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Feygin et al.

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(54) **APPARATUS AND METHOD FOR HIGH THROUGHPUT ANALYSIS OF COMPOUND-MEMBRANE INTERACTIONS**

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This patent is subject to a terminal disclaimer.

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(51) **Int. Cl.**
G01N 33/00 (2006.01)

(52) **U.S. Cl.** **436/8**; 435/297.1; 435/297.5; 422/50; 422/58; 422/68.1

(58) **Field of Classification Search** 435/297.1, 435/297.5; 73/38, 64.47; 422/50, 58, 68.1
See application file for complete search history.

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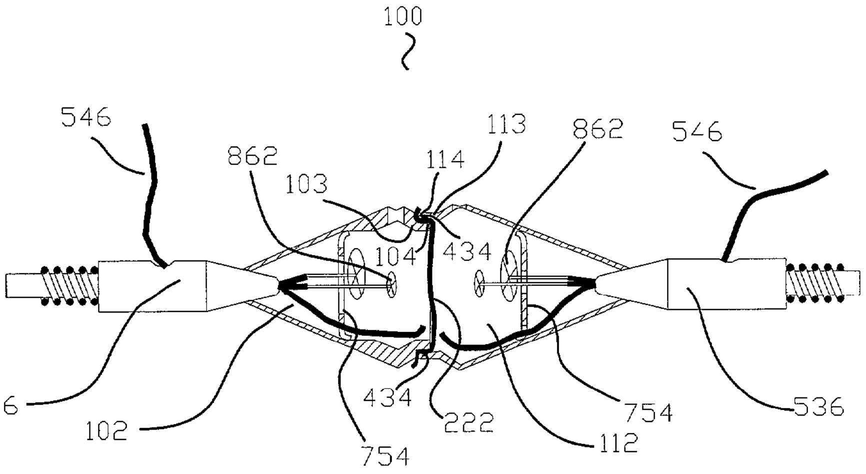
(57) **ABSTRACT**

An article and method for high throughput and a high content investigation of compound interactions with live tissue or its substitutes under controlled conditions during compound absorption and related processes. In some variations of the illustrative embodiment, the article is a multichamber enclosure having at least two chambers separated by a membrane. Membranes can be prepared from live epithelial tissue or from an artificial material with or without attached cells from cell-line cultures. Each chamber is advantageously connected to a fluidic-control system by tubes that pass through a feed fitting. In addition to coupling the chambers with the fluidic-control system, the feed fitting, which is spring-biased, provides a sealing force to seal the enclosure. In some variations, one or more multi-chamber enclosures are installed in a mother chamber, which provides controlled environmental conditions. Operation of the article includes automatic introduction of compounds, buffers, and gases into the chambers, establishing reaction conditions inside the chambers, and individually sampling from the chambers.

24 Claims, 8 Drawing Sheets



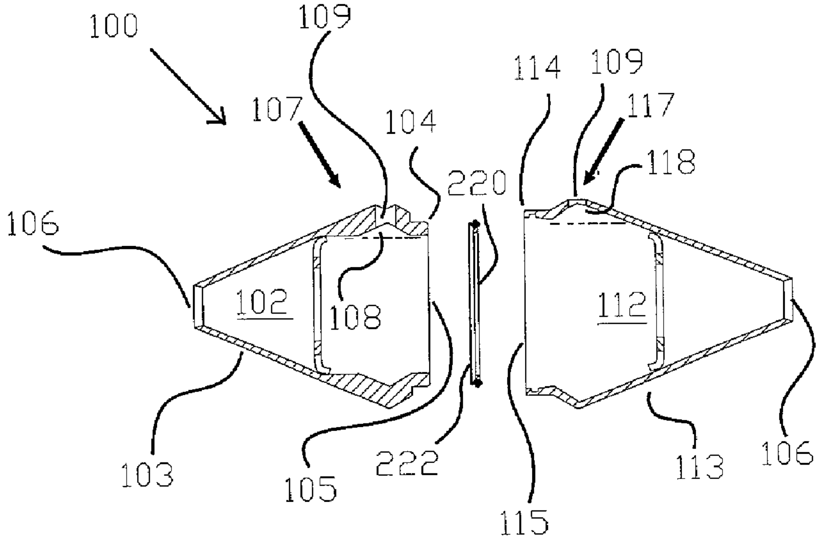


FIG. 1

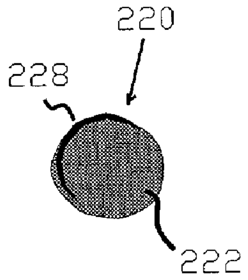


FIG. 2A

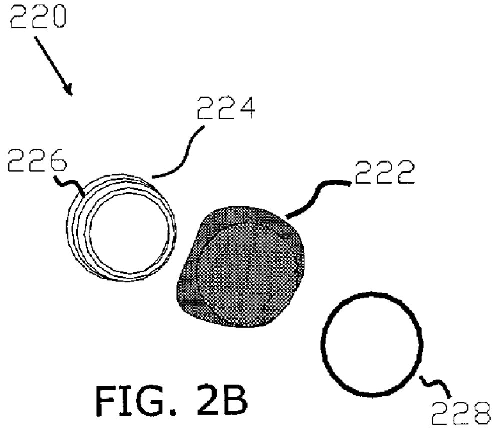


FIG. 2B

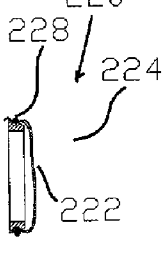


FIG. 2C

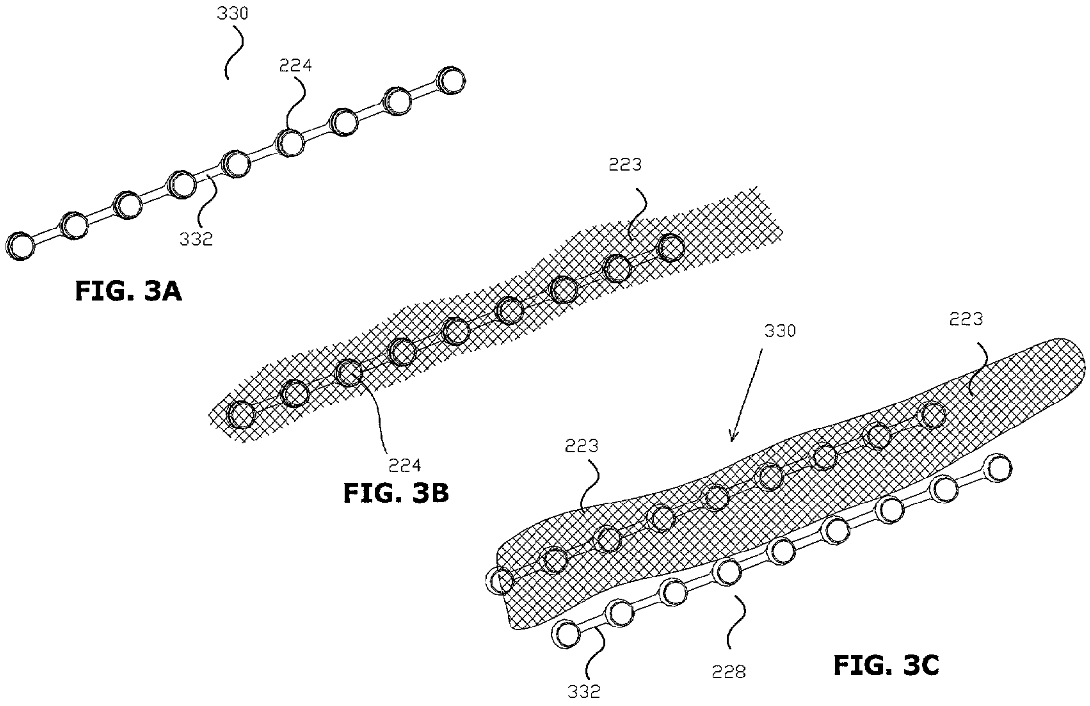


FIG. 3A

FIG. 3B

FIG. 3C

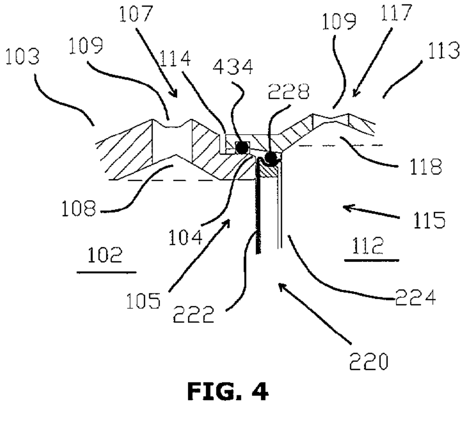


FIG. 4

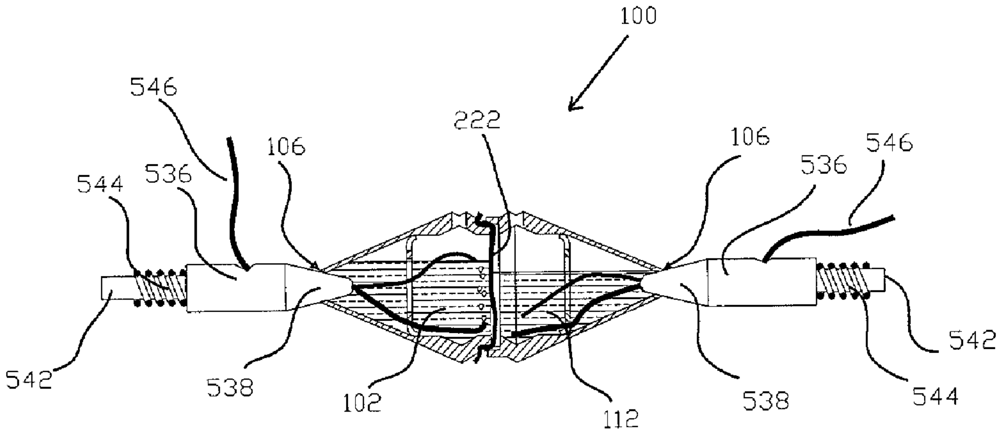

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FIG. 5

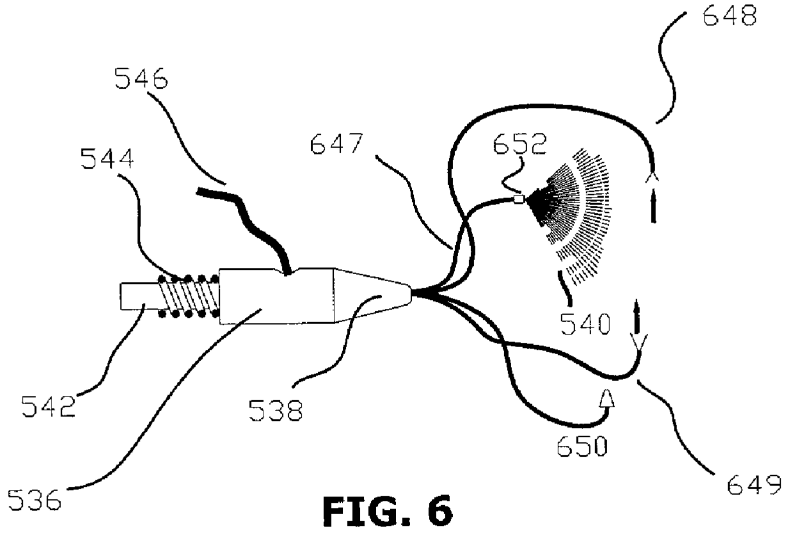
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FIG. 6

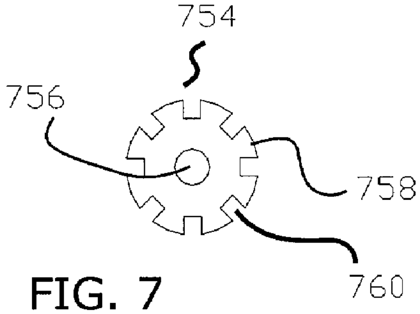
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FIG. 7

