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(54) **Cell culture insert**

Zellkultureinsatz

Insert pour culture de cellules

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(56) References cited:
EP-A- 0 239 697
EP-A- 0 495 213
US-A- 4 871 674

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Description

This invention relates to an apparatus for growing cells or tissue culture in vitro and more particularly comprises a new and improved cell culture insert for supporting tissue cultures in a fluid medium containing nutrients which promote the tissue culture growth.

Cell culture inserts comprise a plastic material with a membrane on the bottom surface thereof so that there is free diffusion and transport of ions and macro-molecules. With the use of a suspended microporous membrane, two cell types, for example, can be cultured, one on each side of the membrane in the same well. Without suspension, cells on the bottom surface of the membrane would be exposed to damage. The microporous membrane allows free passage of macromolecules, proteins and ions. As a result, the interactions of the two cell types can be studied without actual physical contact between the two cell populations in the suspended state of the insert.

Conventional cell culture inserts and devices are described in US-A-4,871,674 and US-A-5,026,649. US-A-4,871,674 discloses a cell culture insert which comprises discontinuous projecting parts for hanging the cell culture insert provided on an upper circumferential part. US-A-5,026,649 discloses a cell culture insert which comprises a projecting part for hanging the culture cell which is provided over the whole upper circumferential part, with openings in the sidewalls for exchanging gas and for pipeting sample.

In the use of these cell culture inserts, gases may not be exchanged sufficiently because the area between the sidewall of the insert and the culture plate is too small. Furthermore, as described in US-A-5,026,649, portions of the cell culture insert could possibly become contaminated because openings are provided in the sidewall of the cell culture insert.

It is an object of the present invention to improve the known cell culture inserts.

This object is solved, according to the invention, with the features of claim 1.

The present invention is a cell culture insert comprising a sidewall bordered by a top end surface and a bottom end surface wherein the bottom end surface has attached thereto a flat porous, permeable membrane, a glass cover slip or the like. The top end surface of the insert carries at least one outwardly extending flange which serves to support the insert in the well of a tissue culture vessel. The geometric configuration of the insert is desirably a continuous circumference, however, any geometric configuration such as but not limited to, round, sphere, circle, oval, rectangle, square, octagon and the like may be used. In addition, the sidewall of the cell culture insert may taper from the top end to the bottom end.

Desirably, there at least two outwardly extending flanges on the top end surface of the cell culture insert. These flanges may be spaced along the top end surface of the cell culture insert.

The exterior dimensions of the portion of the cell culture insert within the well of a tissue culture vessel are sufficiently less than the well interior diameter to allow a pipet or similar device to be positioned between the tissue culture vessel and the cell culture insert for fluid filling or aspiration. The space allows the pipet to reach the bottom of the vessel and introduce or remove medium from beneath the membrane and about the outer surface of the sidewall of the cell culture insert without contaminating the upper surface of the membrane.

Preferably, the outer surface of the sidewall of the cell culture insert includes means for restricting or preventing the flange of the cell culture insert from falling into the well of the tissue culture vessel. The flange may shift from excess movement of the cell culture insert within the well. Such movement of the cell culture insert within the well facilitates pipet access. The movement can be limited in one axis by the means for restricting or preventing the flange of the cell culture insert from falling into the well. The movement of the cell culture insert, however, may be such that the cell culture insert wall and the substantially parallel well interior wall can touch at one or more locations.

The cell culture insert may further comprise at least one support footing on the bottom end surface of the cell culture insert. The footing provides a degree of clearance between the membrane and the well bottom of the tissue culture vessel in the event the outwardly extending flange is not compatible with the tissue culture vessel and cannot support the cell culture insert. Most preferably there are at least two support footings on the bottom end surface of the cell culture insert.

The cell culture insert of the present invention may be sized to be used with a multi-well tissue culture vessel having wells of a specific size. The cell culture insert may be made in different sizes and geometric configurations so as to be used with different sizes and geometric configured tissue culture vessels, such as but not limited to plates, dishes and the like.

An important feature of the cell culture insert of the present invention is the means for preventing or restricting the outer surface of the cell culture insert sidewall from moving close to the inner wall of the well of the tissue culture vessel in which it is placed so that capillary action of the fluid in the well is minimized.

An additional feature of the present invention is that a pipet may be inserted between the outer surface sidewall of the cell culture insert and the inner wall of the tissue culture vessel in which the cell culture insert is placed. The pipet may be inserted without disturbing or removing the cell culture insert from the well of the tissue culture vessel so that fluid may be introduced to or removed from the space beneath the membrane of the cell culture insert and from the bottom of the well of the tissue culture vessel.

