(19) DANMARK

(10) **DK/EP 3766582 T3**

Patent- og Varemærkestyrelsen

(12) # Oversættelse af europæisk patentskrift

(51) Int.Cl.: ***B 01 F 31/00 (2022.01)*** ***B 01 F 31/23 (2022.01)*** ***B 01 L 3/00 (2006.01)*** ***C 12 M 1/00 (2006.01)*** ***C 12 M 1/32 (2006.01)*** ***C 12 M 1/34 (2006.01)*** ***C 12 M 1/42 (2006.01)*** ***C 12 M 3/06 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2022-06-20**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2022-05-25**

(86) Europæisk ansøgning nr.: **20194991.4**

(86) Europæisk indleveringsdag: **2016-10-21**

(87) Den europæiske ansøgnings publiceringsdag: **2021-01-20**

(30) Prioritet: **2015-10-22 GB 201518767**

(62) Stamansøgningsnr: **16787549.1**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Fibrofind IP Limited, 12 Regent Terrace, Gateshead, Tyne and Wear NE8 1LU, Storbritannien**

(72) Opfinder: **OAKLEY, Fiona, University of Newcastle upon Tyne, Kings Gate, Newcastle upon Tyne, NE1 7RU, Storbritannien**
BORTHWICK, Lee, University of Newcastle upon Tyne, Kings Gate, Newcastle upon Tyne, NE1 7RU, Storbritannien
GRIFFITHS, Clive, University of Newcastle upon Tyne, Kings Gate, Newcastle upon Tyne, NE1 7RU, Storbritannien
DRINNAN, Michael, University of Newcastle upon Tyne, Kings Gate, Newcastle upon Tyne, NE1 7RU, Storbritannien

(74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**

(54) Benævnelse: **CELLEKULTUR**

(56) Fremdragne publikationer:

WO-A1-03/078565
WO-A1-2006/097749
WO-A1-2011/161480
WO-A1-2016/166315
WO-A2-03/083044
US-A- 5 422 270
US-A1- 2003 017 582

DESCRIPTION

Field of the Invention

[0001] The present invention relates to a culture apparatus for culturing cells and/or a tissue or portion thereof.

Background

[0002] Cell and tissue culture plays a considerable role in both basic and applied life science research. A standard device used in cell or tissue culture or for performing chemical or cellular assays is the multi-well plate. Multi-well plates are available in a variety of formats. For many general cell culture and tissue engineering applications, 6-, 12-, 24-, 48-, and 96-well formats are most commonly used although higher-density plates, e.g., 384, 1536, or higher are also utilised. Multi-well plates are used in a number of biological assay formats as they are well suited for screening a number of samples simultaneously. Automated laboratory equipment such as plate readers, high throughput screening apparatus and the like has been developed specifically to be used in association with the multi-well format. As a result, the multi-well plate has become a standard format for biological assays.

[0003] A disadvantage of using conventional multi-well plates is that the complexity of the physiological environment is not replicated. For example, tissues and organs of the body *in vivo* are continuously perfused by the blood and lymphatic systems. This perfusion enables constant removal of cell waste products and new nutrients to be provided. Typically static cell culture does not accurately represent this system, even if media is regularly removed and replaced. Furthermore, known multi-well plates do not provide any form of dynamic chemical or physical stimulus such as concentration gradients, flow, pressure or mechanical stress to cells situated in the wells. As a result, *in vitro* testing using conventional multi-well plates often does not represent *in vivo* environments.

[0004] As a result, there is a growing interest in developing more dynamic culture systems which will enable the environment surrounding the cells or tissue in culture to be altered during the course of experiment.

[0005] A number of bioreactors have been designed with an aim to more accurately represent the *in vivo* environment. These "dynamic" bioreactors are intended to provide more accurate models of human disease on which to test efficacy and toxicity of candidate drug molecules for example.

[0006] An example of a bioreactor system which relies on a pumping mechanism is provided by Kirkstall Limited, United Kingdom. This system utilises a plurality of "modular" cell culture

chambers, an electronic control circuit and a peristaltic pump which pumps fluid e.g. cell culture media through a chamber.

[0007] The field of drug development is an example of a field in which the development of dynamic *in vitro* systems may be advantageous. Drug development projects are often terminated after expensive human clinical trials when unacceptable side effects, toxicity or lack of therapeutic efficacy are evident. The development of *in vitro* systems which accurately predict those drugs which will be safe and efficacious in man before *in vivo* clinical trials are commenced is desired.

[0008] A therapeutic area of interest is the development of anti-fibrotic compounds to treat fibrotic disorders. The current gold standard research tools used to understand fibrotic disease and test anti-fibrotic candidate molecules are limited to 2D cultures of scar forming cells and *in vivo* fibrosis molecules in rodents. These approaches have a number of weaknesses associated with them. Firstly, fibrosis is a complex disorder involving multiple cell types, many of which are lacking in 2D culture systems. Furthermore, pre-clinical rodent models do not accurately represent human disease as they lack some of the important features of human clinical pathology.

[0009] Ideally, human organ slices e.g. liver slices would be used in *in vitro* culture systems. However, techniques which involve the use of organ slices are currently limited due to a limited life span in culture. The life span can be extended using existing dynamic cell culture systems which utilise complex pumping mechanisms to generate fluid movement in the system. However, the existing systems have limitations due to their expense and the time required to set up and run the system. In addition, the number of organ slices which can be cultured simultaneously is limited.

[0010] US5422270A discloses a one-step tray test for the release of soluble mediators and an accompanying apparatus. US2003017582A1 discloses a device and method for monitoring leukocyte migration. WO03078565A1 discloses a cell motility and chemotaxis test device and methods of use. WO2011161480A1 discloses multi-well assay plate where each well is in fluid communication with a neighbouring well via a gateway. WO2006097749A1 discloses a fluidic device for cell and embryo culture. WO03083044A2 discloses tissue analogs for in vitro testing and a method of use. WO2016166315A1 discloses a system for propagating cells.

[0011] It is an aim of aspects of the present disclosure to at least partially mitigate the problems associated with the prior art.