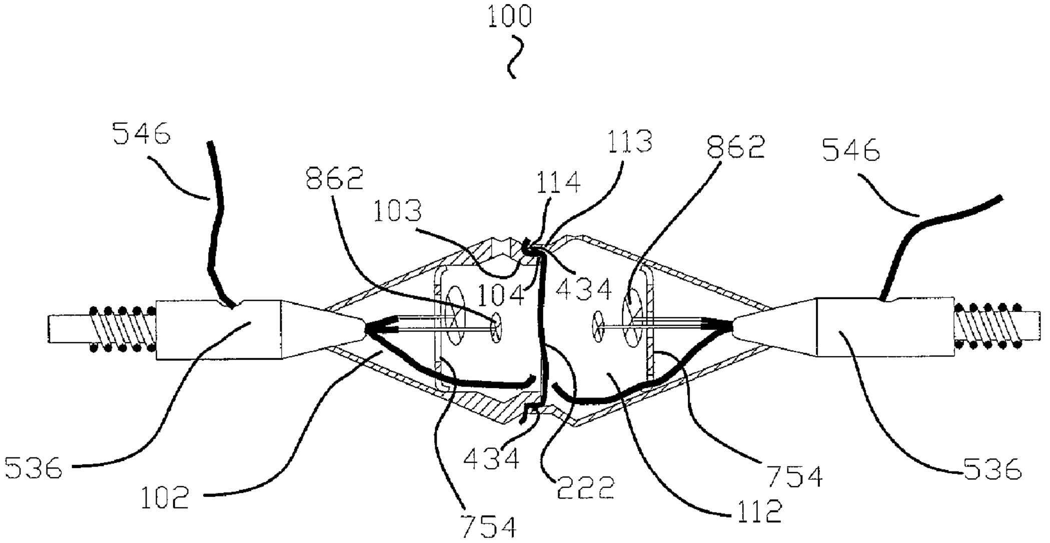
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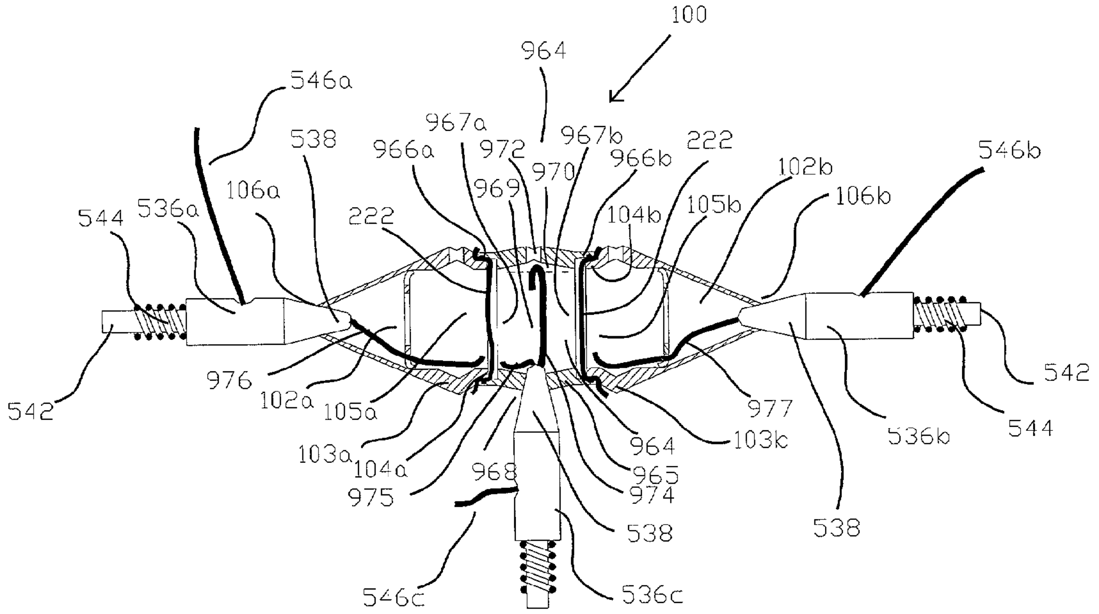
FIG. 8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966b
546b
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536a
222
104b
105b
542
976
102a
105a
977
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968
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546c
536c

FIG. 9

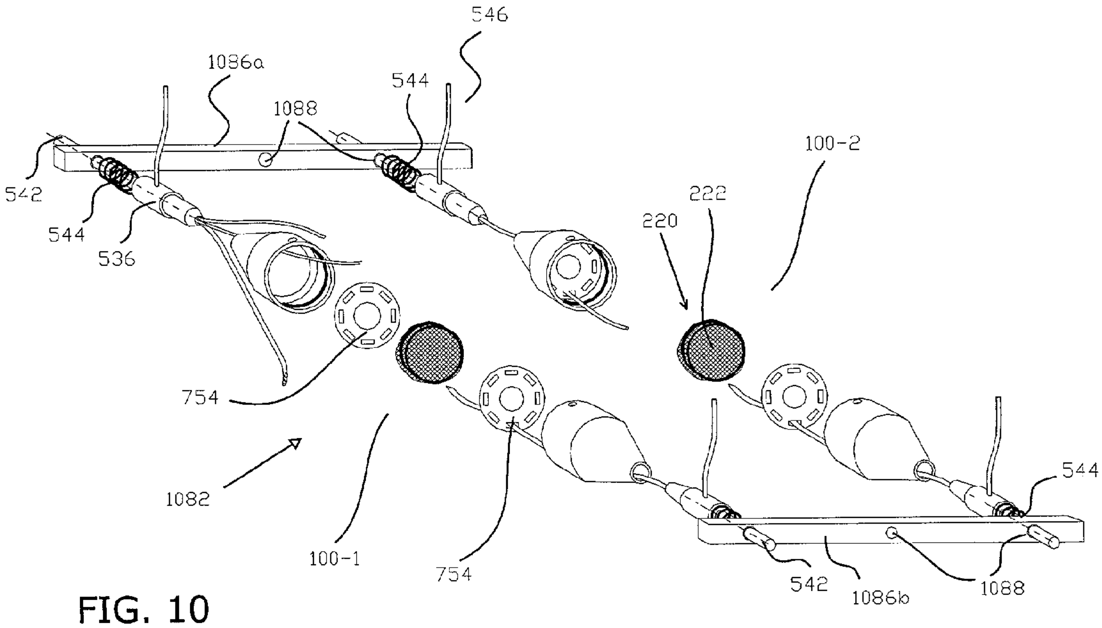
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FIG. 10

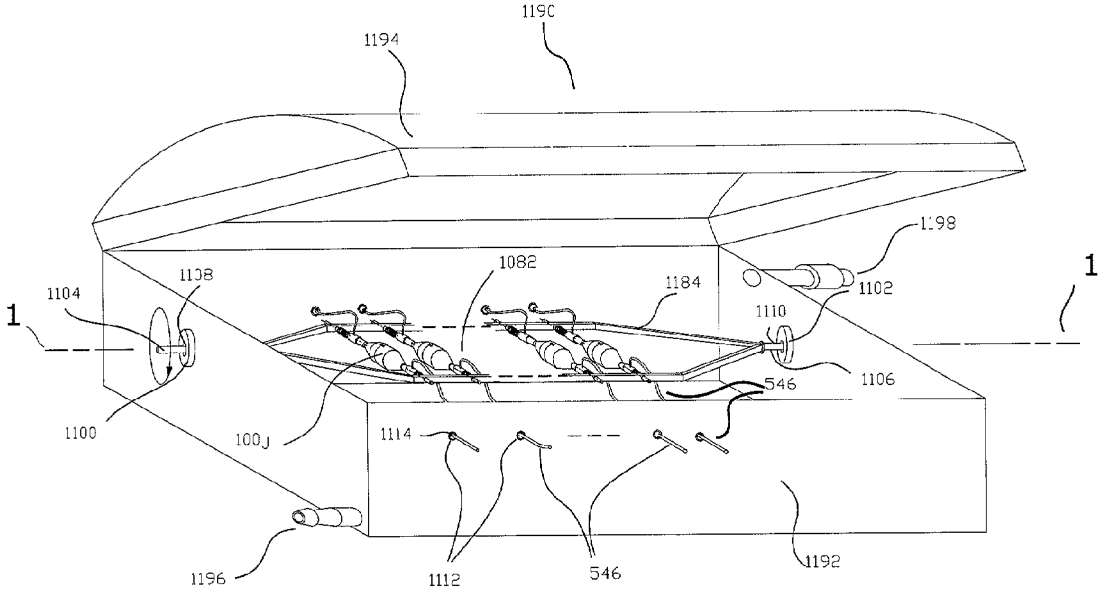
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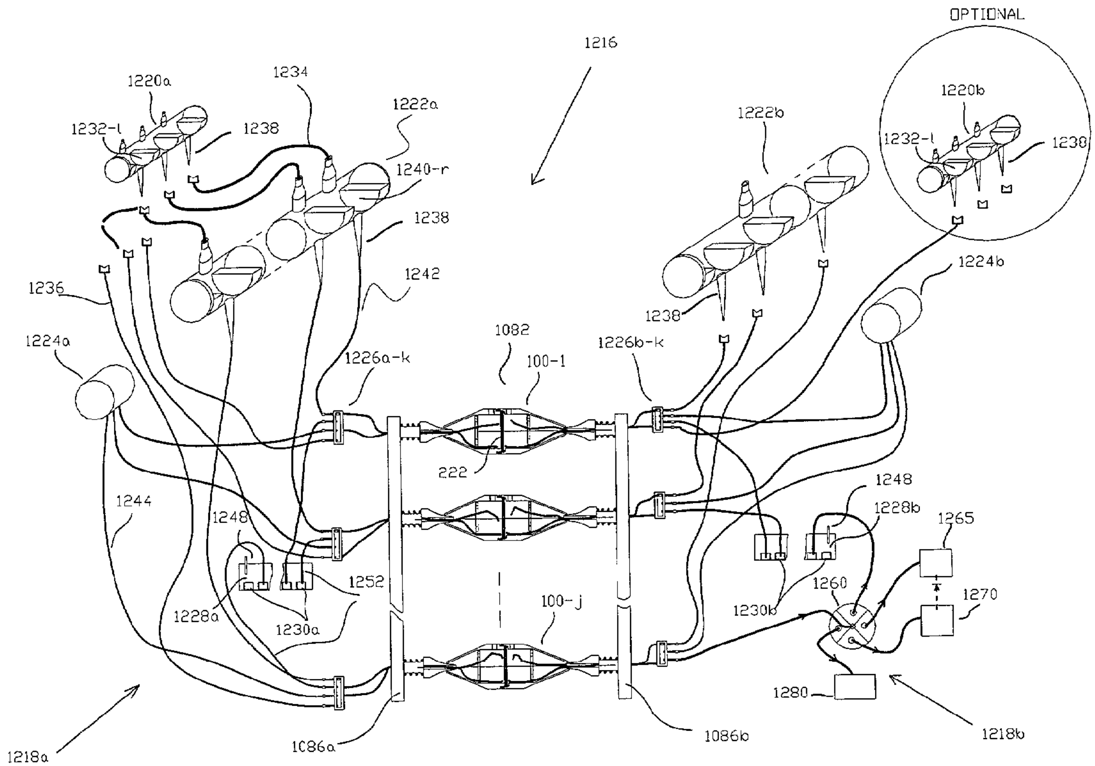
FIG. 11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1238
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1226a-k
1226b-k
222
1244
1248
1248
1228b
1265
1252
1228a
1230a
1230b
1260
1270
100-j
1280
1086a
1086b
1218a
1218b

FIG. 12

APPARATUS AND METHOD FOR HIGH THROUGHPUT ANALYSIS OF COMPOUND-MEMBRANE INTERACTIONS

STATEMENT OF RELATED APPLICATIONS

This case claims priority of U.S. Provisional Application Ser. No. 60/334,332, filed Nov. 29, 2001, entitled "Article and Method for High-Throughput Analysis," which is also incorporated by reference herein.

FIELD OF INVENTION

The present invention relates to high-throughput investigation and screening of compound interactions with live tissue or tissue substitutes.

BACKGROUND OF THE INVENTION

Aggressive research in genomics, functional proteomics and drug discovery has resulted in a large increase in the number of chemical entities ("leads") that have a potential for therapeutic activity. The leads are typically pruned in "pre-clinical screening" studies to select promising candidates for final "clinical studies." Due to the large number of leads to be screened, the pre-clinical screening process has become a bottleneck in the drug discovery process.

During pre-clinical screening, sequential pharmacological transformations of the leads, in conjunction with an organism (e.g., cells, tissues, model animals, etc.,) are evaluated. The evaluation that is typically performed during pre-clinical screening is known as "ADMET" or sometimes "ADME Tox," which is an acronym for Absorption, Distribution, Metabolism, Excretion and Toxicology. The absorption properties of leads are particularly important, and, as discussed later in this section, are particularly problematic to test.

