# A Newly Designed Dish for the Universal Uses of Tissue and Organ Culturing

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## SUMMARY

A newly designed dish for tissue and organ culturing is demonstrated. This dish consists of two sections (cover and container) and one accessory (rubber stopper). The dish can be used for culturing in both closed and open systems. Easy manipulation is demonstrated in feeding, media changing, harvesting and washing. Therefore, this dish is a possible substitute for many other items now used in tissue and organ culturing.

## INTRODUCTION

Many types of glassware have been used for tissue culturing since the establishment of the technique. Of these, the most important and familiar items are culture vessels (containers) such as the prescription bottle, Petri dish, Carrel flask[1], Earle T-flask[2], Leighton tube[3][4], etc. According to Paul[5], the original and exclusive vessel in tissue culturing is the Carrel flask. Other items, such as the Pareker flask (1936), Porter's roller-flask (1947), and Earle's shaker flask, were designed under the influence of the Carrel flask. Each of them has its own characteristics and uses.

These vessels can be classified into two major groups from the viewpoint of pH control. The first group consists of glassware used in an open system, which involves incubation in the presence of 5 per cent $CO_2$ and high humidity. The other group consists of glassware used in a closed system where incubation in $CO_2$ is unnecessary. The Petri dish represents a member of the former group, while all others mentioned belong to the latter.

A new type of dish for tissue and organ culturing which can be used in both systems was recently designed by the author, and proved to be convenient and useful in many ways.

## STRUCTURE

The diagram and photograph of the newly designed dish are shown in Figure 1 and 2, respectively. The dish consists of two main sections and one accessory. One section is the container and the other is the cover containing a tubular arm which is joined at the rim, and the accessory is the rubber stopper. Although the diameter of both container and cover of the standard type is 50 mm, the sizes of those can vary as shown in Table 1. The total height of the container and the cover is 30 mm, including the thickness of glass (1.0 mm × 2). The height of the container is somewhat greater than that of the cover ; the former is 20 mm high and the latter is 15 mm high.

The container has an inner lip with a height of 5 mm and a thickness of 2 mm, locking the cover in place. When the dish is closed, the outer surfaces of the cover and the container are flush, facilitating placement of a tape for a gas tight seal. Thickness of the container wall is 4 mm ; that of cover wall is 2 mm.

The shape and size of the arm are fit to the No.00 or No.000 rubber stopper. The angle of the arm is on an extension of the diagonal line between inside corners of the container and the cover as shown in Figure 1. The arm is designed to be suitable for media changing or feeding with fresh media. Manipulation inside the dish can be accomplished by use of an ordinary 5 ml pipet through the arm. The arm is constructed on the rim of the cover, not on the side, in order to make a complete seal around the sides of both cover and container, and to be able to overlap the container-cover junction with adhesive or nylon tapes.

## USES

This dish has many uses :

(1). **As a type of Carrel flask :** In this case, the dish is sealed with adhesive or nylon tape and is closed with a rubber stopper. Uses of silicon grease was

Figure 1. Diagram of the structure of the new culture dish.

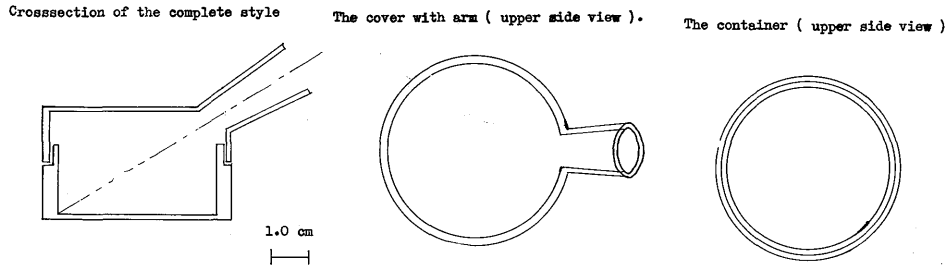
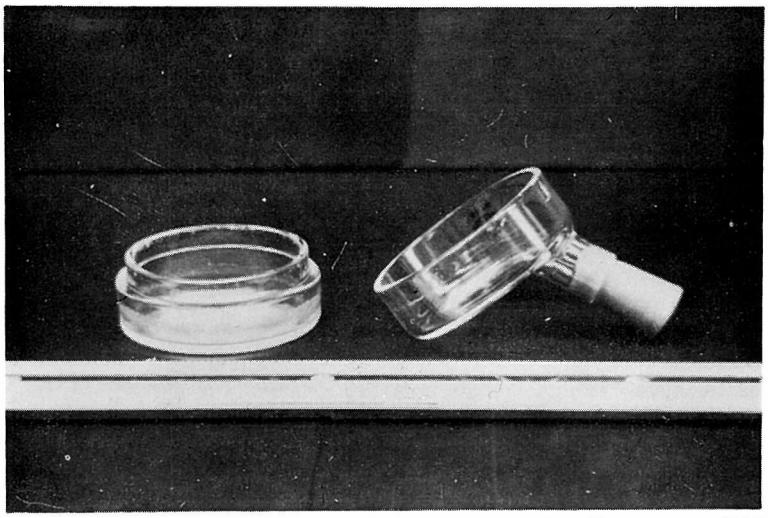
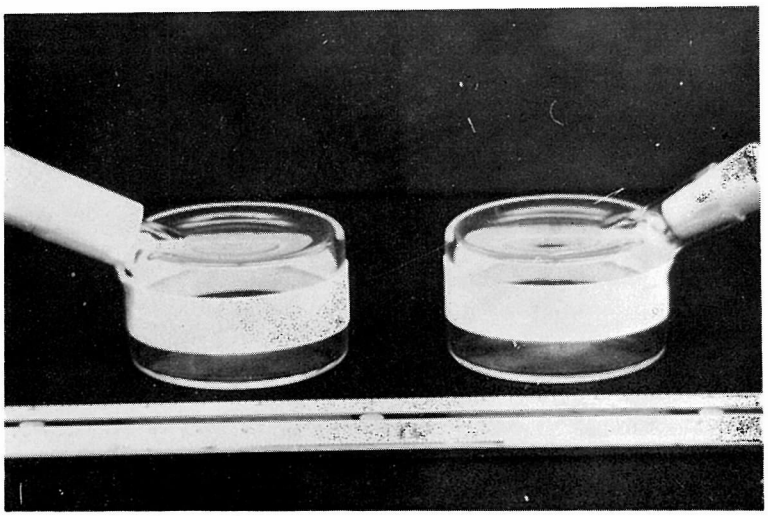