A further feature of the cell culture insert of the present invention is that it may be supported in a tissue

culture vessel with flanges so that there is clearance between the bottom of the membrane and the bottom of the well of the tissue culture vessel. The degree of clearance allows the fluid in the tissue culture vessel to substantially achieve a controlled static head and diffusion so that cells may be properly cultured on the bottom surface of the membrane. In addition, the feet may support the cell culture insert when it is removed from the tissue culture vessel and placed on a flat surface.

The invention will be more fully understood from the following detailed description thereof taken in connection with the accompanying drawings in which:

FIG. 1 is an exploded perspective view of a tissue culture vessel and cell culture insert.

FIG. 2 is an enlarged perspective view of the cell culture insert of the present invention without the membrane.

FIG. 3 is a top view of FIG. 2.

FIG. 4 is a top view of FIG. 1 illustrating the cell culture insert supported in the well of a tissue culture vessel.

FIG. 5 is an alternate top view of the cell culture insert of FIG. 2 wherein the outwardly extending flange is formed with an opening for pipetting or for facilitating placement and removal of the cell culture insert in a tissue culture vessel.

FIG. 6 is a cross-sectional view taken along lines 6-6 of FIG. 4 illustrating where a pipet tip may. enter the space between the cell culture insert and the well of the tissue culture vessel.

The apparatus for growing tissue cultures as shown in FIG. 1 includes a culture vessel **10** and a cell culture insert **40**. Although only a six well culture vessel is shown, it should be appreciated that the culture vessel may have eight, twelve, twenty-four or some other number of wells selected for the particular purpose for which the apparatus is used.

Culture vessel **10** includes a base **11** comprising a number of wells **14** each comprising a sidewall **20** closed at the bottom by a wall **18** and open at the top end **19**. Base **11** typically is transparent and may be molded, for example, of polyvinylchloride.

While in the foregoing paragraphs, the details of the culture vessel illustrated are described, it is to be appreciated that the vessel itself does not form part of the present invention, and the cell culture insert of the present invention may be sized to fit and be used with other culture vessels.

FIGS. 2 and 3 further illustrate cell culture insert **40** of the present invention comprising a body wall **42** having an outer wall surface **44** and inner wall surface **46**. Body wall **42** extends from an upper portion **48** to a lower portion **50**. The body wall may taper from the upper portion to the lower portion.

Upper portion **48** comprises a top surface **52** which carries an outwardly extending flange **54** which provides support to the insert when placed in the well of a culture vessel. Preferably, flange **54** comprises a horizontal orientation for resting on the top of the well of the culture vessel. Lower portion **50** comprises a bottom surface **56** to which a microporous membrane **58** may be adhered. The membrane may be made of suitable material including, but not limited to, perforated inert film, hydrated gel, or a layered combination.

On outer wall surface **44** of the insert is located at least one projecting or extending tab 60. Projecting tab **60** provides stability to the insert and prevents or restricts the flange from falling into the well of the culture vessel. The flange may shift from excess movement of the cell culture insert within the well. Movement of the cell culture insert within the well facilitates pipet access in the space between the outer wall surface of the insert and the sidewall of the well. Projecting tab **60** may limit the movement of the cell culture insert in one axis. The movement of the cell culture insert, however, may be such that the cell culture outer wall surface and the sidewall of the well can touch at one or more locations. The projecting tabs can be any geometric configuration. Most preferably, the projecting tabs may be located on the outer wall surface about half way between the top surface and the bottom surface, although this location is not critical to the invention.

As shown in FIGS. 2, 3, 4 and 5 when more than one outwardly extending flange **54** is used, they are spaced to allow for insertion of probes or pipets during the culturing procedure. The configuration of flange **54** may be structured to fit most situations. As shown in FIG. 5 flange **54** may be formed with an opening for inserting a pipet or probe in the space between the outer wall of the insert and the well. The flange configuration may facilitate the aseptic placement and removal of the cell culture insert into or out of the well of the culture vessel with tweezers or forceps, and allow for air circulation to minimize compartmentalization as well as providing support to the cell culture insert when placed in the well of a culture vessel.

It is most desirable that a space be maintained between the membrane on the bottom surface of the insert and the well of the culture vessel so that cells may be cultured on both sides of the membrane. Therefore, the cell culture insert of the present invention may further include feet or supports **61** located on the bottom surface of the cell culture insert. Feet **61** may be used when the extending flanges may not provide adequate support of the insert in a particular culture vessel arrangement. Feet **61** may further provide adequate support to the cell culture insert when placed on a flat surface.