There are generally two approaches to the pre-clinical screening of leads—in vivo testing and in vitro testing using artificial membranes (immobilized artificial membranes) or cell-based permeability methods. In vivo testing is performed within a living organism, while in vitro testing is performed outside of a living organism. Of these two approaches, in vivo testing provides a more accurate analysis of compound absorption and bio-availability during pre-clinical pharmaco-kinetic studies. Unfortunately, the logistics of animal-based studies makes them extremely expensive and time consuming. Furthermore, in vivo studies cannot provide the speed necessary to support high-throughput screening of drug candidates. Even the recently developed "cassette method," wherein multiple compounds (about five to ten) are combined and administered to a single animal, cannot provide the desired productivity. (See, J. Berman et al., J. Med. Chem. 40:827-829 (1997); Dietz et al., U.S. Pat. No. 5,989,918.)

Consequently, the focus in high-throughput screening of drug candidates is on various in vitro techniques (and even computer "in silico" modeling methods). Unfortunately, absorption is a difficult process to model and evaluate using in vitro testing. Specifically, absorption deals with the transportation of compounds through live membranes (e.g., tissues, etc.)—a situation that is difficult to re-create outside of a living organism under test conditions. Absorption studies, therefore, have been at the forefront of current drug-discovery efforts. These efforts have been directed at the development of instrumentation and methodologies that will accelerate the pace at which absorption studies can be performed.

Specifically, the thrust is to accelerate absorption studies to the speed at which the other steps of the drug discovery process are being conducted.

One of the first methods developed for in vitro absorption studies was the "everted sac" technique (T. H. Wilson & G. Wiseman, J. Physiol. 123:116-125 (1954)). The everted sac is an everted (i.e., mucosal surface turned inside-out) segment of intestine, typically 3 to 5 centimeters in length, that is filled with oxygenated buffer solution (i.e., serosal solution contacting the serosal surface) and tied at both ends with sutures. The everted sac is placed in a similar solution (i.e., mucosal solution) and is incubated at 37° C. with continuous aeration. The compound under test may be added to mucosal or serosal solutions depending on what type of transport is being studied (i.e., mucosa→serosal or serosal→mucosal). After incubation is completed, the concentration of the transported compound is estimated in the solutions on both sides of the intestine and in the intestinal mucosa. This simple, reproducible and inexpensive method is used for studying mechanisms of compound transport through the intestine in various regions, as well as for studying compound metabolism by intestinal mucosa (E. S. Foulkes, Proc. Soc. Exper. Biol. Med. 211:155-162 (1996)).

There are, however, certain disadvantages to the everted-sac technique, including low tissue viability and rapid (thirty minute) onset of histological damage in salt mixtures (R. R. Levine et al., Eur. J. Pharm. 9:211-219 (1970)). Another drawback of the technique is that while the serosal chamber, being a closed system, is appropriate for short-term studies, it might not be suited for the evaluation of molecular kinetics during longer-term studies or when investigating drugs that have a high absorption rate. Furthermore, the everted-sac technique is not suited to high throughput analysis.

Another well-known technique and apparatus for in vitro study of absorption is the "Ussing chamber." The Ussing Chamber, like the everted intestinal sac, can be used for investigating the transport of molecules and for measuring electrical parameters at specific sites of the intestine, as well as for the evaluation of intestinal metabolism.

Originally developed for measuring the electric potential across frog skin, the Ussing Chamber consists of two chambers—a donor chamber and a receiver chamber. To measure absorption of a compound, a tissue (e.g., whole intestinal tissue or tissue that is stripped from muscular and serosal layers) is placed between the chambers. The chambers are filled with buffer solutions, wherein the compound under investigation is added to the solution within the donor chamber. After incubation (i.e., exposure of the tissue to the compound-containing solution for a certain length of time and at certain conditions of temperature, pH, etc.), aliquots are taken from the receiving chamber or from both chambers, and then analyzed. (H. H. Ussing & K. Zehran. Acta Physiol. Scand. 23:110-127 (1951)). Many modifications of the classical Ussing chamber are used for oral permeability studies. (See, e.g., U.S. Pat. Nos. 4,667,504, 5,183,760, 5,591,636, and 5,599,688.)

There are a variety of drawbacks to the Ussing Chamber. One drawback is the uncertain tissue viability during incubation in simple salt buffers. In particular, it has been demonstrated that after thirty minutes incubation of intestinal mucosa in buffer solution, fifty percent to seventy-five percent of epithelium disappears and, after one hour, total disruption of the epithelial border can occur. (See, Levine et al., Eur. J. Pharm. 9:211-219, (1970)). Even after only twenty minutes of intestinal tissue incubation in a simple salt medium, severe intestinal edema and disruption of epithelium has been observed (M. Mayersohn et al., J. Pharm. Sci. 60:225-230 (1971)).

A second disadvantage of the Ussing Chamber is that it is inappropriate for high-throughput studies. In particular, when using excised tissue samples (as opposed to using cell-culture inserts, e.g., Transwells™), each intestinal strip dissection and mounting takes between two to four minutes (M. Field et al., Amer J. Physiol. 220:1388-1396 (1971)). This makes simultaneous preparation of multiple tissue samples for absorption problematic.

A third disadvantage of the Ussing Chamber is that the ability to obtain samples from the donor chamber and the receiver chamber is limited. In particular, the chambers are filled with fluid (i.e., gas or liquid) that is sampled from each of the chambers as desired. There is no ability to sample fluids from, or deliver fluids to, specific regions within the chambers (e.g., near the tissue sample, etc.).

A relatively new technique, called the "cell-culture" technique, is capable of study absorption at substantially higher throughputs. This technique has already been adopted for automated high-throughput compound screening and optimization. Unlike most in vitro models, the cell culture method does not require the use of the animals, but, rather, uses specific cell lines that are grown in sterile conditions.

One of the key cell lines used for absorption studies is the Caco-2 cell line. Originating from human colon adenocarcinoma, the Caco-2 cells, after confluency, possess many of the functional and morphological characteristics of normal differentiated enterocytes. Multiple studies show that this method provides comparative information on absorption of different drug molecules.

The Caco-2 cells are cultured in a specially-constructed cell-culture plate. The plate consists of an inner well that is disposed within an outer well. The bottom of the inner well is a semi-permeable membrane. The Caco-2 cells are grown to confluency on the semi-permeable membrane. To evaluate transport, a compound is added to a medium above the Caco-2 cells (i.e., the compound is added to the inner well). Uptake of the compound is determined by quantifying the amount of the compound in the medium on the opposite side of the semi-permeable membrane (i.e., in the outer well).

There are a number of drawbacks to the cell-culture technique using Caco-2 cells. One drawback is the cancerous nature of these cells, which might be indicative of altered cellular properties. Furthermore, it takes several weeks to grow the cells. This delays the beginning of absorption tests and increases the risk of bacterial or fungal contamination of the culture (see, L. Barthe et al., Europ. J. Drug Metab. Phatmacokinet. 23:313-323 (1998); A. P. Li et al., High Throughput Screening. Jan. 6-9 (2001)). Caco-2 cells might also be phenotypically unstable and change their enzyme activity and transporter expression with passage number (K. M. Hillgren et al., Med Res Rev 15:83-109 (1995)).

Another major disadvantage of the cell-culture technique is the slow compound absorption rate. Caco-2 cells are between twenty to forty times less permeable than normal human colon cells (P. Artursson et al., Pharm. Res. 10:1123-1129 (1993)). Other studies showed that for mannitol, the rate of permeation is fifty to three hundred and sixty times lower through the cell mono-layer than through the gut (L. Barthe et al., Europ. J. Drug Metab. Phatmacokinet. 23:313-323 (1998)).

Furthermore, the usefulness of Caco-2 cells has been limited because they do not express appreciable quantities of bio-transformation enzymes, which are present in human small-bowel epithelial cells. This drawback was overcome recently by treating Caco-2 cells with vitamin D analog (see U.S. Pat. No. 5,856,189). Some believe, however, that regardless of cell line, "the constraints in the methodology surrounding preparation and use of these cells prevent them from being classified as truly high-throughput screens for absorption" (M. H. Tarbit & J. Berman. Curr. Opin. Che. Biol. 2:411-416 (1998)).

The predominantly manual techniques described above cannot support high-throughput programmable operations, nor provide ease of setup and control functions. Yet, there is a pressing need to implement high-speed screening of compound-to-membrane interactions in many industries and many areas of research. In the context of pharmacological studies, it represents a large area of research in oral, dermal, pulmonary, nasal, buccal, corneal, and vaginal drug absorption.

Notwithstanding the many screening techniques available, a need therefore remains for a device and method that provides at least some of the following advantageous characteristics:

Provides high-throughput screening ("HTS") with low cost of preparation, operation and maintenance.

Provides high-content screening ("HCS"), which allows multiple in-process sampling and testing that is necessary for time-based kinetic studies.

Enables the use of live tissue as the most relevant substrate for absorption/penetration studies.

Enables the use of artificial membranes of various types when necessary.

Preserves tissue viability by accelerating all steps of the experiment including loading, conditioning and testing.

Provides simultaneous sample loading of all test chambers in order to reduce time and assure valid comparative analysis.

Utilizes small tissue samples, allowing parallel preparation of large numbers of samples from close locations of the same organ, thereby decreasing the effect of absorption gradients due to tissue variation along the small intestine and reducing the required number of donor animals.

Provides selectable and stable testing conditions, which are closely matched in all test chambers of the device.

Maintains, if necessary, an accepted industry-standard number of independent investigative chambers in multiples of 8 or 12 (e.g., 24, 48, 96, etc.).

Maintains in vitro conditions that closely match in vivo conditions, and provides the ability to monitor, alter and "in-process" control these conditions (e.g., temperature, pH, oxygen, etc.).

Has a small physical size for saving bench-top real estate, and is physically adapted for expansion and for the multiplexing of testing chambers.

Provides ease of operation including loading, sampling, cleaning, servicing and maintaining.

SUMMARY OF THE INVENTION

The present invention provides an apparatus and method that avoids some of the drawbacks of the prior art and possesses at least some of the advantageous characteristics listed above. Among other capabilities, the illustrative embodiment of the present invention provides high-throughput and high-content investigation of compound interactions with live tissue or tissue substitutes during compound absorption and related processes. The interactions occur in a controlled environment that closely resembles in-vivo conditions. The invention facilitates the development of new compounds (e.g., drugs, nutrients, nutraceuticals, cosmetics, cosmeceuticals, etc.) for oral, dermal, pulmonary, nasal, buccal, scleral or vaginal application, as well as toxicological evaluation of xenobiotics.

An apparatus for high-throughput analysis in accordance with the illustrative embodiment includes at least one membrane-holding multi-chamber enclosure that is capable of interfacing (e.g., via transfer of fluids, electronically, etc.) with a variety of preparative devices and analytical instrumentation.

Membranes for use in conjunction with the illustrative embodiment of the present invention include a thin sheet of live epithelial tissue such as gastrointestinal, buccal, nasal, corneal, vaginal, lung, and skin or an artificial membrane with or without attached cells from various cell-line cultures. Each individual chamber within the multi-chamber enclosure is advantageously connected to a fluidic-control system by means of conduits and tubes.

In some variations, a multi-chamber enclosure in accordance with the illustrative embodiment of the present invention includes two individual chambers that are interfaced with each other across the membrane. In some other variations, more than two chambers are present.

In some variations of a two-chamber multi-chamber enclosure, the enclosure is formed of two generally conical-shape housings. The housings are open at both ends. At one of the ends of each housing, there is a relatively larger opening ("larger aperture,") and at the other end there is a relatively smaller opening ("small aperture,") consistent with the generally conical shape of the housings. The housings are coupled to one another proximal to the large apertures. The membrane is disposed between the large apertures. With the membrane in place, two chambers are formed within the enclosure, wherein the interior volume that is circumscribed by each housing defines each chamber.

A feed fitting forcefully mates with the small aperture of each of the housings, advantageously disposed at opposite ends of the enclosure. The feed fitting is preferably spring-biased, such as by coupling a spring-biasing element to it. This arrangement provides a number of beneficial features including, among others:

- A way to seal the interface between the feed fitting and the relatively smaller opening in each conical-shaped member.
- A way to introduce one or more tubes into each chamber (i.e., through the feed fittings). The tubes, which enter the feed fitting bundled within a conduit, support fluid (i.e., liquid and/or gas) exchange between the chambers and the fluid control system.
- Mechanical and fluidic integrity to the entire chamber enclosure.