Figure 2. Photographs of the new culture dish



(a) Separated two sections: container (Left) and cover with rubber stopper (Right).



(b) Complete style with sealing by tape: Paper capping on the arm (Left) and use of rubber stopper (Right).

Table 1. Variable sizes of the new type dishes and their uses

| | Diameter ($\phi$) mm | Rubber sopper | Uses |
|---|---|---|---|
| Small size | 35 | No. 000 | Cloning and Separation |
| Medium size (Standard) | 50 | No. 00 | General uses as in Fig. 4. |
| Large size | 70–80 | No. 00 | Harvesting of a large amount of cells |

avoided because of the difficulty of the subsequent maintenance of glasswares. That is, silicon grease sometimes soils fingers, glasswares, and other instruments. Its resistance to washing may leave glasswares contaminated and repellent to liquid. Also, remaining silicon grease is harmful for cell growth from the viewpoint of adhesiveness and wetability.

(2). **As a type of Leighton tube :** The glass cover slips for cultivation of cells and for microscopic observation by staining can be inserted and easily removed by simply lifting the cover.

(3). **As a type of Petri dish** : If the culture must be incubated in the presence of $CO_2$, this dish can be used with a cotton stopper or paper cap in place of the rubber stopper, and without sealing of tape. In this case, the gas ($CO_2$) circulates as within a Petri dish. Continuous feeding of cells with fresh media can be done through the arm. Plating of cells, picking up the colonies at cloning, and erasing cells by scratching at separation[6], are possible by lifting the cover. Observation is also possible in all situations.

These uses are compared with those of other types of glassware in Figure 4.

## EXPERIMENTAL ANALYSIS

The many uses of this newly designed dish were tested with the following cells and media :

Cells used : (1) HeLa cell
(2) Green monkey kidney cell
(3) Mink cell from peritoneal exudates

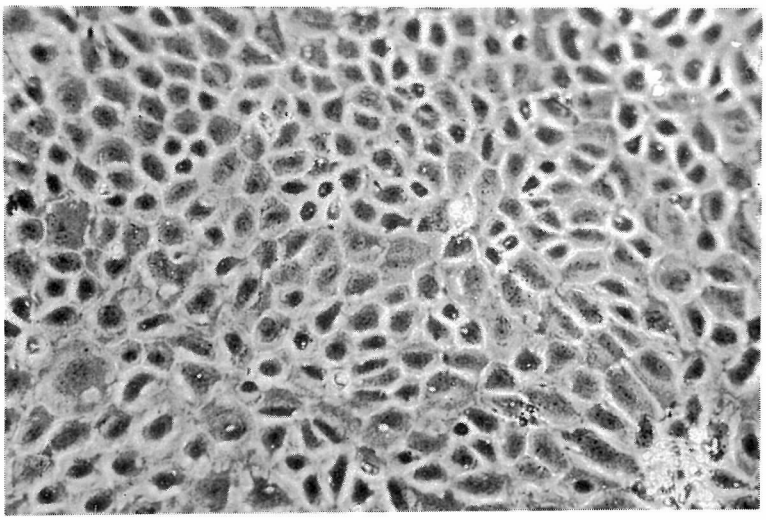
Media used : (1) Basic media
(a) No. 199 and (b) MEM
(2) Sera
(a) Calf serum and (b) Fetal calf serum

The combination of both basic media and sera was used as required. As glassware controls, the Carrel flask, Earl flask and Leighton tube were also used for cultivation of the above cells. All experiments were carried out under the conditions of a closed system at 37°C.