[0012] It is an aim of certain examples of the present disclosure to provide a cost-effective bioreactor system which is suitable for culturing cells and/or tissues e.g. tissue slices.

[0013] It is an aim of certain examples of the present disclosure to provide a system and a method which more accurately reflect the *in vivo* environment of disease for example.

[0014] It is an aim of certain examples of the present disclosure to provide apparatus which utilise small volume of cell culture media.

Summary of Certain Examples

[0015] The invention is set out in the appended claims.

Detailed Description

[0016] Examples of the present disclosure will now be described hereinafter, by way of example only, with reference to the accompanying drawings in which:

"CMR" refers to a bidirectional system according to certain examples of the present disclosure.

"CMR2" refers to a bidirectional system according to certain examples of the present disclosure.

Figure 1 illustrates a multi-well plate apparatus according to certain examples of the present disclosure;

Figure 2 is a top view of adjacent chamber of a multi-well plate apparatus according to certain examples of the present disclosure;

Figure 3a is a schematic representation of the rocket apparatus holding a multi-well plate apparatus according to certain examples of the present disclosure. As illustrated by arrows, media exchange may occur via insert pores within wells of the plate apparatus. In addition, media exchange may occur via a channel between wells.

Figure 3b illustrates a rocker apparatus ("CMR") according to certain examples of the present disclosure;

Figure 4a and 4b illustrates a cell scaffold insert element for use in certain examples of the present disclosure;

Figure 5 is a schematic representation of the rocker apparatus of Figure 3;

Figure 6 is a graph illustrating a comparison of albumin production (ng/ml) using a 12 chambered apparatus as described herein (herein referred to as "CMR") as compared to albumin production using a static Transwell® insert (referred to as "insert") of precision cut liver slices. The viability of the cells is increased using the apparatus of examples of the disclosure. N=8. The left hand bars represent the use of the apparatus of examples of the present disclosure whilst the right hand bars illustrate the static Transwell® insert. The data shows that the apparatus of certain examples maintains the ability of the slice to synthesize and secrete albumin for up to 4 days, which is suggestive of an improved liver function and extended

longevity of the tissue slice. Rocking the bioreactor plate promotes media exchange between the two chambers connected by the channel. Each chamber contains an insert, which holds the tissue slice in an inner well. Rocking not only allows media exchange between the two chambers but also permits media exchange via the pores in the culture insert membrane which separate the inner and outer wells. The latter media exchange generates flow around/over the tissue slice, which will aid oxygenation and removal of toxic metabolites, which in turn is likely to increase viability and function (albumin production) of the tissue.

Figure 7 is a graph illustrating that cell viability and albumin-synthesizing ability using a cell scaffold insert element as described herein having an average membrane pore size of 3μm are of insufficient size to allow effective media exchange through the insert pore between the inside of the insert and the outer chamber, thus resulting in no improvement in slice function compared to static inserts. "CMR" refers to the use of apparatus of examples of the present disclosure, whilst "Ins" refers to a static (i.e. non-rocking) set-up;

Figure 8 is a graph illustrating albumin production (ng/ml) from cells cultured using a cell scaffold insert element having an average membrane pore size of 8μm. 8μm pore inserts allow effective media exchange through the insert pore between the inside of the insert and the outer chamber, thus resulting in an improvement in slice function compared to static inserts.

[0017] Furthermore, rocking of the CMR bioreactor plate ("CMR") retains liver function and albumin secretion. Albumin production is significantly reduced in the static bioreactor plate ("CMR Static"), on static inserts (Ins R), or inserts in standard 12 well plate that are rocked ("Ins"). This suggests that media exchange between the two wells of the chamber in the CMR plate caused by rocking is necessary for retaining albumin secretion.

[0018] Figure 9 is a graph illustrating albumin production from cells cultured using a cell scaffold insert element having an average pore size of 100μm. 100μm pore inserts provided in an apparatus of certain examples (CMR) are of sufficient size to allow effective media exchange through the insert pore between the inside of the insert and the outer chamber, thus resulting in an improvement in slice function compared to static inserts. Again, media exchange between the two wells of the chamber is required to retain albumin secretion ("CMR"). Conversely, albumin production is significantly reduced in the static bioreactor plate with 100μm insert pores (CMR Static), on static inserts with 100μm insert pores ("Ins R"), or inserts with 100μm insert pores in standard 12 well plate that are rocked ("Ins").

[0019] Figure 10 is a graph illustrating that the effect of cell scaffold insert pore size on and rocking the bioreactor plate to introduce a bidirectional flow and media exchange on albumin secretion (ng/ml). Conclusion: albumin production and tissue viability is improved with bidirectional flow on inserts pore sizes between 8μm - 100μm. 3μm pore inserts did not improve slice function, n=1.

[0020] Figure 11 is a graph comparing that the effect of unidirectional flow using a system

available from Kirkstall Limited, UK, ("Kirkstall") versus bidirectional flow (rocked CMR plate, "CMR") with 3μm, 8μm or 100μm inserts on albumin secretion (ng/ml). Conclusion: bidirectional flow results in albumin secretion which is stable for longer. The minimum pore size needed to improve function is required to improve albumin synthesis and secretion.

[0021] Figure 12 is a graph comparing that the effect of unidirectional flow using a unidirectional flow system, (uniflow, black bars) versus bidirectional flow (rocked CMR plate, with 8μm pores) on albumin secretion (ng/ml). Unidirectional flow system, (uniflow) versus CMR I and static insert data indicates that when bidirectional flow is provided, albumin secretion is stable for longer.

[0022] Figure 13 is a graph showing albumin production (ng/ml) in the CMR bioreactor plate with 8μm inserts and rocking with increasing volumes of culture media within the chamber from 0.5ml/well (total chamber volume 1ml) to 4mls/well (total chamber volume 8mls). The media volume range that improves liver function and retains albumin secretion is between about 1.5ml -4mls.