Individual chambers are advantageously internally equipped with a device or arrangement that guides and fixes the plurality of tubes at desired locations in the vicinity of the membrane. Positioning the end of the tubes near the membrane provides efficient delivery and sampling of solvents and buffers, as well as perfusion, gas and fluidic purging and other processes. The other end of each tube is individually connected to the fluidic-control system. The tubes are designed to quantitatively deliver liquids, gases and aerosols of choice, to and from each individual chamber. The chambers can be fully filled with solvent, or the membrane can be sprayed and coated with a high viscosity compound that is introduced as aerosol.

The fluidic control system, which is advantageously programmable, interconnects individual chambers with supply reservoirs and sampling receivers, and/or directly with various analytical instruments and preparative devices. The movement of fluids and gases through the tubes can be controlled by means of pressurization, vacuum or positive-displacement forces.

In some variations of the illustrative embodiment, individual chambers are equipped with one or more electrodes for introducing and/or measuring electric potential across the membrane. The electrodes are electrically isolated and introduced into individual chambers through the openings along with the tubes. The electrodes are advantageously terminated with pliable noble metal (e.g. platinum wire, etc.) and can be shaped and located in accordance with the desired function of applying or measuring electrical potentials or currents.

In some variations of the illustrative embodiment, a plurality of dual-chamber multi-chamber enclosures are mechanically coupled, side-by-side, to a frame. The frame advantageously has two spaced-apart rails against which the spring-biasing elements for each dual-chamber multi-chamber enclosure are referenced. Since the spring-biasing elements are mechanically coupled to the feed fittings, and the feed fittings are received, in at least some variations, on opposite ends of the enclosure, the enclosure is placed in compression by the opposing spring-biasing elements.

In some variations of the illustrative embodiment, a frame having a plurality of multi-chamber enclosures is installed into a mother chamber. The frame is advantageously rotatable, within the mother chamber, about an axis that is orthogonal to the long axis of each multi-chamber enclosure (i.e., each multi-chamber enclosure is capable of being "tilted"). This enables horizontal or vertical alignment of the membranes, as well as gravitational and centrifugal leveling of compounds if required by the investigative protocol (e.g., in a dermal application that models accelerated compound permeation through skin, etc.).

The mother chamber advantageously controls internal environmental conditions using, for example, a flow of one or more liquids, a flow of one or more gases, infrared heating, or other techniques that are customarily employed in various incubation and environmental chambers, or any combinations of all of the above.

In some embodiments, operation of the multi-chamber enclosure involves fully-controlled introduction of compounds, buffers, gases, or aerosols into individual chambers, as well as fully-controlled sampling of materials from any individual chamber. Sampling and inter-connecting the fluidic control system with analytical instrumentation is performed in known fashion, by any of a number of techniques, apparatuses and arrangements that are known to those skilled in the art.

The filling and evacuation of solvents, as well as agitation, purging and related functions that occur inside the multi-chamber enclosures are conducted by controlling the flow of solvents and gases through the tubes into and out of the individual chambers. In some embodiments, each individual chamber is equipped with gas/solvent escape vents, advantageously located at the top of each chamber, that enable free filling and evacuation of gases and solvents. As a function of the investigative process, individual chambers function either as a supply chamber or as a receiving chamber.

Rapid preparation and mounting of selected types of live tissues is a result of the relative ease with which membranes can be simultaneously installed into multiple individual chambers. Thus, for example, adjacent areas of intestine or other areas of gastro-intestinal tract, which have minimum tissue-property variations, can be investigated in parallel fashion. High-speed preparation is advantageous, if not essential, for sustaining high throughput for the entire absorption-testing process. High-speed preparation of test membranes also aids in maintaining tissue viability, which is necessary for obtaining reliable results with minimum variations due to altered or diminishing tissue properties. Tissue viability is inversely proportional to the time of its autonomous existence.

The multi-chamber enclosure enables the evaluation of compound fluxes through the membrane from an arbitrarily designated "supply" chamber to a "receiving" chamber and vice versa, and the evaluation of compound transformations within the individual chambers and the membrane. The multi-chamber enclosure is further useful for the analysis of, among other phenomena:

1. The mechanism of compound uptake (accumulation) in tissue.
2. The mechanism of compound transport through the tissue.
3. The kinetics of compound uptake and transport.
4. First-pass metabolism in tissue under investigation.
5. The cell excretion system: P-glycoprotein and its expression in the tissue.
6. Trans-membrane current and potential.
7. The effect of absorption inhibitors and enhancers.
8. Pharmacologic antagonism in toxicity studies.
9. Various compound (e.g., nutrients, drugs, nutraceuticals, cosmetics, cosmeceuticals, other xenobiotics) interactions during their absorption.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** depicts an exploded, cross-sectional side-view of a multi-chamber enclosure having two housings, which, in conjunction with a membrane, define two chambers, in accordance with the illustrative embodiment of the present invention.

FIG. **2**A depicts a view of a membrane-holding assembly, with membrane attached, for use in conjunction with the illustrative embodiment of the present invention.

FIG. **2**B depicts an exploded view of the membrane-holding assembly of FIG. **2**A.

FIG. **2**C depicts a cross-sectional view of the membrane-holding assembly of FIG. **2**B with O-ring fastener.

FIG. **3**A depicts a cassette having a plurality of individual membrane-holding frames.

FIG. **3**B depicts the cassette of FIG. **3**A with a strip of unprepared membrane overlying the membrane-holding frames.

FIG. **3**C depicts the cassette of FIG. **3**A wherein a plurality of linked fasteners are positioned to secure the membrane to cassette.

FIG. **4** depicts an enlargement of the chamber-chamber interface of the dual-chamber, multi-chamber enclosure of FIG. **1**.

FIG. **5** depicts an assembled, cross-sectional view of the dual-chamber, multi-chamber enclosure of FIG. **1** including feed fittings and conduits containing tubes for delivering fluid to and receiving fluid from individual chambers of the multi-chamber enclosure.

FIG. **6** depicts a feed fitting and conduit containing a plurality of tubes.

FIG. **7** depicts a guide for supporting and positioning tubes with a chamber.

FIG. **8** depicts a dual-chamber, multi-chamber enclosure having electrodes, and a directly-mounted (no frame) membrane, in accordance with a variation of the illustrative embodiment of the present invention.

FIG. **9** depicts a triple-chamber, multi-chamber enclosure that uses two membranes, in accordance with a variation of the illustrative embodiment of the present invention.

FIG. **10** depicts an array of multi-chamber enclosures within a frame.

FIG. **11** depicts a mother chamber containing a plurality of multi-chamber enclosures.

FIG. **12** depicts a fluid control system for use in conjunction with a multi-chamber enclosure.

DETAILED DESCRIPTION

The terms listed below are given the following definitions for use in this specification.

Absorption enhancer is a chemical entity (compound) that facilitates the absorption of a tested compound.

Absorption inhibitor is a chemical entity (compound) that inhibits the absorption of a tested compound.

Apical membrane is a part of the cell plasma membrane of polarized cells facing the external environment.

Baso-lateral membrane is a part of the cell plasma membrane covering the base and sides of the polarized cells.

Buccal means related to the mouth or hollow part of the cheek.

Communicates means that one element is able to receive information or material from another element. For example, an opening that communicates with a chamber is capable of receiving fluid from or delivering fluid to the chamber.

Confluency means that all cells in a tissue culture are in complete contact with other adjacent cells and no available substrate is left uncovered with cells.

Cosmeceutical is a cosmetic product whose active ingredients have a beneficial physiological effect in comparison with inert cosmetic. Examples of cosmeceuticals include, without limitation, vitamins, minerals, antioxidants and other biologically-active substances.

Coupled means directly or indirectly connected. In some cases, coupled elements do not physically contact one another. For example, two housings that do not touch one another because they are separated by a gasket, but otherwise collectively form an enclosure, are properly characterized as "coupled" under this definition. In other cases, two elements that are in physical contact with one another are properly characterized as "coupled" for the purpose of this specification.

Epithelial tissue is the tissue covering the surface of the skin and lining every canal, tract (e.g., gastrointestinal tract, etc.) and cavity that communicates with the external environment.

Enterocytes are absorptive cells of the small intestine.

First-pass metabolism is drug bio-transformation during its passage through the intestinal epithelium. This process can markedly decrease the amount of an administered drug that is actually available to the body.

Incubated means kept at constant conditions such as, for example, temperature, oxygenation, etc.

In vivo refers to biological processes that occur inside the body of a living organism.

In vitro refers to biological processes that occur outside the body (e.g., in a test tube, etc.).

Mechanically coupled means that a first element is capable of imparting a force to a second element, regardless of whether or not the two elements are in physical contact with one another. For example, consider a spring that is connected to a fitting that is, in turn, connected to a housing. It is proper, under this definition, to characterize the spring as being mechanically coupled to the housing.

Mucosal tissue (mucosa) is the moist membrane lining the surface of many tubular structures and cavities (e.g. mouth, esophagus, small intestine). Along with other types of cells, mucosal tissue contains cells that secrete mucus.

Nutraceutical is a nutritional supplement. Examples of nutraceuticals include, without limitation, vitamins, minerals, polyunsaturated fatty acids, antioxidants, probiotics and other biologically active substances.

P-glycoprotein is a membrane-localized drug-transport mechanism that has an ability to actively pump drugs out of a cell.

Pharmacologic antagonism means that one drug opposes the action of another drug (e.g., preventing it from combining with its receptor, etc.).

Sclera is a white fibrous outer layer of the eyeball.

Serosal membrane (serosa) is a smooth transparent membrane that lines certain large cavities of the body and that covers some of its organs (e.g., gastrointestinal tract, etc.).

Synthetic tissue or tissue substitute is a thin layer of artificial membrane having a selected permeability. It is used for compound permeability studies or as a substrate for cell attachment in tissue culture. Examples of synthetic tissue (membranes—I.F.) include, without limitation, nitrocellulose, nylon, polypropylene, other synthetic polymers, etc.

Xenobiotic is a foreign compound that is toxic, at some dose, to at least some living organisms. Examples of xenobiotics include, without limitation pesticides, herbicides, fungicides, and the like.

FIG. **1** depicts an exploded, cross-sectional side-view of a multi-chamber enclosure **100** having two chambers **102** and **112**, in accordance with the illustrative embodiment of the present invention. In the illustrative embodiment, chambers **102** and **112** are defined by respective, generally conical-shape housings **103** and **113**, in conjunction with membrane **222**. Each of housings **103** and **113** has two open ends. More particularly, conical-shape housing **103** includes large aperture **105** and small aperture **106**. Large aperture **105** is formed at marginal region **104** of conical-shape housing **103**. Similarly, conical-shape housing **113** has large aperture **115** and small aperture **106**. Large aperture **115** is formed at marginal region **114** of conical-shape housing **113**.

Membrane **222**, which is advantageously secured in membrane-holding assembly **220**, is disposed between large aperture **105** of conical-shape housing **103** and large aperture **115** of conical-shape housing **113**. Membrane **222** therefore separates and seals chambers **102** and **112** from one another. (Recall that FIG. **1** depicts an exploded view; when assembled, the gaps that are depicted in FIG. **1** between membrane-holding assembly **220**/membrane **222** and the large apertures **105** and **115** do not exist.)

In some variations of the illustrative embodiment, membrane **222** is live tissue. For example, in oral absorption studies, intestinal tissue is used, or alternatively, the mucosa of intestinal tissue, stripped from underlying layers, can suitably be used. In other absorption studies, skin, buccal, nasal, pulmonary, corneal, vaginal or other tissues can be used. In further variations, the membrane is a tissue substitute (i.e., synthetic tissue), such as nitrocellulose, nylon, polypropylene, etc. In yet additional variations of the illustrative embodiment, the membrane is a mono-layer of cells from a cell line that is grown on a surface of a membrane.

Referring now to FIGS. **2**A (assembled view), **2**B (exploded view), and **2**C (cross-section of FIG. **2**A), membrane-holding assembly **220** includes membrane-holding frame **224** and fastener **228** (e.g., O-ring, etc.). Membrane-holding assembly **220** receives membrane **222** in a diaphragm-like manner (e.g., as a drum head is received by the body of a drum, etc.). The membrane is held in place via fastener **228**, which references itself through membrane **222** into groove **226** on membrane-holding frame **224**.