FIG. 6 illustrates the insert as supported in culture vessel **10** and where a pipet tip **64** may enter the space between outer wall surface **44** of the cell culture insert and sidewall **20** of the culture vessel. Further shown are the projecting tabs **60** which may prevent or restrict the flange of the insert from falling into the well of the culture vessel.

It will be appreciated that when cell culture insert **40** is set within well **14** and spaced sufficiently from the well

sidewall **20** as facilitated by projecting tabs **60**, no capillary action will occur to cause solution or media in space **62** from wicking up outer surface **44** and entering the interior of the insert or spilling from well **14**. Furthermore, because flange **54** supports the device, the culture may be treated if desired in a deeper well than suggested so as to provide more solution beneath the membrane. While it is customary to position the membrane a distance above bottom wall **18** if desired, a well of greater depth may be used so as to provide additional space between the membrane and bottom wall **18**.

As practitioners-in-the-art will understand, the cell inserts of the present invention may be comprised of simple moldable parts which can be mass produced from a variety of materials, including, for example, polyethylene, polystyrene, polyethylene terephthalate, and polypropylene.

Claims

1. A cell culture insert having a hollow chamber comprising a top surface (52), a bottom surface (56), a sidewall (42) comprising an inner (46) and outer (44) surface extending from said top surface (52) to said bottom surface (56), a porous membrane (58) aligned with said bottom surface (56), means for suspending (54) said cell culture insert (40), extending from said top surface (52), and means for restricting movement (60) of said cell culture insert (40), extending from said outer surface (44) of said side wall (42),
characterized by
said means for restricting movement (60) being located on said outer surface (44) spaced away from the top surface (52) and from the means for suspending (54).

2. The insert of claim 1, wherein said means for restricting movement (60) being located about halfway between the top surface (52) and the bottom surface (56).

3. The insert of claim 1 or 2, wherein said means for restricting movement (60) of said insert (40) being two projecting tabs arranged on opposite sides of the insert (40).

4. The insert of one of claims 1-3, wherein said means for suspending (54) said insert (40) being a plurality of discontinuous projecting flanges.

5. The insert of claim 4, wherein said flange has a horizontal orientation for resting on the top (19) of a well (14) of a culture vessel (10).

6. The insert of one of claims 1-5, further comprising means for supporting (61) said insert (40) on said bottom surface (56).

7. The insert of one of claims 1-6, wherein said sidewall (42) tapers from said top surface (52) to said bottom surface (56).

8. The insert of one of claims 1-7, wherein said means for restricting movement (60) of said insert (40) provides access of sufficient size between said outer sidewall (48) of said insert (40) and the inner wall (20) of said well (14) of said culture vessel (10) so as to substantially prohibit capillary activity.

9. Apparatus for use in growing tissue cultures in vitro comprising:

   a tissue culture vessel (10) having a well (14) with inner (20) and bottom (18) walls for receiving media, and

   a cell culture insert (40) according to one of claims 1-8 in said well (14).

Patentansprüche

1. Zellkultureinsatz, der eine hohle Kammer aufweist, mit einer Oberseite (52), einer Bodenfläche (56), einer Seitenwand (42) mit einer Innen- (46) und einer Außenfläche (44), die sich von der Oberseite (52) zur Bodenfläche (56) erstreckt, einer zu der Bodenfläche (56) ausgerichteten porösen Membran (58), einer sich von der Oberseite (52) aus erstreckenden Einrichtung (54) zum Stützen des Zellkultureinsatzes (40), und einer Einrichtung (60) zum Begrenzen der Bewegung des Zellkultureinsatzes (40), die sich von der Außenfläche (44) der Seitenwand (42) aus erstreckt.
dadurch gekennzeichnet, daß
die Einrichtung (60) zum Begrenzen der Bewegung von der Oberseite (52) und der Stützeinrichtung (54) beabstandet an der Außenfläche (44) angeordnet ist.

2. Einsatz nach Anspruch 1, bei dem die Einrichtung (60) zum Begrenzen der Bewegung ungefähr in der Mitte zwischen der Oberseite (52) und der Bodenfläche (56) angeordnet ist.

3. Einsatz nach Anspruch 1 oder 2, bei dem die Einrichtung (60) zum Begrenzen der Bewegung des Einsatzes (40) aus zwei abstehenden Ansätzen besteht, die auf gegenüberliegenden Seiten des Einsatzes (40) angeordnet sind.