[0023] Figure 14 is a graph illustrating that liver damage quantified by leakage of the liver enzyme aspartate aminotransferase from damaged hepatocytes (arbitrary units) is reduced in CMR cultured slices compared to static conditions or conditions provided by a unidirectional flow system, (uniflow). Rocking the CMR bioreactor plate with 8μm inserts ("CMR 8") improves slice viability compared to static conditions ("CMR 8S") or unidirectional flow system, (uniflow), suggesting bidirectional flow is important to prevent death of the liver slice.

[0024] As indicated, serum transaminases are reduced using the CMR plate according to certain examples of the present disclosure (8μm compared to static inserts (Ins 1.5) or unidirectional flow system (uniflow)). N=3. Statistics are unpaired t-test compared to static inserts;

[0025] Figure 15 is a graph illustrating that liver damage quantified by leakage of the liver enzyme aspartate aminotransferase from damaged hepatocytes (arbitrary units) is reduced in CMR cultured slices compared to static conditions when the media volume of the CMR is between 1.5mls - 2mls. Media volumes below 1.5mls do not prevent tissue death. 8μm pore inserts were used. Conclusion: Serum transaminases are reduced when the hepatocytes are cultured using CMR (1.5mls - 2mls media), suggest less damage to the slice. The bars run left to right as labelled from top to bottom.

[0026] Figure 16 is a graph illustrating urea production from liver slices cultured in static inserts (Ins 1.5), a well having pores of 8μm of a plate according to certain examples of the present disclosure and those cultured in a unidirectional flow system (uniflow). Conclusion: Liver function is improved in CMR cultured slices compared to static conditions.

[0027] UREA production (mg/dL) is increased on CMR (8μm) compared to static inserts (Ins 1.5) or unidirectional flow system, (uniflow). n=2 (CMR and static insert) n=1 uniflow.

[0028] Figure 17 is a graph illustrating urea production (mg/dL) on the CMR with wells having 8μm pore inserts versus static inserts over a media range of 0.5mls - 4mls. The data suggest a dynamic range of 1.5mls - 4 mls/well, n=1 and media volumes between 1.5mls - 4mls on a rocked CMR plate preserve urea synthesis and secretion. The bars run left to right as labelled from top to bottom;

[0029] Figure 18: is a graph illustrating the tissue area stained with α-smooth muscle actin (a-SMA) a marker of hepatic myofibroblasts in histological sections of liver slices at t-0 (post slice without culture) or cultured on the CMR (8μm pore inserts) (CMR bars) versus static inserts (Static bars) over a media range of 0.5mls - 4mls.
The data suggest that hepatic myofibroblasts are activating by day 4 in CMR in media volumes of 1.5mls - 4mls, n=1.

[0030] Figure 19 illustrates representative images of histological sections of liver slices at t-0 (post slice without culture) or cultured on the CMR (8μm pore inserts) in 1.5mls or 3mls of media versus static inserts and stained with α-smooth muscle actin (a-SMA) a marker of hepatic myofibroblasts. This suggests that hepatic myofibroblasts are activating by day 4 in CMR, n=1. This may be of use for modeling liver disease and fibrosis.

[0031] Figure 20 is a graph illustrating the tissue area stained with cytokeratin 19 (CK19) a marker of ductular cells in histological sections of liver slices at t-0 (post slice without culture) or cultured on the CMR (8μm pore inserts) versus static inserts over a media range of 0.5mls - 4 mls. The data suggests that a ductular expansion may occur by day 4 in >1.5ml in the CMR, n=1. This may be of use for modeling liver disease and fibrosis.

[0032] Figure 21 is a graph comparing is a graph illustrating the tissue area stained with CD68 a marker of Kupffer cells (liver macrophages) in histological sections of liver slices at t-0 (post slice without culture) or cultured on the CMR (8μm pore inserts) (blue bars) versus static inserts (green bars) over a media range of 0.5mls - 4mls. The data suggests that macrophages are retained in the slices up to day 4 in CMR and Static inserts (static), n=1. This may be of use for modeling liver disease and fibrosis.

[0033] Figure 22 shows representative images of histological sections of liver slice at t-0 (post slice without culture, left) or cultured on the CMR (8μm pore inserts) in 1.5mls of media (right) and stained with CD68 a marker of Kupffer cells (liver macrophages). Conclusion: macrophages are retained in the slices up to day 4 in CMR.

[0034] Figure 23 is a graph illustrating the production of albumin (ng/ml) using a static cell culture plate and apparatus according to certain examples of the disclosure. Conclusion: CMR plate version comprising through holes between chambers (CMR) and an example which comprises slots between chambers (CMR III) work equally well and preserves stable albumin synthesis and secretion for up to 7 days, n=1. 8μm pore inserts was used.

[0035] Figure 24 is a graph comparing albumin production of 6mm liver slices cultured in the CMR (12 well) with 8µm inserts with CMR (24 well) 8µm inserts. The data shows that the 24 well CMR system works as well the 12 well CMR system.

[0036] Figure 25 is a graph showing quantification of albumin production in precision cut liver slices (PCLS) cultured on either the CMR versus static trans-well insert cultures (25A) or CMR2 versus static trans-well insert cultures (25B) for up to 7 days with daily media changes. Similar to CMR, the CMR2 bioreactor maintained albumin production, compared to static trans-well insert cultured slices. The bars run left to right as labelled from top to bottom.

[0037] Figure 26 is a graph showing albumin production by PCLS cultured on CMR2 and stimulated +/- 3ng/ml transforming growth factor beta 1 (TGFb1) for 48h after a 24h rest period (Figure 26A). Figure 26B shows that treatment of PCLS with 3ng/ml TGFb1 for 48h induced expression of the fibrosis genes collagen I, alpha-smooth muscle actin (aSMA) and tissue inhibitor of metalloprotease 1 (TIMP1) compared to control (untreated). The bars run left to right as labelled from top to bottom.

[0038] Figure 27 shows albumin production by PCLS cultured on CMR2 and stimulated +/- fat, fat + Lipopolysaccharide (LPS) or Bovine Serum Albumin (BSA, vehicle) for 48h after a 24h rest period. Treatment with FAT or LPS did not affect albumin production.