Membrane **222** is advantageously prepared by positioning a tissue, such as an intestinal segment (free of Payer's patches) over one or more membrane-holding frames **224** and then fastening, cutting and trimming the intestinal segment to size. In the illustrative embodiment, membrane **222** is depicted as having a circular shape; however, in other embodiments, membrane **222** can have any shape that is suitable for cooperation with membrane-holding assembly **220**. The size of membrane **222** depends on the investigative process. Typically, membrane **222** has a diameter that is typically in a range from about 0.25 inches to about 0.5 inches.

FIG. **3**A depicts cassette **330**, which has a plurality of membrane-holding frames **224** that are linked to one another by holding bridge **332**. As suits the requirements of a given application, unprepared membrane (e.g., not yet sized, etc.) **223** is positioned over the plurality of membrane-holding frames **224**, as depicted in FIG. **3**B. The membrane is then attached to each of membrane-holding frames **224** by fasteners **228** (not depicted in FIG. **3**B). In some other variations of the illustrative embodiment, prefabricated (e.g., sized, etc.) segments of membrane are positioned and attached to each of membrane-holding frames **224**.

In yet some further variations, membrane is simultaneously attached to each of the membrane-holding frames **224** in cassette **330**. This is depicted, via an exploded view, in FIG. **3**C, wherein after unprepared membrane is positioned over membrane-holding frames **224**, a plurality of fasteners **228**, which are linked together by holding bridge **332**, secure the membrane to cassette **330**.

The membrane-holding frames can be separated during installation into multi-chamber enclosure **100**, as described later in this specification. Cassette **330** thereby facilitates rapid preparation of a plurality of membranes **222** and rapid attachment of the membranes to membrane-holding frames **224**. And this rapidity, in turn, aids in the preservation of biological activity of membrane **222**. Those skilled in the art of high-speed assembly will appreciate that this method of placing and fixing a relatively softer material (i.e., membrane **222**) onto a rigid frame (i.e., membrane-holding frame **224**) is indicative of a process that can be readily automated.

As desired, after membrane **222** has been mounted onto a single membrane holding frame **224** or cassette **330**, it can be prepackaged in preservation solutions under appropriate conditions for future insertion into multi-chamber enclosure **100**.

FIG. **4** depicts an enlarged view of the interface between chamber **102**, chamber **112** and membrane-holding assembly **220**. As depicted in FIG. **4**, large aperture **105** of housing **103** is slightly smaller than large aperture **115** of housing **113**. Specifically, the inner diameter of marginal region **114** of housing **113** is sized to receive the outer diameter of marginal region **104** of housing **103**. Chambers **102** and **112** can then be isolated from one another by a fluid-tight seal that is created via fastener **228** and chamber seal **434** (e.g., O-ring, etc.) in conjunction with membrane **222**. In particular, membrane-holding frame **224** (and membrane **222**) is sealed by fastener **228** against inner diameter of marginal region **114** (of housing **113**). And, also, chamber seal **434** seals the outer diameter of marginal region **104** (of housing **103**) against the inner diameter of marginal region **114**.

With continued reference to FIG. **4**, and with reference to FIG. **1**, the diameter of each member increases moving from marginal region **104** to region **107** (on housing **103**), and from marginal region **114** to region **117** (on housing **113**). This increase in diameter in each member provides a region within each chamber that has a relatively expanded volume. This configuration enables membrane **222** to be fully submerged while maintaining a small, liquid-free region **108** in chamber **102** and a small, liquid-free region **118** in chamber **112**. Vent **109** is advantageously located in the "uppermost" location within regions **108** and **118**, thereby enabling free exchange of fluids out of chamber **102** and chamber **112**, as appropriate.

FIG. **5** depicts a cross-sectional side view of multi-chamber enclosure **100** of FIG. **1** in an assembled state. As depicted in FIG. **5**, each small aperture **106** receives tapered region **538** of feed fittings **536**, and is suitably sized for this purpose. A spring-biasing element advantageously mechanically couples to each feed fitting **536**. In the illustrative embodiment, the spring-biasing element is holding spring **544**; however, other arrangements capable of providing a force that is directed toward multi-chamber enclosure **100**, as are known to those skilled in the art, can suitably be used. This force maintains the mechanical integrity and fluidic seals between all interfaces throughout multi-chamber enclosure **100**. Holding spring **544** is received by spring-holding extension **542**.

Feed fittings **536** hermetically accommodate conduits **546**. A seal (not depicted) prevents leakage of any fluid in the space between conduit **546** and a hole (not depicted) in feed fitting **536** that receives conduit **546**. Conduits **546**, in conjunction with a fluidic-control system described later in this specification, introduce liquid and gases into chamber **102** and **112**, as desired, and also retrieve liquids and gases from these chambers. In the variation depicted in FIG. **5**, chamber **102** serves as a feed chamber, which receives a compound. Chamber **112** is a receiving chamber that receives however much of the compound that passes from chamber **102** through membrane **222** and into chamber **112**.

With continuing reference to FIG. **5**, and with reference to FIG. **6**, conduit **546** advantageously carries a plurality of tubes, such as tubes **647-650**, which are shown emerging from tapered region **538** of feed fitting **536**. An opening (not depicted) is located at the end of tapered region **538** for this purpose. Although four tubes are depicted in FIG. **6**, it will understood that in other variations of the illustrative embodiment, more than four tubes or fewer than four tubes, as suits a particular application, are suitably contained in each conduit **546**.

In some variations of the illustrative embodiment of the present invention, each tube (e.g., tubes **647-650**, etc.) performs a different function. For example, in the variation depicted in FIG. **6**, tube **648** removes fluid from a chamber, tube **649** introduces fluid into a chamber; and tube **647**, which is terminated with nebulizing nozzle **652**, produces an aerosol or other spray pattern. Various methods of generating spray patterns using, for example, nebulizing nozzles, are well known in the art. In one method, specially-shaped nozzles spray pressure-fed liquids. In another method, a pressurized gas is used in conjunction with an adjacent liquid-supplying nozzle. Miniature spray nozzles, which are typically made from metal, glass, ceramic and other materials, are commercially available from Microglass Co. of Keene, Tex., Misty Mate Inc. of Gilbert, Ariz., and others.

In some variations of the illustrative embodiment, it is advantageous to position the various tubes (e.g., tubes **647-650**) in different locations and/or in different orientations within a chamber (e.g., chambers **102** and **112**). The position and orientation of the tube will, in some cases, be dictated by the desired function of the tube. Some of the functions that are provided by the tubes include, without limitation:

Filling individual chambers with selected solvents at selected rates while maintaining the desired position of the tube.

Evacuating any amount of fluid from either or both of the individual chambers **102** and **112**.

Injecting gas or gas mixtures as might be required to sustain a proper environment (e.g., an oxygen/carbon dioxide mixture), to remove stagnant layers, to agitate, etc.

Generating an aerosol-type spray to evenly coat membrane **222**.

Consequently, as might be required for some variations of the illustrative embodiment, it is advantageous to provide a device for fixing each tube in a desired location and orientation within the chamber.

One device suitable for accomplishing this function is guide **754**, which is depicted in FIG. **7**. In use, guide **754** is disposed within chamber **102** and/or **112** (see, e.g., FIG. **8**; also depicted in FIGS. **1**, **5** without identification). Guide **754** has central opening **756**, and has a castellated perimeter that is defined by spaced tabs **758**. Central opening **756** and slots **760** between adjacent tabs **758** can be used to position a tube in a desired location and orientation within chambers **102** and **112**. Tabs **758** of guide **754** engage the inside surface of the wall that defines the chambers **102** or **112**, thereby fixing guide **754** in place.

A second device for positioning and orienting individual tubes (e.g., tubes **647-650**) is a "shape memory" insert (not depicted). This type of insert can be formed from a soft metal (e.g. copper, lead, etc.) that is coated by an inert material (e.g., teflon, polypropylene, etc.) or that is sealed inside soft (e.g., Teflon™, etc.) tubing and attached to one or more locations along the exterior surface of the tubes.

FIG. **8** depicts a further variation of the illustrative multi-chamber enclosure. In multi-chamber depicted in FIG. **8** (which is again a dual-chamber arrangement), membrane **222** is not fitted to a membrane-holding frame (e.g., membrane-holding frame **224**). Rather, membrane **222** is mounted directly between chambers **102** and **112**. This can be done, for example, by positioning membrane **222** so that it overlaps the outer diameter of marginal region **104** of conical-shaped housing **103**. As marginal region **114** (of conical-shape housing **113**) receives marginal region **104** (of conical-shaped member **102**) to join housings **103** and **113**, chamber seal **434**, which is disposed against the inner diameter of marginal region **114**, presses against membrane **222**, thereby creating a seal.

In the variation depicted in FIG. **8**, a multi-chamber enclosure in accordance with the illustrative embodiment includes electrodes **862**. Electrodes **862** supply and/or measure electrical potential and the resulting current across said membrane **222**, thereby providing a capability to investigate electrical characteristics of membrane transport.

Electrodes **862** are hermetically introduced into individual chambers **102** and **112**, as desired, through feed fittings **536**. Electrodes **862** are electrically insulated from all other parts of the multi-chamber enclosure. Electrodes **862** can be, for example, platinum wire that is shaped (e.g., manually) into a desired configuration for generating or measuring an electrical field. In one such configuration that is depicted in FIG. **8**, electrodes **862** have an "umbrella" configuration. The umbrella configuration is desirable for distributing an electrical field across the entire surface of membrane **222**. Distributing the electrical field across the entire surface of membrane **222** aids in obtaining results that are independent of local fluctuations in conditions along the membrane.

In the variations of a multi-chamber enclosure **100** that are depicted in FIGS. **1**, **5** and **8**, the multi-chamber enclosure has two chambers **102** and **112** (i.e., it is a "dual-chamber multi-chamber enclosure"). It will be understood that in some other variations of the illustrative embodiment, more chambers are present.

For example, FIG. **9** depicts a tri-chamber multi-chamber enclosure in accordance with a variation of the illustrative embodiment. The multi-chamber enclosure depicted in FIG. **9** has two conical-shape housings **103***a* and **103***b* that flank cylindrical-shape housing **965**.

Conical-shape housings **103***a* and **103***b* are configured like conical-shape housing **103** that is depicted in FIGS. **1**, **5** and **8**. In the tri-chamber multi-chamber enclosure depicted in FIG. **9**, two membranes **222** are used to form and seal the chambers. Like the variation of the illustrative embodiment that is depicted in FIG. **8**, membranes **222** are mounted, without a membrane-holding frame, at each conical-shape housing/cylindrical-shape housing interface.

In conjunction with one of the membranes **222**, conical-shape housing **103***a* defines chamber **102***a*. In conjunction with the other of membranes **222**, conical-shape chamber **103***b* defines chamber **102***b*. In conjunction with both of the membranes **222**, cylindrical-shape housing **965** defines chamber **964**. Thus, one of the membranes **222** isolates chamber **102***a* from chamber **964**, and the other of the two membranes **222** isolates chamber **102***b* from chamber **964**.

In some variations, chamber **964** functions as a feed chamber to introduce fluids into the tri-chamber multi-chamber enclosure, and chambers **102***a* and **102***b* are receiving chambers that receive compounds that cross membranes **222**.

Cylindrical-shape housing **965** has two large apertures **967***a* and **967***b* that are formed at respective marginal regions **966***a* and **966***b*. Large aperture **967***a* of cylindrical-shape housing **965** is slightly larger than large aperture **105***a* of conical-shape housing **103***a*. Similarly, large aperture **967***b* of cylindrical-shape housing **965** is slightly larger than large aperture **105***b* of conical-shape housing **103***b*. More particularly, the inner diameter of marginal region **966***a* (of cylindrical-shape housing **965**) has a size that is appropriate for receiving the outer diameter of marginal region **104***a* (of housing **103***a*). And, likewise, the inner diameter of marginal region **966***b* (of cylindrical-shape housing **965**) has a size that is appropriate for receiving the outer diameter of marginal region **104***b* (of housing **103***b*).