4. Einsatz nach einem der Ansprüche 1 - 3, bei dem die Einrichtung (54) zum Stützen des Einsatzes (40) aus mehreren unterbrochenen abstehenden

Flanschen gebildet ist.

5. Einsatz nach Anspruch 4, bei dem der Flansch eine horizontale Ausrichtung aufweist, um auf dem oberen Ende (19) der Aufnahme (14) eines Kulturgefäßes (10) aufzuliegen.

6. Einsatz nach einem der Ansprüche 1 - 5, ferner mit einer Einrichtung (61) zum Stützen des Einsatzes (40) auf der Bodenfläche (56).

7. Einsatz nach einem der Ansprüche 1 - 6, bei dem die Seitenwand (42) sich von der Oberseite (52) zur Bodenfläche (56) hin verjüngt.

8. Einsatz nach einem der Ansprüche 1 - 7, bei dem die Einrichtung (60) zum Begrenzen der Bewegung des Einsatzes (40) ausreichenden Abstand zwischen der äußeren Seitenwand (44) des Einsatzes (40) und der Innenwand (20) der Aufnahme (14) des Kulturgefäßes (10) aufweist, um kapillare Aktivitäten im wesentlichen zu verhindern.

9. Vorrichtung zur Verwendung beim In-Vitro-Züchten von Gewebekulturen, mit:

   - einem Gewebekulturgefäß (10) mit einer eine Innen- (20) und eine Bodenwand (18) aufweisenden Aufnahme (14) zum Aufnehmen von Medien, und
     einem in der Aufnahme (14) vorgesehenen Zellkultureinsatz (40) nach einem der Ansprüche 1 - 8.

Revendications

1. Insert pour culture de cellules ayant une chambre creuse comprenant une surface supérieure (52), une surface inférieure (56), une paroi latérale (42) comprenant une surface intérieure (46) et une surface extérieure (44) s'étendant depuis ladite surface supérieure (52) vers ladite surface inférieure (56), une membrane poreuse (58) alignée avec ladite surface inférieure (56), des moyens pour mettre en suspension (54) ledit inseit pour culture de cellules (40), s'étendant depuis ladite surface supérieure (52), et des moyens pour limiter le mouvement (60) dudit insert pour culture de cellules (40), s'étendant depuis ladite surface extérieure (44) de ladite paroi latérale (42), caractérisé par le fait que
   lesdits moyens pour limiter le mouvement (60) se situent sur ladite surface extérieure (44) espacée de la surface supérieure (52) et des moyens de mise en suspension (54).

2. Inseit selon la revendication 1, dans lequel lesdits moyens pour limiter le mouvement (60) se situent environ à ni-chemin entre la surface supérieure (52) et la surface inférieure (56).

3. Insert selon la revendication 1 ou 2, dans lequel lesdits moyens pour limiter le mouvement (60) dudit insert (40) sont deux pattes faisant saillie agencées sur les côtés opposés de l'insert (40).

4. Insert selon l'une des revendications 1 à 3, dans lequel lesdits moyens de mise en suspension (54) dudit insert (40) se composent d'une pluralité de brides discontinues faisant saillie.

5. Insert selon la revendication 4, dans lequel ladite bride a une orientation horizontale pour reposer sur le dessus (19) d'un puits (14) d'une cuve de culture (10).

