensions have been given, it is to be understood that these are not critical but are given for the purpose of illustration only.

While only a single example of the invention has been given for the purpose of illustration, changes in detail will suggest themselves to those skilled in the art. Accordingly, it is desired to be limited only by the spirit of the invention as defined by the scope of the appended claims.

What is claimed is:

1. A tissue culture device comprising, first and second rigid retaining plates in parallel spaced relationship each having an aperture therethrough in mutual alignment, a gum latex gasket between the retaining plates with its outer edges exposed, said gasket having an aperture therethrough aligned with the other apertures and said gum latex gasket being nontoxic to tissue to be used, transparent cover slips between the gasket and the adjacent retaining plates, said cover slips being in mutual alignment over the aperture in the gasket and each cover slip contacting a retaining plate, one of said plates having threaded openings on opposite sides of the aperture, and threaded means connected to the other retaining plate on opposite sides of the aperture and threadable in the threaded openings thereby releasably and adjustably holding the retaining plates, cover slips, and gasket sealingly together whereby said assembled culture device is adapted to be placed under a microscope for observance of the tissue culture therein.

2. The tissue culture device of claim 1 in which the cover slips are of standard No. 1 optical thickness.

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