As in the variations of multi-chamber enclosure **100** that depicted in FIGS. **1**, **5** and **8**, conical-shape housings **103***a* and **103***b* have respective small apertures **106***a* and **106***b*. Apertures **106***a* and **106***b* accommodate tapered region **538** of respective feed fittings **536***a* and **536***b*. Cylindrical-shape housing **965** includes small aperture **968** that is oriented along an axis that is orthogonal to the axis that aligns with small apertures **106***a* and **106***b*. Small aperture **968** accommodates tapered region **538** of feed fitting **536***c*. Each of the three feed fittings **536***a* through **536***c* receives respective conduits **546***a*, **546***b* and **546***c*. As described in conjunction with previous variations of the illustrative embodiment, the conduits **546***a* through **546***c* advantageously each carry a plurality of tubes. In FIG. **9**, conduit **546***c* delivers tubes **974** and **975** to chamber **964**. Tube **974** is for aspirating (removing) fluid from chamber **964**, and tube **975** is for delivering fluid to chamber **964**. Conduit **546***a* delivers tube **976** to chamber **102***a* and conduit **546***b* delivers tube **977** to chamber **102***b*. Tubes **976** and **977** are for aspirating fluid from chambers **102***a* and **102***b*, respectively.

The diameter of cylindrical-shape housing **965** increases to a maximum at region **969** near the mid-point between the two large apertures **967***a* and **967***b*. This increase in diameter provides a region having a relatively expanded volume. As previously described in conjunction with other variations of the illustrative embodiment, this configuration enables membranes **222** to be fully submerged while maintaining a small, liquid-free region **970** in chamber **964**. Vent **972** is advantageously located in the "uppermost" location within region **970** to enable free exchange of fluids out of chamber **964**, as appropriate.

FIG. **10** depicts, via an exploded view, an array **1082** of multi-chamber enclosures **100**-*j*, where j=1, n, where n is an integer. The multi-chamber enclosures depicted in array **1082** are the dual-chamber multi-chamber enclosure depicted in FIGS. **1**, **5** and **8**. For clarity, only two multi-chamber enclosures **100-1** and **100-2** of the array **1082** are depicted in FIG. **10**. It will be understood that array **1082** can comprise any desired number, n, of multi-chamber enclosures **100**. General rules of the biochemical industry usually dictate, however, that this number, n, should be a multiple of eight or twelve for ease of interfacing with multi-well plates. Furthermore, it is understood that in other variations of the illustrative embodiment, array **1082** consists of other variations of multi-chamber enclosure **100**. In still further embodiments, different variations of multi-chamber enclosure **100** are included within a single array.

The variations of multi-chamber enclosure **100** in accordance with the illustrative embodiment of the present invention that have been described herein and depicted in the accompanying drawings (e.g., FIGS. **5** and **8**) show small apertures **106** (of housings **103** and **113**) receiving spring-biased feed-fittings **536**. This arrangement is particularly advantageous. Specifically, in addition to providing a way to introduce tubes into the multi-chamber enclosure, and in addition to any other benefits, this arrangement provides a way to easily:

seal the multi-chamber enclosure; and

install the multi-chamber enclosure into a surrounding structure.

These points are illustrated in FIG. **10**. In FIG. **10**, multi-chamber enclosures **100-1** and **100-2** are depicted in "exploded" fashion, showing feed fittings **536**, the conical-shape housings, guides **754**, membrane-holding assembly **220**, and membrane **222**. Conduits **546**, which are received by feed fittings **536**, deliver tubes to the multi-chamber enclosures.

As depicted in FIG. **10**, each multi-chamber enclosure **100**-*j* is mounted to two spaced-apart rails **1086***a* and **1086***b*. The rails compose frame **1184** (see FIG. **11**). Rails **1086***a* and **1086***b* each include a plurality of openings **1088**. Openings **1088** receive a spring-holding extension **542** that depends from each feed fitting **536**. Spring-holding extensions **542** accommodate a spring-biasing element, such as holding springs **544**, which are referenced against rails **1086***a* and **1086***b* and exert a force that is directed towards multi-chamber enclosures **100**-*j*. It is seen that for each multi-chamber enclosure **100**-*j*, the spring-biasing elements (e.g., springs **544**, etc.), feed fittings **536**, small apertures **106** are all aligned with an axis passing through spring-holding extensions **542** (see, e.g., FIG. **5**). This arrangement assures the mechanical integrity and hermetic seal of each multi-chamber enclosure **100**-*j*. Also, the use of holding springs **544** enables the installation of fully assembled individual multi-chamber enclosures **100** in frame **1184** (see, e.g., FIG. **11**).

Furthermore, this arrangement (i.e., frame **1184**, holding springs **544**, feed fittings **536**, the enclosure halves, etc.) is advantageously used in conjunction with cassette **330** (see FIGS. **3**A through **3**C) to rapidly install membranes **222** into each multi-chamber enclosure **100**-*j*. In particular, each membrane-holding frame **224**, with membrane **222** attached, is sandwiched between the housings. Once multi-chamber enclosures **100**-*j* are sealed via the force applied by holding springs **544**, holding bridge **332** that is disposed between adjacent membranes **222** is severed, as desired.

For a tri-chamber multi-chamber such as is depicted in FIG. **9**, a third rail (not depicted) is advantageously provided in frame **1184**. The third rail is configured in the manner of rails **1086***a* and **1086***b* to provide openings that receive spring-holding extension **542** that depends from each feed fitting **536**. Each spring-holding extension **542** accommodates a spring-biasing element that is referenced against the third rail so that feed fitting **546***c* (see, FIG. **9**) is forced into small aperture **968**.

FIG. **11** depicts array **1082** of multi-chamber enclosures **100**-*j* (four are shown) retained by frame **1184** and located inside mother chamber **1190**. The mother chamber provides one or more of the following benefits, among any others:

- protects the multi-chamber enclosures;
- provides a fully-controllable environment for the multi-chamber enclosures; and
- orients and rotates said chamber assemblies allowing for the preferred distribution of substances within said chamber assemblies.

Mother chamber **1190** includes base **1192** and cover **1194**. Base **1192** of mother chamber **1190** includes fittings **1196** and **1198**. These fittings function, in some variations, as a liquid inlet (typically fitting **1196**) and liquid outlet (typically **1198**) to enable a temperature-controlled liquid to be circulated through base **1192**. In this way, mother chamber **1190** provides thermal control of submerged array **1082** of multi-chamber enclosures **100**-*j*.

In the variation depicted in FIG. **11**, frame **1184** is received by base **1192** through two openings **1100** and **1102**. More particularly, support members **1104** and **1106** that depend from frame **1184** are received by openings **1100** and **1102**. Seals **1108** and **1110** are provided to prevent leakage of liquid (e.g., temperature-controlled liquid, etc.) from base **1192** through openings **1100** and **1102**.

It is advantageous to have a capability to rotate array **1082** of multi-chamber enclosures **100**-*j* within mother chamber **1190**. For example, in conjunction with some studies, it might be necessary to place multi-chamber enclosures **100**-*j* in a vertical orientation (i.e., orthogonal to the orientation depicted in FIG. **11**), or subject multi-chamber enclosures **100**-*j* to a "centrifugal" force.

To this end, frame **1184** and openings **1100** and **1102** are physically adapted to enable frame **1184** and the captive multi-chamber enclosures **100**-*j* to rotate about axis **1**-**1**. For example, in such variations, support members **1104** and **1106** advantageously have a cylindrical shape and a smooth surface. Additionally, openings **1100** and **1102** can be fitted with nylon grommets, bearings, etc., to promote free rotation. A device, such as a stepper motor (not shown) for rotating frame **1184**, is advantageously provided. Typically, the stepper motor, etc., is mechanically coupled to one of support members **1104** and **1106** to rotate frame **1184**.

In the variation depicted in FIG. **11**, openings **1112** receive conduits **546** which, as previously described, provide for exchange of fluids between multi-chamber enclosures **100**-*j* and the fluidic control system, described below. Seals **1114** prevents liquid from leaking out of base **1192** through openings **1112**.

Conduits **546** enter mother chamber **1190** from a direction that is orthogonal to the axis of rotation **1**-**1**, as depicted in FIG. **11**. Consequently, the rotation of the multi-chamber enclosures **100**-*j* is limited, practically, to less than a full rotation. (That is, the rotation of frame **1184** would require a substantial amount of slack and excess conduit **546** that would wrap around frame **1184** as the frame rotates.) For most applications, this limited amount of rotation will not be a problem. If, however, a capability for farther rotation is desired, a single opening (not shown) that receives all conduits **546** is advantageously provided at a location that is very close to opening **1100** or **1102**. Since, in this variation, conduits **546** are aligned with axis of rotation **1**-**1**, some multiple number of rotations of the frame **1184** can be tolerated without requiring a large excess of slack conduit **546**.

FIG. **12** depicts an embodiment of fluidic control system **1216** for delivering fluid to and receiving fluid from multi-chamber enclosures **100**-*j* (three are shown), and for interfacing with sampling devices and other types of instrumentation. Fluidic control system **1216** places all multi-chamber enclosures **100**-*j* in array **1082** in fluid communication with all liquid and gas sources and receivers that are required for conducting required tests. It will be appreciated that the functionality, as described below, provided by the specific embodiment of fluidic control system **1216** that is depicted in FIG. **12** can be provided by many different arrangements, as can be devised by those skilled in the art in view of the present teachings. For example, precise dosing equipment, well-known in the art, can be used instead of some of reservoirs and fluid switches that compose system **1216** shown in FIG. **12**. Consequently, fluidic control system **1216** that is depicted in FIG. **12** is presented by way of illustration, not limitation.

With reference to FIG. **12**, fluidic control system **1216** is divided into supply-side system **1218***a* and receiving-side system **1218***b*. This division is based on the designation of one of the chambers in the multi-chamber enclosure as a "supply" chamber and the other chamber as a "receiving" chamber. These designations refer to the direction of movement, across membrane **222**, of a compound that is under investigation.

In FIG. **12**, supply-side system **1218***a* includes elements that appear to the "left" of multi-chamber enclosures **100**-*j* and receiving-side system **1218***b* includes elements that appear to the "right" of multi-chamber enclosures **100**-*j*. It should be understood that the arrangement shown (i.e., supply on the "left" and receiving on the "right") is arbitrary (although it is tied to the designation of the chambers within the multi-chamber enclosure as "supply" or "receiving"). The specific location in FIG. **12** in which the individual elements composing supply-side system **1218***a* and receiving-side system **1218***b* appear is more for the sake of clarity than any design considerations. It will be understood that one or more elements of supply-side system **1218***a* and receiving-side system **1218***b* can be co-located, as desired.

With continued reference to FIG. **12**, supply-side system **1218***a* includes supply-side compound reservoir **1220***a*, supply-side buffer-solution reservoir **1222***a*, supply-side gas reservoir **1224***a*, supply-side fluid switches **1226***a-k* (where k=1, p, where p is an integer), supply-side vacuum chamber **1228***a*, and supply-side sample receivers **1230***a*, interrelated as shown.

Supply-side compound reservoir **1220***a* advantageously, but not necessarily, includes a plurality of partially isolated compartments **1232**-*l*, where l=1, q, and where q is an integer. These partially isolated compartments enable q different liquids to be fed to different multi-chamber enclosures **100**-*j* in array **1082**. For this reason, it is advantageous but not necessary for the number of compartments, q, to be equal to the number, n, of multi-chamber enclosures **100**-*j*. If it is desirable to feed the same liquid to each multi-chamber enclosure **100**-*j*, then liquid can simply overflow the barriers that segregate compartments **1232**-*l*.

Supply-side compound reservoir **1220***a* either feeds supply-side buffer-solution reservoir **1222** or is directly connected to supply-side fluid switches **1226***a-k*. To this end, tubing **1234** that leads to supply-side buffer solution reservoir **1222***a*, and tubing **1236** that leads to supply-side fluid switches **1226***a-k*, are advantageously, but not necessarily, provided with disconnect fittings **1238**.

Supply-side buffer-solution reservoir **1222***a* includes a plurality of isolated compartments **1240**-*r*, where r=1, s, and where s is an integer. Advantageously although not necessarily, the number, s, of compartments **1240**-*r* in supply-side buffer-solution reservoir **1222***a* is equal to the number, q, of compartments **1232**-*l* in supply-side compound reservoir **1220***a*. And, as previously noted, it is advantageous but not necessary for the number of compartments, q, to be equal to the number, n, of multi-chamber enclosures **100**-*j*.

Liquid is delivered through tubing **1242** from supply-side buffer-solution reservoir **1222***a* to supply-side fluid switches **1226***a-k*. Liquid is fed to supply-side fluid switches **1226***a-k*, from either supply-side compound reservoir **1220***a* or supply-side buffer-solution reservoir **1222***a*, by either pressure or gravity feed.