All the cells showed good growth in all glasswares, and no difference between new culture dish and other items could be observed. Consequently, microphotographs of the mink cells grown in the new culture dish are shown as the representative of those cells in Figure 3.

Figure 3. Microphotographs of mink cells using new culture dish

(a) Fibroblastic cells grown on the cover slip, 3 days incubation : May-Grünwald & Giemsa stain, Magnification 135×.



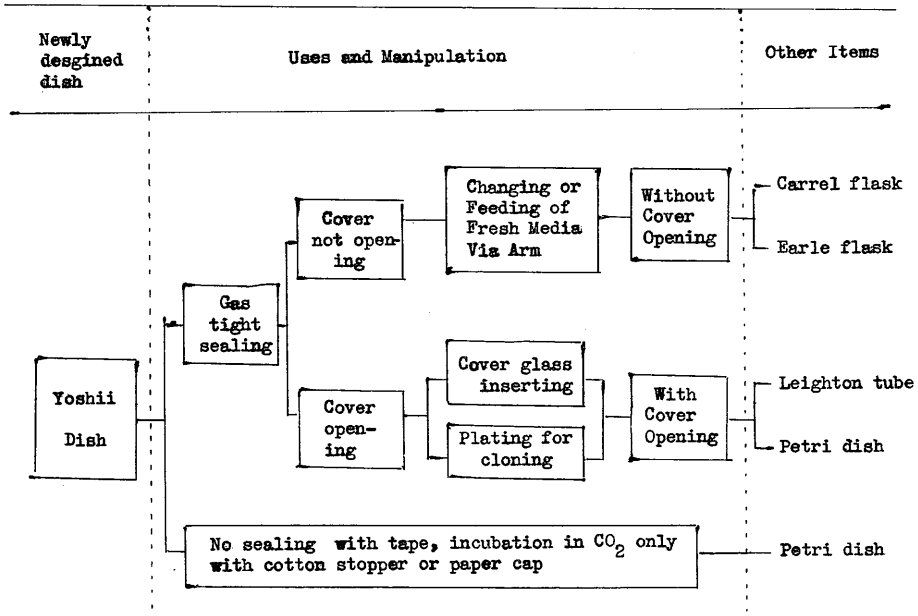
(b) Epitheloid cells grown on the inside surface of the container, 4 days incubation : Phase contrast microscopy, Magnification 135×.

## DISCUSSIONS

The most valuable characteristic of the newly designed dish is its multi-purpose variability ; that is, it combines into one flask the many uses of all other flasks and dishes now used in tissue and organ culture. This dish can therefore be

substituted for any of the others. The advantage that the dish offers over the other types is the easy manipulation of the cover in opening and closing according to experimental purposes as described in the Fig. 4. The details of uses are discussed as follows :

Figure 4. Uses and manipulation of the new cuture dish in comparison with presently used items.




(1) When sealed tightly with adhesive or nylon tape and closed with a rubber stopper, the dish works well as a Carrel flask or as an Earle flask. Sealing with tape is effective for making the vessel gas-tight. Changing or feeding with fresh media can be done easily via the arm. Harvesting of grown cells is possible in both open and closed situations ; fine manipulation is easily achieved in the former case after removal of the tape. In the case of the Carrel flask and the Earle flask, fine manipulation is more difficult.

Warren and his coworkers[7] developed a kind of sealable dish, but it has no arm. Therefore, whenever media change or feeding is necessary, the cover of their dishes must be opened. Moreover, silicon grease is applied for sealing. Silicon grease usually resists complete washing, and is regarded as a burden from the viewpoint of maintaining wetness on the glass surface.

(2) As the dish has a removal cover, glass cover slips or pieces of slide glass may be inserted as in Leighton tube before the cultivation of cells. The new dish has a larger capacity, and more cover slips or slide pieces can be accomodated

than with ordinary Leighton tubes. Harvesting of cover slips or slide pieces can be accomplished easily after removing the cover.

(3) A removal cover provides the same usefulness as that of a Petri dish for cloning of cells. After plating, the dish can be recovered, sealed, and incubated. Continuous observations of the colonial growth of the cells can be made under closed system conditions. The picking up of a colony can be done by opening the dish like a Petri dish.

(4) This dish can also be used in the $CO_2$ incubator without sealing with adhesive or nylon tape or rubber stopper. The rubber stopper can be replaced by a sterilized cotton ball stopper or paper cap.

(5) As this dish can be separated similarly to a Petri dish, washing and rinsing of it are very easy and convenient in comparison with other glassware for closed systems, such as the Carrel flask, Earle flask, and so forth.

(6) Size and shape of the standard type of this dish are shown in Figure 1 and 2, however, these can be changed following requests of experimental purposes.

(7) No organ culturing experiment was performed with this dish, however, the basic design similarity to the Petri dish leads the author to believe that the new dish would also be found suitable in that field of endeavor.

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