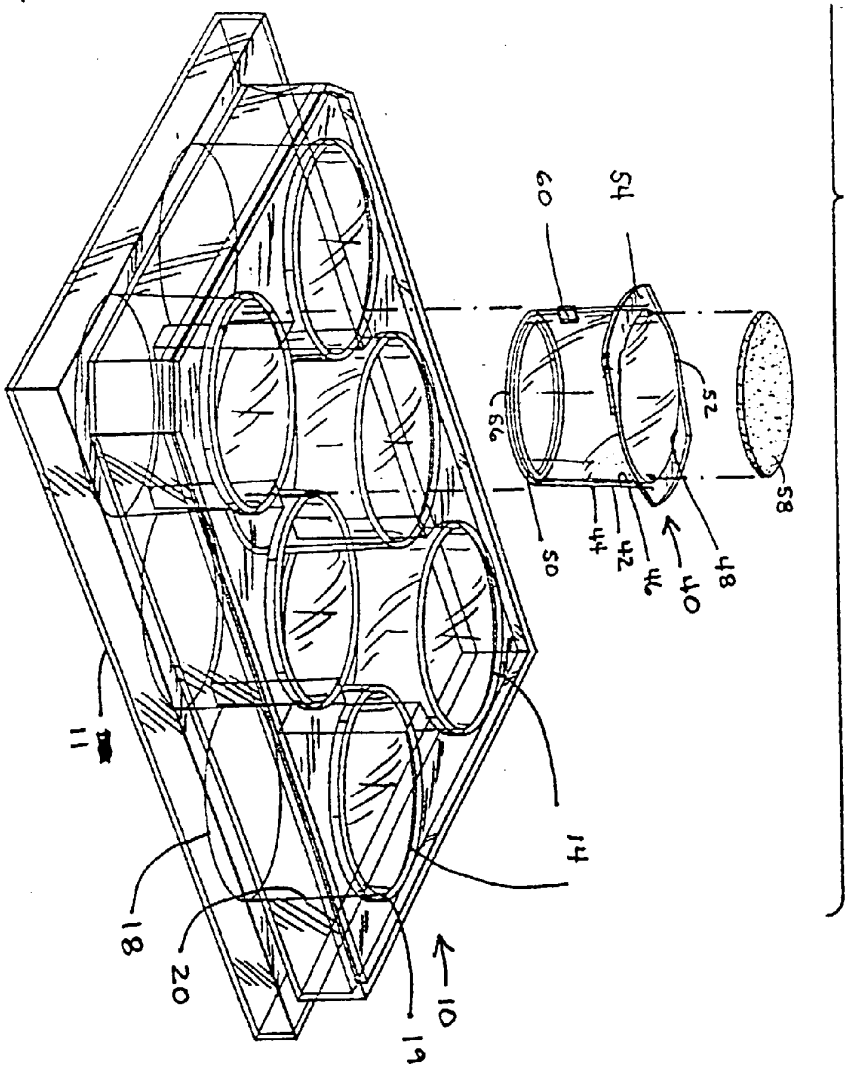
6. Insert selon l'une des revendications 1 à 5, comprenant en outre des moyens pour supporter (61) ledit insert (40) sur ladite surface inférieure (56).

7. Insert selon l'une des revendications 1 à 6, dans lequel ladite paroi latérale (42) s'amincit depuis ladite surface supérieure (52) vers ladite surface inférieure (56).

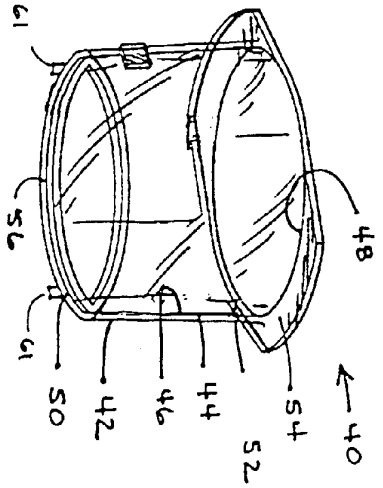
8. Insert selon l'une des revendications 1 à 7, dans lequel lesdits moyens de limitation de mouvement (60) dudit insert (40) fournissent un accès de dimensions suffisantes entre ladite paroi latérale extérieure (48) dudit insert (40) et la paroi intérieure (20) dudit puits (14) de ladite cuve de culture (10) afin d'empêcher sensiblement l'activité capillaire.

9. Appareil destiné à être utilisé pour élever des cultures de tissus in vitro comprenant :

   une cuve de culture de tissus (10) ayant un puits (14) avec des parois intérieure (20) et inférieure (18) destinés à recevoir les milieux, et un insert pour culture de cellules (40) selon l'une des revendications 1 à 8, dans ledit puits (14).