Supply-side gas reservoir **1224***a* supplies gas (or gas mixtures) to supply-side fluid switches **1226***a-k* via tubing **1244**.

Thus, all supply-side fluid (i.e., gas and liquid) is fed to supply-side fluid switches **1226***a-k*. The fluid switches provide proper logic, volume and timing of the exchange of all liquid and gas between multi-chamber enclosures **100**-*j* and rest of fluid control system **1216**. In the illustrative embodiment, there is one supply-side fluid switch **1226***a-k* per multi-chamber enclosure **100**-*j*. Consequently, the number, p, of supply-side fluid switches **1226***a* is advantageously, but not necessarily, equal to the number, n, of multi-chamber enclosures **100**-*j*. In some other variations, a single fluid switch handles fluid exchange for more than one multi-chamber enclosure **100**, such that p<n. Switches suitable for this service include, among others, multi-position valves from Valco Instruments Company of Houston, Tex.

Fluids are sampled from the "supply" chamber of multi-chamber enclosures **100**-*j* by, for example, gravity or negative pressure. In the illustrative embodiment, supply-side vacuum chamber **1228***a* having vacuum pull **1248** provides a negative pressure to aspirate fluid from the supply chamber of each the multi-chamber enclosures **100**-*j*. Receivers **1230***a* are disposed within supply-side vacuum chamber **1228***a*. Receivers **1230***a*, are, in various embodiments, wells of a multi-well plate, vials, bottles, slides, substrate, "bio-chips," etc., as appropriate. Tubing **1252** delivers aspirated fluid from supply-side fluid switches **1226***a-k* to supply-side vacuum chamber **1228***a*. Sample analysis is described below in conjunction with the description of receiving-side system **1218***b*.

With continued reference to FIG. **12**, receiving-side system **1218***b* includes the same reservoirs and switches as supply-side system **1218***a*, although not all tubing is shown (e.g. tubing **1234** connecting compound reservoir **1220***b* to buffer-solution reservoir **1222***b*, etc) for the sake of clarity. In particular, receiving-side system **1218***b* includes receiving-side compound reservoir **1220***b*, receiving-side buffer-solution reservoir **1222***b*, receiving-side gas reservoir **1224***b*, receiving-side fluid switches **1226***b-k* (where k=1, p, where p is an integer), receiving-side vacuum chamber **1228***b*, and receiving-side sample-receivers **1230***b*, interrelated as shown.

Receiving-side system **1218***b* functions in the same manner as supply-side system **1218***a* to deliver or receive fluids from receiving side chambers of each multi-chamber enclosure **100**-*j* in the array **1082**.

Also shown on receiving-side **1218***b* are illustrative connections to analytical devices. For clarity, these connections are depicted only for the receiving chamber of the "nth" multi-chamber enclosure (i.e., **100**-*n*). It should be understood that all chambers (i.e., both feed chambers and receiving chambers) are advantageously, although not necessarily, connected to analytical instrumentation.

With continuing reference to FIG. **12**, tubing connects the "nth" receiving-side fluid switch **1226***b-k* to distribution valve **1260**. The distribution valve can be, for example, a multi-position valve such as is available from Valco Instruments Company, Houston, Tex. Distribution valve **1260** is in line with one of the positions of the receiving-side fluid switch. Consequently, through the action of the receiving-side fluid switch, a sample is selectively sent to distribution valve **1260**.

Distribution valve **1260** is connected to various analytical devices. In the illustrative embodiment, distribution valve **1260** is connected to (1) receivers **1230***b*; (2) mass spectrometer **1265**; (3) high precision liquid chromatograph ("HPLC") **1270**; and (4) other analytical devices **1280**, such as, without limitation, capillary electrophoresis and a fraction collector. Additionally, liquid chromatograph **1270** and mass spectrometer **1265** can be connected for tandem analysis in known fashion. In operation, distribution valve **1260** is selectively addressed to the desired analytical device.

Sampling from the feed or receiving chamber(s) in multi-chamber enclosures **100**-*j* can be performed in a continuous manner to provide information concerning process kinetics. Distribution of a sample can be performed sequentially, such as by using the arrangement depicted in FIG. **12**, or in parallel, using parallel sets of lines and valves. Those skilled in the art will know how to use one or more multi-position valves **1260** to parallel comparative analyses.

In summary, multi-chamber enclosure **100** and fluidic-control system **1216** in accordance with the illustrative embodiment of the present invention provide, collectively, a system that is useful, among other functions, for investigating compound absorption (i.e., compound-membrane interactions). Some variations of this system provide a capability of investigating compound-membrane interactions under programmable and controlled-variable sequences, timing, rate/intensities and chemical/physical conditions, including, without limitation:

Range of pH	Temperature of fluids, gases and environment
Range of compound concentrations	Mixture of gases for perfusion
Rate of perfusion	Sequence of fluid/compound exchange
Rate of fluid/compound exchange	Rate of purging of compounds and mixtures
Rate of aeration	Rate of spraying of tissue with compounds or mixtures
Rate of washing of tissue	

Illustrative compound-membrane interactions, and a method for studying them using the illustrative system, are described below.

Studies of compound absorption are advantageously conducted in the dual-chamber variation of multi-chamber enclosure **100**. Epithelial tissue, for example, prepared as previously described, is used as membrane **222** and is mounted between chamber **102** and chamber **112** (see, e.g., FIGS. **1**, **5** and **8**). The mucosal side of the tissue, for example intestinal tissue, faces the "supply" chamber of multi-chamber enclosure **100**. (As previously indicated, either chamber in a dual-chamber multi-chamber enclosure can be designated as the supply chamber. For the purposes of the present discussion, chamber **102** is designated as the supply chamber and chamber **112** is designated as the receiving chamber.) When the mucosal side faces the supply chamber **102**, the flux of the compound being studied propagates from the mucosal side of membrane **222** to the serosal side of membrane **222**. This direction of compound flux is the same as occurs in vivo, wherein the transported compound moves through the brush border membrane and baso-lateral membrane of the cells of the intestinal mucosa before reaching the underlying capillaries of the submucosa. The same tissue orientation is valid for other epithelial tissues such as, for example, buccal, nasal, corneal, pulmonary or vaginal. In the case of dermal tissue, however, the epidermis faces the supply chamber **102**.

To study compound absorption, the compound under investigation is provided in a buffer solution and is delivered, from supply-side compound reservoir **1220***a*, to supply chamber **102** via supply-side fluid switch **1226***a*. Alternatively, the compound, in pure form, can be directed to supply-side buffer-solution reservoir **1222***a* and then delivered, via supply-side fluid switch **1226***a*, into supply chamber **102**. Simultaneously, receiving chamber **112** is filled with the same buffer solution, without the compound. This arrangement is used to study compound flux from supply chamber **102** into receiving chamber **112**.

The pH of buffer solutions that are delivered to supply chamber **102** and/or chamber **112** can be changed. This facilitates the investigation of compound absorption at conditions that correspond to the pH prevailing in different regions of the gastro-intestinal system (ie., ranging from a pH 1.5 in the stomach to a pH 7.5 in the intestine).

The supply of solutions with controlled pH is provided through one of the tubes within conduit **546** (e.g., see FIGS. **5** and **6**: tubes **647**-**650**). Another of the tubes supplies a gas mixture (usually oxygen and carbon dioxide at a volumetric ratio of 95:5), which is necessary for tissue oxygenation and mixing the buffer solutions.

In some other variations of a method in accordance with the illustrative embodiment, the compound under investigation is introduced only into receiving chamber **112** for a back-flux study into supply chamber **102**.

Mother chamber **1190** (see FIG. **11**) maintains constant temperature, which is usually in the range of body temperature, during experimentation. Incubation time, which depends on the nature of compound under investigation, its transport mechanism, binding, metabolic, and other characteristics, should be optimized experimentally for each compound in known fashion.

At the end of incubation and/or during the incubation cycle, samples of the buffer/compound solution are taken from receiving chamber **112** (and feed chamber **102**, if desired), through a tube that is aspirating. The samples are then analyzed using known analytical methods. An increase in compound concentration in receiving chamber **112** characterizes the compound's absorption or trans-membrane transport. This method can be used for absorption screening of different types of compounds (e.g., drugs, nutrients, nutraceuticals, cosmetics, cosmeceuticals, or other xenobiotics, etc.) that might use different mechanisms for their transport through live tissues.

To evaluate compound accumulation in tissue, membrane **222** is removed from multi-chamber enclosure **100**, rinsed with buffer solution, and homogenized in an appropriate solution or dissolved in 1 M sodium hydroxide. The compound is then further extracted and tested using appropriate analytical methods in known fashion. After completion of each individual study, multi-chamber enclosures **100** are taken apart, washed and, if necessary, sterilized following standard GLP requirements. When testing lipophilic compounds, a wash with 10 percent or 50 percent soap solution (e.g., Ivory® brand liquid soap, Proctor and Gamble Co., etc.) is recommended. In some variations of the illustrative embodiment, disposable multi-chamber enclosures are used to simplify and accelerate testing processes.

As indicated earlier, illustrative multi-chamber enclosure **100** and methods in accordance with the present invention, as described herein, can be used to study compound absorption in different types of tissues. In cosmetology and dermatology, for example, multi-chamber enclosure **100** can be used to conduct single and/or multiple compound applications onto epidermal tissue (i.e., skin). When conducting multiple applications of compound to the skin, the surface of the skin is gently washed between applications with distilled water or soap to remove deposits that might be adsorbed on the surface of the skin. Additionally, in some applications, multi-chamber enclosure **100** is used to test solutions of different densities (including highly-viscous creams).

As previously described, membrane **222** can be prepared from model animal tissues, artificial tissues, and tissues with cells attached from different cell lines. Live tissues can be used immediately after the extraction from the animal or, alternatively, prepackaged in preservation solution (buffer, cell or tissue medium) and kept at or below 4° C. (see, H. Burgmann et al., Transplant. Proc. 24:1085-1086 (1992); A. R. Muller et al., Transplantation. 57:649-655 (1994); F. G. Rodriguez et al., J. Invest. Surg. 7:439-451 (1994); S. Massberg et l., Brit. J. Surg. 85:127-133 (1998)).

Some tissues, such as skin for example, can be used for studies after long-term storage at −20° C. (see, B. W. Barry. Dermatological Formulations. Percutaneous Absorption. Marcel Dekker, Inc. New York, Basel, 1983; S. M. Harrison et al., J. Pharm. Pharmacol. 36:261-262 (1984)). This presents the opportunity for streamlining high-throughput-testing processes on preserved samples of these tissues.

When considering cell lines for oral absorption studies, the Caco-2 cell line is usually a preferred choice. Cells are routinely cultured for compound uptake studies in cell culture plates on semi-permeable membranes (see, L. S. L. Gan & D. R. Thakker. Adv. Drug Deliv. 23:77-98 (1997); F. Delie & W. Rubas. Crit. Rev. Ther. Drug Carrier Syst. 14:221-286 (1997)). After cell confluency, a membrane having attached cells is installed between adjacent individual chambers (e.g., either by direct mounting or via a membrane-holding assembly, as previously described). In both cases, cells should face supply chamber **102**, thereby imitating the compound transport route that occurs in the intestine from the mucosal surface to the serosal surface.

Using electrodes (e.g., see FIG. **8**, electrodes **862**) that are disposed in both supply chamber **102** and receiving chamber **112**, a potential difference and currents can be measured across membrane **222** using the well-known Ussing/Zehrans voltage/current clamping technique.

Possessing a thorough understanding of the site specificity of absorption is important for optimizing oral drug delivery. For site-specificity studies, it is necessary to use tissues from different locations in the same experiment. For example, to study the gradient of compound-membrane interactions in the gastrointestinal tract, tissue ought to be prepared from different parts of the gastro-intestinal tract. Multi-chamber enclosure **100** can accommodate tissue samples from all portions of the gastro-intestinal tract, including the esophagus, stomach, small intestine and large intestine.

On the other hand, to reduce the effect of tissue variability [due to intestinal absorption gradient], small-size samples of tissue are advantageously used in multi-chamber enclosure **100**. As previously described, membrane (tissue) samples, which have a diameter that is in the range of about 0.25 inches to about 0.5 inches, can be prepared in large quantities (simultaneously), using, for example, membrane holding cassette **330** (see, FIG. **3**).