[0039] Figure 28 is images show that the fat treatment induces fat accumulation and deposition of collagen in the PCLS.

[0040] Figures 29A, 29B and 29C are perspective views of an apparatus according to certain examples of the present disclosure (referred to herein as CMR2).

[0041] Figure 30A and Figure 30B depict various components of the apparatus of Figure 29.

[0042] Figure 31 is a graph showing expression of the fibrotic genes pro-collagen 1, alpha-smooth muscle actin (aSMA) and Tissue inhibitor of metalloproteinase 1 (TIMP1) in precision cut liver slices (PCLS) cultured on CMR2 and stimulated +/- transforming growth factor beta (TGFb) and +/- platelet derived growth factor b (PDGF-bb) for 72h after a 24h rest period. Treatment with both stimuli induce expression of fibrosis genes.

[0043] Figure 32 is a graph showing quantification of lactate dehydrogenase (LDH) release in precision cut kidney slices (PCKS) cultured on either the CMR versus static trans-well insert cultures for up to 5 days with daily media changes. PCKS damage as determined by LDH release is reduced on CMR cultured slices compared to static Transwell® insert cultured slices.

Definitions

[0044] While the terminology used in this application is standard within the art, definitions of certain terms are provided herein to assure clarity and definiteness to the meaning of the claims. Units, prefixes, and symbols may be denoted in their SI accepted form. Numeric ranges recited herein are inclusive of the numbers defining the range and include and are supportive of each integer within the defined range. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:
It will be appreciated that the terms "chamber" and "well" as used herein are interchangeable and are exemplary and non-limiting. The mention of one term does not exclude substitution of the other terms in the described example. As used herein, a chamber is an orifice in a holder body which typically comprises a flat planar base and an upper opening. The chamber may be cylindrical in shape and have one or more side walls. Further details of exemplary chambers are provided herein.

[0045] As used herein, the term "cell culture" or "culture" refers to the maintenance, growth, differentiation and/or continued viability of cells in an artificial, in *vitro* environment. The cells may be comprised in a tissue or portion thereof e.g. a tissue or organ slice. The cell culture may be a two dimensional cell culture or a three dimensional cell culture. In one example the organ slice may be for example a liver slice, a kidney slice or a lung slice.

[0046] An "organ slice" or "tissue slice" is an *in vitro* model which represents the multi-cellular, structural and functional features of *in vivo* tissue. Tissue slices may provide a model for characterising mechanisms of drug-induced injury and for identifying biomarkers of organ injury, which can be a significant clinical issue.

[0047] Certain examples may have particular utility culturing tissue slices comprising different cell types for extended periods of time and therefore may provide an *in vitro* model of an *in vivo* tissue or organ environment. Regional differences and changes in morphology can be readily evaluated by histology and special stains, similar to tissue obtained from *in vivo* studies.

[0048] The tissue slice may be of any suitable size. In certain examples, the tissue slice is between about 4mm and 10mm in diameter. Aptly, the tissue slice may be about 6mm in diameter. Aptly, the tissue slice is a liver or a kidney slice having a thickness of 200-300μm and cores of between about 4 - 8mm diameters. Aptly, the tissue slice is a lung slice having a thickness of 200-500μm and cores between about 4 - 8mm in diameter.

[0049] In certain examples, liver tissue slices can be a beneficial model as they retain liver structure, contain all the cell types found *in vivo,* have good *in vitro* / *in vivo* correlation of xenobiotic metabolism, and maintain zone-specific cytochrome activity (allowing for cellular and zonal toxicity) and mechanisms of toxicity.

[0050] A three-dimensional cell or tissue culture may be differentiated from a two-dimensional cell or tissue culture which is typically provided by a flat layer of cells supported by a base surface of a chamber or well. Three dimensional cell cultures are aptly cellular networks in which cells are round and organised in three dimensions, an environment and cell morphology

that are more similar to that found *in vivo.* A 3-D cell culture may be provided by a scaffold.

[0051] As used herein, the terms "cell scaffold" and "tissue scaffold" refer to an artificial three-dimensional porous solid structure. These scaffolds serve to mimic the actual *in vivo* microenvironment where cells interact and behave according to the mechanical cues obtained from the surrounding 3D environment. A variety of cell scaffold materials are available. Suitable materials include for example, polymer microfibers or nanofibers e.g. electrospun nanofibers. Suitable polymers include e.g. poly(L-lactide) (PLLA) and poly(D,L-lactide) (PDLLA). The scaffold can be made using conventional techniques such as for example silicon processing technology, micromachining, injection moulding and rapid additive manufacturing techniques.

[0052] As used herein, the phrases "medium", "cell culture medium", "tissue culture medium," "culture medium" (plural "media" in each case) and "medium formulation" refer to a nutritive solution suitable for cultivating cells or tissues e.g. mammalian cells. These phrases can be used interchangeably. Cell culture media formulations are well known in the art. Typically, a cell culture medium is composed of a number of ingredients and these ingredients can vary from medium to medium. Cell culture media are typically comprised of buffers, salts, carbohydrates, amino acids, vitamins, and trace essential elements. The selection of cell culture media will be dependent on e.g. cell type and other factors.

[0053] The cell culture medium may or may not contain serum, peptone, and/or proteins. Various tissue culture media, including serum-free and defined culture media, are commercially available, for example, any one or a combination of the following cell culture media can be used: RPMI-1640 Medium, RPMI-1641 Medium, Dulbecco's Modified Eagle's Medium (DMEM), Minimum Essential Medium Eagle, F-12K Medium, Ham's F12 Medium, Iscove's Modified Dulbecco's Medium, McCoy's 5A Medium, Williams E Medium, Leibovitz's L-15 Medium, and serum-free media such as EX-CELL™ 300 Series (JRH Biosciences, Lenexa, Kans.), among others. Cell culture media may be supplemented with additional or increased concentrations of components such as amino acids, salts, sugars, vitamins, hormones, growth factors, buffers, antibiotics, lipids, trace elements and the like, depending on the requirements of the cells to be cultured and/or the desired cell culture parameters.