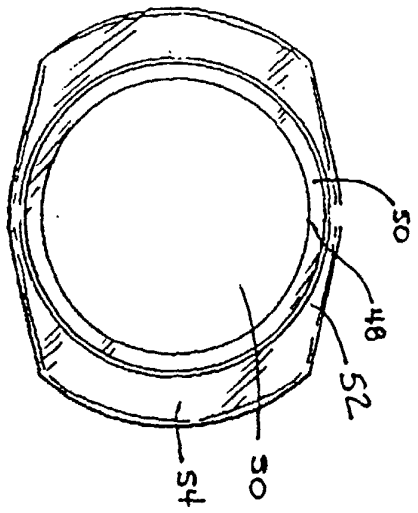

60
54
52
56
58
50
44
42
46
48
40
11
14
18
20
10
16

FIG—1


16
56
48
16
50
42
46
44
54
52
40

FIG—2

FIG—3



FIG—4

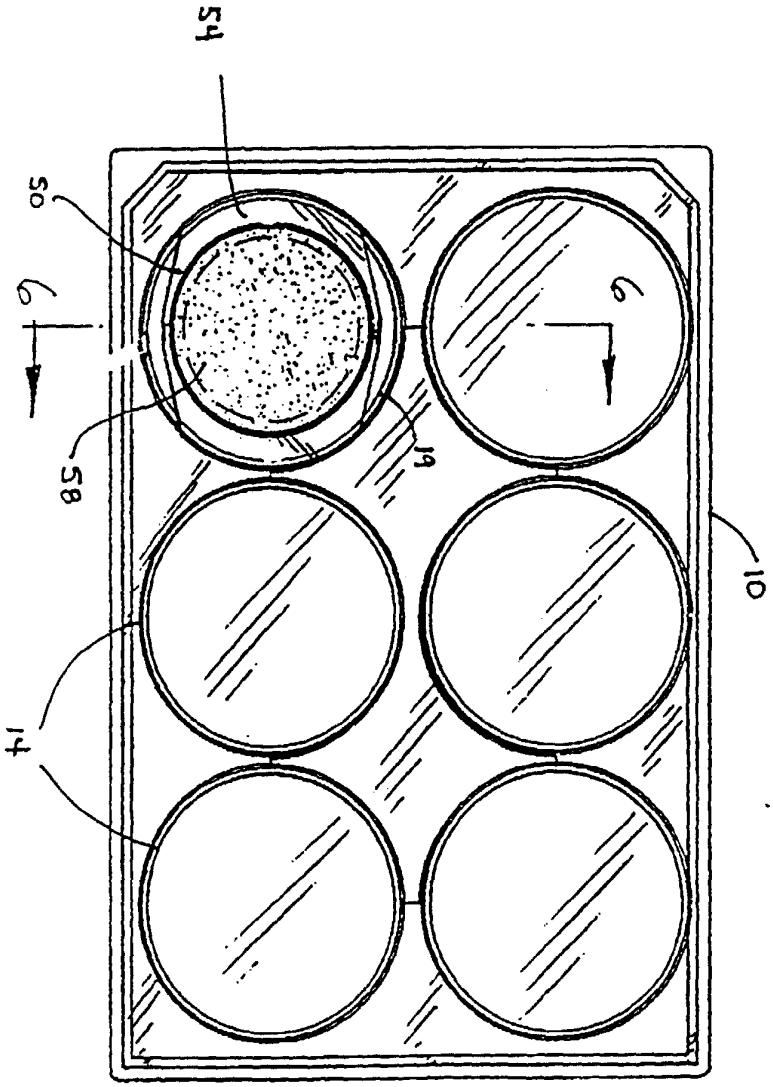
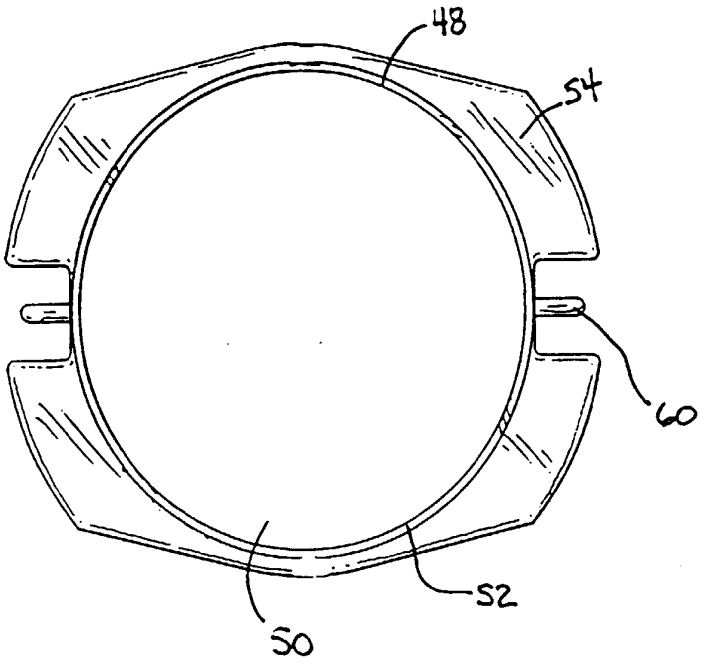


FIG-5



FIG—6