It will be appreciated that tissue can be attached to multiple membrane holding frames **224** simultaneously along cassette **330** using a technique such as stamping, forming or cutting. In this manner, the absorption parameters of various compounds can be determined for almost every centimeter of intestine.

Using multi-chamber enclosure **100**, compound-absorption studies can be conducted on tissues from various species (e.g., rodents, farm animals, etc.) and from animals of different age groups. The only parameter that is likely to change is the diameter of the membrane holding frame **224**, as might be required to accommodate tissues from different-size animals. If it is desirable or otherwise necessary to reduce the thickness, for example, of intestinal tissue, its mucosa can be stripped from underlying tissue layers before the tissue is mounted onto membrane holding frame **224**.

In addition to its utility for studying compound-tissue interactions, multi-chamber enclosure **100** in accordance with the illustrative embodiment of the present invention is also useful for studying compound-compound interactions during absorption. These interactions are important since they are known, in some cases, to inhibit or enhance absorption. (For nutrient-nutrient, and nutrient-nutraceutical interactions and their affect on compound absorption, see, e.g., A. M. Ugolev et al., Brit. J. Nutr. 34:205-220 (1976); H. McCoy & M. A. Kenny. Magnesium Res. 9:185-203 (1996); C. R. Lynch. Nutr. Rev. 55:102-1010 (1997)). Furthermore, compound-compound interactions can affect organism immune functions (see, e.g., K. S. Kubena & D. N. McMurray. J. Amer. Diet. Assoc. 96:1156-1164 (1996)).

Perhaps more important than nutrient-nutrient and nutrient-nutraceutical interactions are drug-nutrient, drug-nutraceutical and especially drug-drug interactions (i.e., pharmaceutical synergism or antagonism). These types of interactions can decrease drug absorption, make some portion of the drug unavailable for absorption, or even increase a drug's toxic effects (see, e.g., R. R. Levine et al., Pharmacology. Drug Actions and Reactions. 6^{th} edition. The Parthenon Publishing Group. New York, London, 2000; B. N. Singh. Clin. Pharmacokinet. 37:213-255 (1999)).

To study compound interactions during their absorption using multi-chamber enclosure **100**, the tested compound (e.g., nutrient, drug, nutraceutical, cosmetic, cosmeceutical, or other xenobiotics, etc.) is injected alone or in different combinations with another compound (modifier) into supply chamber **102** and the concentration of the compound under investigation is monitored in receiving chamber **112**. The compounds under investigation and modifiers can belong to the same or different classes. That is, the compounds and modifiers are individually selected from the group consisting of, for example, drugs, nutrients, nutraceuticals, cosmetics, cosmeceuticals and xenobiotics, etc. In this manner, multi-chamber enclosure **100** can be used to study the following interactions:

drug-drug	drug-nutrient
drug-neutraceuticals	nutrient-nutrient,
nutraceutical-nutraceutical	nutrient-neutraceuticals
cosmetics-cosmetics	cosmeceutical-cosmeceutical
cosmetics-cosmeceuticals	xenobiotic interactions

Using a plurality of chambers, such as with the variations of the illustrative embodiment depicted in FIGS. **9** through **12**, enables simultaneous screening of different compound-compound combinations.

The same multi-chamber enclosure can be used for testing compound absorption sans modifier and in the presence of a modifier. In the latter case, compound absorption sans modifier is typically evaluated first as described above, then, after an appropriate wash, compound absorption in the presence of a modifier is tested. This methodology reduces the effect of membrane variability on compound absorption.

Multi-chamber enclosure **100** in accordance with the illustrative embodiment of the present invention has a variety of uses in addition to those already described. For example, a detailed analysis of compound concentration in supply chamber **102**, in tissue (i.e., membrane **222**), and in receiving chamber **112** is important to evaluate compound transport mechanisms, especially to differentiate between passive and active transport mechanisms. These analyses are readily performed using fluid control system **1216** in conjunction with multi-chamber enclosure **100**.

Furthermore, analysis of compound concentrations in the supply and receiving compartments, as well as in tissue (i.e., membrane **222**), is necessary to separately evaluate compound transport through apical membranes and baso-lateral membranes in polarized epithelial cells. The ratio between compound concentration in tissue and in supply chamber **102** characterizes the intensity of compound transport through the apical membrane, and the ratio between compound concentrations in tissue and in receiving chamber **112** is used to evaluate baso-lateral membrane transport.

Additionally, multiple samples can be obtained from multi-chamber enclosure **100** during an absorption cycle, as is necessary for kinetics studies of compound absorption. Also, the ability to run a plurality of multi-chamber enclosures **100** in parallel is advantageous for a comparative analysis of the effect of compound concentration and incubation time on the compound interaction with membrane **222**.

Yet another use for multi-chamber enclosure **100** is to evaluate first-pass metabolism in metabolically-active tissues. First-pass metabolism follows absorption and is believed to contribute to the poor oral bio-availability of some drugs in the intestine after their oral administration. To perform this type of evaluation using multi-chamber enclosure **100**, media from both the supply and receiving chambers, as well as the tissue (ie., membrane **222**) is removed after incubation and analyzed for metabolite concentration.

Moreover, multi-chamber enclosure **100** can be used to study the role of cell excretion systems in compound absorption, given the presence of a P-glycoprotein pump located in the brush border of intestinal epithelial cells (see, e.g., F. Thiebaut et al., Proc. Natl. Acad. Sci. USA 84:7735-7738 (1987); L. Barthe et al., Fundam. Clin. Pharmacol. 13:154-168 (1999)). Specifically, the kinetics of compound absorption can be investigated in the absence and presence of P-glycoprotein inhibitors in supply chamber **102**. The same approach is used to screen absorption enhancers and for conducting compound toxicity analysis.

It is to be understood that the above-described embodiment is merely illustrative of the present invention and that many variations of the above-described embodiment can be devised by one skilled in the art without departing from the scope of the invention. For example, rather than using two separate housings (e.g., **103** and **113**, etc.) to form, in conjunction with a membrane, two separate chambers, a single housing can be used. In such an embodiment, a membrane, attached to a membrane-holding assembly, is inserted through a slot in the housing. It is therefore intended that such variations be included within the scope of the following claims and their equivalents.

We claim:

1. An article comprising:

an enclosure defining a sealable volume that receives fluid, wherein said enclosure has a first aperture and a second aperture;

a membrane. wherein said membrane is disposed in the enclosure, and further wherein said membrane segregates the volume defined by the enclosure into a first chamber and a second chamber;

a first feed fitting, wherein said first feed fitting is received by said first aperture;

a first spring-biasing element, wherein said first spring-biasing element mechanically couples to said first feed fitting;

a second feed fitting, wherein said second feed fitting is received by said second aperture; and

a second spring-biasing element, wherein said second spring-biasing element mechanically couples to said second feed fitting; wherein:

said first spring-biasing element, said first feed fitting, said first aperture, said second aperture, said second feed fitting and said second spring-biasing element are aligned along a first axis.

2. The article of claim **1** wherein:

said first spring-biasing element exerts a first force;

said second spring-biasing element exerts a second force; and

said first force and said second force collectively place said enclosure in compression.

3. The article of claim **1** wherein said enclosure comprises a first housing and a second housing, wherein said first and second housing are coupled to one another.

4. The article of claim **3** wherein:

said first housing has a first end and a second end;

said first housing is relatively larger at said second end and relatively smaller at said first end;

said second housing has a first end and a second end;

said second housing is relatively larger at said second end and relatively smaller at said first end; and

said first housing and said second housing engage one another at said relatively larger second ends.

5. The article of claim **4** wherein:

said first aperture is disposed at said first end of said first housing; and

said second aperture is disposed at said first end of said second housing.

6. The article of claim **5** wherein:

said second end of said first housing is substantially open thereby defining a relatively large aperture compared to said first aperture at said first end of said first housing; and

said second end of said second housing is substantially open thereby defining a relatively large aperture compared to said second aperture at said first end of said second housing.

7. The article of claim **4** wherein each of said first housing and said second housing have a conical shape.

8. The article of claim **6** wherein:

said membrane is disposed between said first housing and said second housing proximal to said relatively large aperture of each of said first housing and said second housing; and

said first chamber is defined within said first housing and said second chamber is defined within said second housing.

9. The article of claim **8** wherein said membrane is selected from the group consisting of live tissue, synthetic tissue and synthetic tissue with cells attached.

10. The article of claim **8** wherein:

said first feed fitting has a first end and a second end;

said first spring-biasing element couples to said first end of said first feed fitting;

said second end of said first feed fitting has a first opening; and

said second end of said first feed fitting is received by said first aperture of said enclosure so that said first opening communicates with said first chamber.

11. The article of claim **10** further comprising a tube, wherein said tube extends into said first chamber through said first opening of said first feed fitting.

12. The article of claim **11** further comprising a fluid control system, wherein:

said fluid control system is coupled to said tube;

said fluid control system supplies fluid to and receives fluid from said first chamber;

and said fluid control system is coupled to analytical devices.

13. The article of claim **1**, further comprising a frame, wherein:

said frame has two spaced-apart rails;

said first spring-biasing element is coupled to one of said spaced-apart rails; and

said second spring-biasing element is coupled to the other of said spaced-apart rails.

14. An article comprising:

a first housing and a second housing, wherein said first housing and said second housing are coupled to one another, wherein:

said first housing has a first interior volume, a small aperture, and a large aperture;

said second housing has a second interior volume, a small aperture, and a large aperture; and

said first housing and said second housing are coupled proximal to said large apertures;

a first feed fitting, wherein said first feed fitting has an opening and said first feed fitting is received by said small aperture of said first housing;

a first plurality of tubes, wherein said first plurality of tubes pass through said opening in said first feed fitting and extend into said first interior volume of said first housing;

a second feed fitting, wherein said second feed fitting has an opening and said second feed fitting is received by said small aperture of said second housing;

a second plurality of tubes, wherein said second plurality of tubes pass through said opening in said second feed fitting and extend into said second interior volume of said second housing;

a first spring-biasing element that mechanically couples to said first feed fitting; and

a second spring-biasing element that mechanically couples to said second feed fitting.

15. The article of claim **14** further comprising a membrane, wherein:

said membrane is disposed between said first housing and said second housing;

said membrane and said first housing define a first chamber that comprises said first interior volume;

said membrane and said second housing define a second chamber that comprises said second interior volume; and

said membrane forms a seal between said first chamber and said second chamber.

16. The article of claim **15** further comprising:

a first vent, wherein said first vent is disposed in said first housing and vents said first chamber;

a second vent, wherein said second vent is disposed in said second housing vents said second chamber.

17. The article of claim **15** further comprising an electrode, wherein said electrode passes through said opening in said first feed fitting and extends into said first chamber.

18. The article of claim **15** further comprising a fluid control system, wherein:

said first plurality of tubes and said second plurality of tubes are coupled to said fluid control system;

said fluid control system supplies fluid to at least one of said first and second chambers;

said fluid control system receives fluid from at least one of said first and second chambers; and

said fluid control system is coupled to analysis devices and control devices.

19. The article of claim **14** further comprising a device for fixing each tube of said first plurality of tubes in a desired location and orientation within said first chamber.

20. The article of claim **14** further comprising a third housing, wherein:

said third housing has an interior volume; and

said third housing is flanked by and couples to said first housing and said second housing.

21. The article of claim **20** further comprising a first membrane and a second membrane, wherein:

said first membrane is disposed between said first housing and said third housing;

said first membrane and said first housing define a first chamber that comprises said first interior volume;

said second membrane is disposed between said second housing and said third housing;

said second membrane and said second housing define a second chamber that comprises said second interior volume;

said third housing, said first membrane, and said second membrane define a third chamber that comprises said third interior volume; and wherein:

said first membrane forms a seal between said first chamber and said third chamber; and

said second membrane forms a seal between said second chamber and said third chamber.

22. The article of claim **15** wherein said first housing, said second housing and said membrane collectively compose a first multi-chamber enclosure, and further comprising:

a second multi-chamber enclosure; and

a frame;

wherein said first multi-chamber enclosure and said second multi-chamber enclosure are mechanically coupled to said frame.

23. The article of claim **22** wherein:

said frame comprises a first rail and a second rail;

said first spring-biasing element is mechanically coupled to said first rail; and

said second spring-biasing element is mechanically coupled to said second rail.

24. The article of claim **22** further comprising a mother chamber, wherein said mother chamber receives said frame having said first multi-chamber enclosure and said second multi-chamber enclosure.

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