[0054] Aptly, the methods of certain examples may be for culturing cells from any source including eukaryotic cells and prokaryotic cells, e.g. plant cells, mammalian cells, yeast cells, fungal cells and/or bacterial cells. Aptly, the cell culture comprises mammalian cells selected from epithelial cells, tumour cells, hepatocytes, fibroblast cells, stem cells, myocardiocytes, kidney cells, lung cells, neuronal cells, adipocytes, intestinal cells, skin cells, immune cells, either alone or in combination.

[0055] In certain examples, the mammalian cells are selected from tumour cells, stem cells and primary epithelial cells (e.g., keratinocytes, cervical epithelial cells, bronchial epithelial cells, tracheal epithelial cells, kidney epithelial cells and retinal epithelial cells).

[0056] Aptly, the mammalian cells may be human. The mammalian cells may be sourced from

an individual e.g. a patient suffering from a disorder. In certain examples, the cells (e.g. in an organ slice) may be isolated from a patient suffering from, or at risk of, a fibrotic disease. The fibrotic disease may be for example a fibrotic disease affecting the liver, the kidneys or the lungs. The patient may be suffering from a disorder which may progress to a fibrotic disease.

[0057] In certain examples, the cells may be sourced from established cells lines. In certain examples, the cells may be genetically modified. In certain examples, the cells are from established cell lines such as for example, 293 embryonic kidney cells, HeLa cervical epithelial cells and PER-C6 retinal cells, MDBK (NBL-1) cells, CRFK cells, MDCK cells, CHO cells, Chang cells, Detroit 562 cells, HeLa 229 cells, HeLa S3 cells, Huh7, Hep3b, A549, BEAS-2B, Calu-3, Hep-2 cells, KB cells, LS 180 cells, LS 174T cells, NCI-H-548 cells, RPMI 2650 cells, SW-13 cells, T24 cells, WI-28 VA13, 2RA cells, WISH cells, BS-C-I cells, LLC-MK_2cells, Clone M-3 cells, 1-10 cells, RAG cells, TCMK-1 cells, Y-1 cells, LLC-PK_1 cells, PK(15) cells, GH.sub.1 cells, GH_3cells, L2 cells, LLC-RC 256 cells, MH_1C_1 cells, XC cells, MDOK cells, VSW cells, and TH-I, B1 cells, or derivatives thereof), fibroblast cells from any tissue or organ (including but not limited to heart, liver, kidney, colon, intestine, oesophagus, stomach, neural tissue (brain, spinal cord), lung, vascular tissue (artery, vein, capillary), lymphoid tissue (lymph gland, adenoid, tonsil, bone marrow, and blood), spleen, and fibroblast and fibroblast-like cells lines (e.g., CHO cells, TRG-2 cells, IMR-33 cells, Don cells, GHK-21 cells, Dempsey cells, Detroit 551 cells, Detroit 510 cells, Detroit 525 cells, Detroit 529 cells, Detroit 532 cells, Detroit 539 cells, Detroit 548 cells, Detroit 573 cells, HEL 299 cells, IMR-90 cells, MRC-5 cells, WI-38 cells, WI-26 cells, $MiCl_1$ cells, CHO cells, CV-1 cells, COS-1 cells, COS-3 cells, COS-7 cells, Vero cells, DBS-FrhL-2 cells, BALB/3T3 cells, F9 cells, SV-T2 cells, M-MSV-BALB/3T3 cells, K-BALB cells, BLO-11 cells, NOR-10 cells, C.sub.3H/IOTI/2 cells, $HSDM_1C_3$ cells, KLN_2O_5 cells, McCoy cells, Mouse L cells, Strain 2071 (Mouse L) cells, L-M strain (Mouse L) cells, NCTC clones 2472 and 2555, SCC-PSA1 cells, Swiss/3T3 cells, Indian muntjac cells, SIRC cells, C_{II} cells, and Jensen cells, or derivatives thereof.

[0058] In certain examples, the apparatus and/or method may comprise the use and/or culture of tissues or portions thereof. The tissue may be for example an organ or a portion thereof. In one example the tissue portion is a slice of an organ. Aptly, the organ may be for example, a heart, a kidney, a liver, a lung, a pancreas, a stomach, a brain. In certain examples, the tissues may be for example, skeletal tissue, muscle tissue, connective tissue, nervous tissue, epithelial tissue and/or mineralised tissue. In certain examples, the tissue portion is a liver slice or a kidney slice. Aptly, the tissue slice may further comprise multiple cell types including for example immune cells. Aptly the tissue is a human organ and the tissue portion a slice or section thereof.

[0059] In certain examples, there is provided a method for testing efficacy of a candidate therapeutic molecule. A "candidate therapeutic molecule" and "candidate molecule" may act as a modulator of target molecule concentration or target molecule function in a system. A "modulator" may agonize (i.e., up-regulates) or antagonize (i.e., down-regulates) a target molecule concentration partially or completely in a system by affecting such cellular functions

as DNA replication and/or DNA processing (e.g., DNA methylation or DNA repair), RNA transcription and/or RNA processing (e.g., removal of intronic sequences and/or translocation of spliced mRNA from the nucleus), polypeptide production (e.g., translation of the polypeptide from mRNA), and/or polypeptide post-translational modification (e.g., glycosylation, phosphorylation, and proteolysis of pro-polypeptides). A modulator may also agonize or antagonize a biological function of a target molecule partially or completely, where the function may include adopting a certain structural conformation, interacting with one or more binding partners, ligand binding, catalysis (e.g., phosphorylation, dephosphorylation, hydrolysis, methylation, and isomerization), and an effect upon a cellular event.

[0060] In one example, the candidate molecule may be an anti-fibrotic compound as described herein.

[0061] Turning now to the Figures, certain examples of the apparatus according to the present disclosure are illustrated in Figure 1 for example.

[0062] As shown in Figure 1, an apparatus 1 according to certain examples is illustrated. The apparatus 1 is aptly a multi-well plate apparatus as detailed herein. The multi-well plate 1 comprises a holder body 10 which includes a plurality of chambers 12. The holder body is rectangular and has a lower planar surface (not shown).

[0063] In one example, the apparatus is a multi-well plate. Aptly, the apparatus comprises a footprint defined by the standards of the Society for Biomolecular Sciences (e.g. Standards ANSI SLAS-1 to 4). The multi-well plate can be manufactured using known techniques including for example, rapid prototype manufacturing, moulding or the like.

[0064] The chambers may also be referred to as "wells". In the illustrated example, each chamber is cylindrical. Other shapes of chamber e.g. cuboidal, are envisaged and within the scope of certain examples of the disclosure. The chambers may be arranged to receive and/or remove cell culture media. In addition, the chambers may be configured to receive and/or support an insert element, as described in more detail below.

[0065] Each chamber 12 comprises a lower planar base 20 as shown in Figure 2. The lower planar base in certain examples may alternatively be defined by a base (not shown) of the holder body. Each chamber also comprises an upper opening 14 extending through a top surface 18 of the holder body. Aptly, the upper opening is sized to enable cell culture media to be added to the chamber and/or removed therefrom. Thus, cell/ tissue seeding, agent addition, sample removal, media addition and removal can be carried out via the opening of the chamber. Addition of components may be via pipette or robotics for example.

[0066] In certain examples, each chamber has a depth of between about 15mm and 20mm e.g. 15, 16, 17, 18, 19 or 20 mm. Other depths may be useful in certain examples.

[0067] The chamber 12 also comprises a side wall 16 extending between the lower surface

and the upper opening. The wall element may not only define portions of the walls but also act to separate at least a portion of a chamber from at least a portion of an adjacent chamber.

[0068] Depending on the application of the apparatus, the base and side walls of each chamber may be formed from a transparent, translucent or opaque material. Suitable materials include for example Zeonex™, Zeonor™, polystyrene, polycarbonate, polyethylene, polypropylene, PMMA, cellulose acetate and glass.

[0069] The chambers may be provided in any quantity and in any arrays. Aptly, the chambers are uniformly arranged in the holder body i.e. the chambers are arranged in rows and columns. The apparatus may be for example a 12-well plate, a 24-well plate, a 48 well plate or a 96-well plate. The holder body may be formed with any dimensions including for example standard dimensions for use with robotic laboratory equipment.

[0070] As shown in Figure 1, a pair of adjacent chambers 12a and 12b are fluidly connected by a through passageway 22. The through passageway 22 provides a fluid communication pathway between the interior of a first chamber 12a and the interior of a second chamber 12b. The fluid communication pathway is aptly a through passageway and may be for example a through slit, as shown in Figure 1, which has a height which extends between the top surface 18 of the holder body to adjacent to and planar with the lower planar bases of the respective chambers.

[0071] In an alternative example, the through passageway 22 may be a through slit which has a height that extends only part of the distance between the top surface of the holder body and the lower planar bases of the respective chambers.

[0072] In certain examples, the through slit is approximately 2mm wide and approximately 4mm in length. In other examples, the through slit is approximately 2mm wide and approximately 3.5mm in length. Other dimensions of the through slit are envisaged.

[0073] In a yet further example, the through passageway 22 is a through hole which is provided between the side wall 16a of the first chamber 12a and the side wall 16b of the second chamber 12b. The through hole may be provided at a lower portion of the respective side walls.

[0074] The through passageway should be sized so as to allow fluid movement between the respective adjacent chambers. Such fluid includes for example liquid cell culture media. The cell culture media may comprise a plurality of components including for example components secreted by cells and/or tissues provided within one or both chambers. Thus, the through passageway allows for an exchange of cell culture media and components comprised therein between chambers of the multi-well plate apparatus.

[0075] In the illustrated example, a through passageway is provided between two adjacent chambers. It will be understood that in certain examples a through passageway may be

provided between three or more chambers. Aptly, the through passageway comprises a plurality of through slits and/or through holes, a first slit or hole being provided between a side wall or base of a first chamber and an adjacent second chamber, and further slits and/or holes being provided between the first or second chamber and adjacent further chamber and optionally between adjacent further chambers. In such examples, the through passageway is provided in a linear non-angled direction between the plurality of chambers.

[0076] The holder body further comprises an outer perimeter wall 24. The outer perimeter wall may comprise a recessed outer edge portion 26 which provides a surface 28. The lower planar surface of the holder body may comprise a recessed edge region (not shown) which is configured to rest and locate on the surface 28 of the recessed outer edge portion of a further holder body such that the holder bodies are located in a nested relationship. In certain examples, a plurality of holder bodies may be located in a vertical nested relationship.

[0077] In certain examples, the apparatus also comprises one or more insert elements 30 which are configured to support one or more cells. In certain examples, the insert element may be a Transwell® available from Corning, US. The cells may be in the form of a tissue portion e.g. a tissue slice. The insert element may provide a 3D cell scaffold. As shown in Figure 4, the insert element 30 has a body portion 32 which is generally cylindrical in shape. The insert element is sized so as to fit within a chamber. The insert element also comprises a plurality of radially outwardly extending flanges 34a, 34b, 34c which, when the insert element is placed in a chamber, contact and rest on the top surface of the holder body so as to support the insert element within the chamber.

[0078] Aptly, when supported by the flanges resting on the top surface, the insert element is positioned within the chamber such that it does not contact a side wall or the base of the chamber. As a result, when the chamber is filled with fluid e.g. cell culture media, the insert element is at least partially surrounded by the fluid. In other examples, the insert element may contact the base of the chamber for example. The insert element may comprise a lower surface membrane 40 with pores.

[0079] Aptly, the pores having an average diameter of between about around 8μm to about 150μm. in one example, the pores have an average diameter of about 8 μm. The insert element may be coated with a matrix material e.g. collagen or the like.

[0080] In use, the top surface of the holder body may be covered by a common removable lid (not shown). The lid may be removed to add or remove components in the chambers such as cell inserts, cell scaffolds, cell culture media, and the like.

[0081] Turning to Figures 3 and 5, a rocker apparatus 100 according to certain examples of the present disclosure is illustrated.

[0082] Figure 3a represents an apparatus for providing bi-directional fluid flow and which includes a rocker apparatus holding a multi-well plate apparatus according to certain examples

of the present disclosure. As illustrated by arrows, media exchange may occur via insert pores within wells of the plate apparatus. In addition, bi-directional media exchange may occur via a channel between wells. A tissue slice is illustrated in the chambers in Figure 3a.

[0083] The rocker apparatus may be alternatively referred to as a "see-saw" type apparatus. A schematic representation of the rocker apparatus 100 is shown in Figure 5. The rocker apparatus comprises a holder body support 102 which directly or indirectly supports a holder body 10. Aptly, the holder body support includes a fixed pivot or fulcrum on which a moveable platform 104 is supported. The platform 104 includes an upper surface for supporting the holder body 10. The platform and fixed pivot can be formed from any suitable material including for example plastic, metal or wood. The platform comprises a first end region 106 and a second end region 108 spaced apart from the first end.

[0084] The rocker apparatus 100 may be configured to tilt the platform 104 e.g. by raising and lowering spaced apart end regions. Aptly, the platform 104 is biased downwardly at one of the spaced apart end regions e.g. by way of a weight which is provided at the respective end region. The weighted end is biased downwards to a resting position. Aptly, the bias is a minimal bias which is just sufficient to overcome the weight of the opposing end region.

[0085] The apparatus 100 also comprises a drive element arranged to rock the holder body supported via the support to thereby repeatedly raise and lower the spaced apart first and second ends of the holder body. In one example, the rocker apparatus comprises a drive element comprising a linear actuator 110 provided at the first end region 106. In one particular example, a linear actuator is configured to tilt the platform, and therefore the multi-well plate. Under normal circumstances therefore this end of the platform will rest lower. The actuator is arranged to drive against the base, so that when driven it raises that end of the platform and lowers the other spaced apart end.

[0086] In some examples, the linear actuator provides the biasing weight itself and so can be used to return the platform passively to its resting position with first end 106 lowered, such that the drive element need only drive actively in one direction.

[0087] In one particular example, the linear actuator is configured to tilt the platform, and therefore the multi-well plate, at a speed of between about 10 seconds to about 20 minutes e.g. about 2 minutes per rock i.e. approximately two minutes for the first end of the holder body to be moved from a first position e.g. a lowered position to a second position e.g. a raised position.

[0088] The linear actuator may be powered by battery or by mains power for example.

[0089] It will be appreciated that Figure 5 is a schematic representation only and the angle of tilt of the platform may be smaller than depicted in Figure 5. For example, the platform may have a range of tilt from 0 degrees to about +/-20 degrees or less e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or20 degrees.

[0090] The tilting action of the rocker apparatus enables fluid e.g. liquid cell culture media to flow from a first chamber, via the fluid communication pathway, to a second chamber and back again. In certain examples, e.g. when each chamber has a well diameter of approximately 20mm, the rocking of the holder body enables fluid to flow between adjacent chambers at a rate of between about 15 to about 20 µl/second via the through passageway. In certain examples, at all stages of the rocking motion, a cell or tissue culture provided within a chamber remains at least partially covered by the cell culture media.

[0091] Rocking not only allows media exchange between the two chambers but also permits media exchange via the pores in the culture insert membrane which separate the inner and outer wells. The latter media exchange generates flow around/over a tissue slice, which will aid oxygenation and removal of toxic metabolites, which in turn is likely to increase viability and function of the tissue.

[0092] The multi-well plate and rocker apparatus as described herein can be used in a variety of methods to provide a dynamic cell culture environment which may more accurately represent *in vivo* conditions and therefore better support the growth and maintenance of cells and/or tissues in culture.

[0093] Figure 29 and 30 depict an apparatus 1000 according to certain examples of the disclosure. The apparatus 1000 includes a platform element 1010 which is configured to support a plurality of culture apparatus or multi-well plates as described herein. The platform may comprise a plurality of recessed regions 1020a, 1020b, 1020c which are each sized to locate a multi-well plate. A lip region 1030 is provided which extends substantially around the periphery of the platform and separates each recessed region. The lip region acts to prevent the culture apparatus from sliding when the apparatus is being rocked. In some examples, a plurality of multi-well plates may be stacked as shown in Figure 29.

[0094] The apparatus 1000 comprises a pivot element 1040 which sits in a central groove 1050 which is provided in a base portion 1060 of the apparatus. The linear actuator 1070 moves the platform up and down about the pivot element 1040 to tilt the culture apparatus up and down as described above.

[0095] Certain examples of the present disclosure relate to a method of culturing cells and/or tissues as described herein below:

Examples

[0096] Hepatocytes often present a challenge in cell culture and are known to rapidly loose phenotypic expression *in vitro* due to the absence of a suitable microenvironment. The following examples focussed on determining whether the apparatus of certain examples of the present disclosure could be used to prevent or delay phenotypic expression loss in

hepatocytes. The term "CMR", "CMR2" and "CMR tissue culture plates" used herein refers to the plate apparatus of certain examples of the present disclosure. The below examples of this description are to be interpreted as reference to the apparatus of the present invention for use in a method for treatment of the human (or animal) body by therapy (or for diagnosis).

Precision cut slice isolation and culture

[0097] Liver tissue was placed in a 10cm dish containing Hanks Buffered Saline Solution (HBSS+, Lonza, BE10-508F). Four to eight mm cores of liver tissue were made using a Stiefel Biopsy Punch. Cores were then transferred to a metal mould and submerged in 2.5-3.0% low gelling temperature agarose (Sigma, A9414) and then placed on ice for 2-5 minutes.

[0098] Once set, the cores in agarose were super-glued to a vibratome mounting stage and submersed in the media chamber in ice cold HBSS+ prior to cutting on a Leica VT1200 S fully automated vibrating blade microtome. Liver tissue cores were cut at a speed of 0.3mm/sec, and an amplitude 2mm and thickness (step size) of 250µm. Slices having a thickness of between 200-400µm can be used.

[0099] Slices were transferred to 3µm, 8µm or 100µm pore inserts provided in static (Griener) or CMR tissue culture plates. The CMR tissue culture plates of examples of the disclosure comprised either holes between adjacent wells or were rapid prototyped from CAD design to comprise slots between wells. The wells contained slice culture media. The slices were cultured under static, unidirectional or rocked conditions at 37°C in 5% carbon dioxide in a humidified tissue culture incubator. Twelve or twenty-four well CMR tissue culture plates were used, as indicated.

Slice culture media

[0100] The slice culture media comprised the following components:

- Williams Medium E (Sigma, W4128)
- 2% Heat Inactivated Fetal Bovine Serum (Gibco)
- Penicillin-Streptomycin (Sigma, P0781)
- L-glutamine (Sigma, G7513)
- Pyruvate (Sigma, S8636)
- 0.5uM Insulin/ transferrin selenium mix (Gibco 51500-056)
- 0.1uM dexamethasone (Sigma, D4902)

Urea Assay (Universal Biologicals Cambridge)

[0101] 50µl of the slice culture media from slices in static or CMR culture was used to quantify urea release using the QuantiChromTM Urea Assay Kit (catalogue number DIUR-500) following manufacturer's instructions.

Albumin Elisa (bethyl laboratories)

[0102] 100µl of the slice culture media diluted 1:250 was removed from wells comprising slices in either a static culture plate, a unidirectional system or the CMR culture plate apparatus which was rocked using a rocking apparatus as described herein. The media was used to quantify albumin release using the Rat Albumin ELISA Quantitation Set (catalogue number E110-125) or Human Albumin ELISA Quantitation Set (catalogue number E80-129) following manufacturer's instructions.

LDH Cytotoxicity Assay Kit (Pierce)

[0103] 50µl media was removed from wells comprising slices in either a static culture plate or the CMR culture plate apparatus which was rocked using a rocking apparatus as described herein. The media was used to quantify lactate dehydrogenase release (LDH) release using the LDH Cytotoxicity Assay Kit (catalogue number 88953) following manufacturer's instructions.

Aspartate aminotransferase (AST)

[0104] 200µl media from slices in static or CMR culture was sent to the Clinical pathology department, Royal Victoria infirmary, Newcastle Upon Tyne. AST was measured using a clinical colorimetric enzyme assay.

[0105] Results are shown in Figures 6 to 28 and 31 to 32.

[0106] Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of them mean "including but not limited to" and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

[0107] Features, integers, characteristics or groups described in conjunction with a particular example of the disclosure are to be understood to be applicable to any other example described herein unless incompatible therewith. All of the features disclosed in this

specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of the features and/or steps are mutually exclusive.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US5422270A [0010]
- US2003017582A1 [0010]
- WO03078565A1 [0010]
- WO2011161480A1 [0010]
- WO2006097749A1 [0010]
- WO03083044A2 [0010]
- WO2016166315A1 [0010]

PATENTKRAV

1. Dyrkningsanordning til dyrkning af celler og/eller et væv eller en del deraf, og som omfatter et holderlegeme, hvilket holderlegeme omfatter en flerhed af kamre til at indeholde et celledyrkningsmedium og mindst én fluidforbindelsesbane, der strækker sig mellem mindst to tilsvarende kamre af flerheden af kamre, hvor hver fluidforbindelsesbane muliggør fluidstrømning i to retninger mellem de mindst to kamre, og hvor

fluidforbindelsesbanen har en bredde på mellem 1,5 mm og 3,5 mm; og

hvor hvert kammer af flerheden af kamre er konfigureret til at rumme et kammerindsætningselement, der er konfigureret til at understøtte et cellestøtteelement,

hvor kammerindsætningselementet endvidere omfatter et cellestøtteelement med en flerhed af porer, hvor flerheden af porer har en gennemsnitlig diameter på mellem ca. 8 µm til ca. 150 µm.

2. Anordning ifølge krav 1, hvor hvert kammer af flerheden af kamre omfatter et basiselement og mindst ét sidevægelement.

3. Anordning ifølge krav 2, hvor fluidforbindelsesbanen omfatter en gennemgående kanal, der strækker sig mellem et basiselement eller sidevægelement af et første ét af flerheden af kamre til et basiselement eller sidevægelement af et yderligere ét af flerheden af kamre.

4. Anordning ifølge krav 2, hvor fluidforbindelsesbanen omfatter en gennemgående spalte, der strækker sig mellem et basiselement eller sidevægelement af et første ét af flerheden af kamre til et basiselement eller sidevægelement af et yderligere ét af flerheden af kamre.

5. Anordning ifølge krav 3 eller krav 4, hvor det første kammer er tilvejebragt stødende op til det yderligere kammer.

6. Anordning ifølge et hvilket som helst foregående krav, hvor holderlegemet omfatter en flerhed af fluidforbindelsesbaner, hvor hver fluidforbindelsesbane strækker sig mellem mindst to kamre af flerheden af kamre.

7. Anordning ifølge krav 6, hvor hver fluidforbindelsesbane af flerheden af fluidforbindelsesbaner i alt væsentligt er parallel med og adskilt fra hver anden fluidforbindelsesbane.

8. Anordning ifølge et hvilket som helst foregående krav, hvor hvert kammer af flerheden af kamre omfatter en åbning til modtagelse og/eller fjernelse af vævsdyrkningsmedier og/eller et tilsvarende kammerindsætningselement.

5

9. Anordning ifølge et hvilket som helst foregående krav, der endvidere omfatter mindst ét generelt cylindrisk kammerindsætningselement, der omfatter en flerhed af flanger, der strækker sig radialt udefter, til at understøtte kammerindsætningselementet inde i et tilsvarende kammer af flerheden af kamre.

10

10. Anordning ifølge et hvilket som helst foregående krav, hvor det totale volumen af den mindst én fluidforbindelsesbane og de mindst to tilsvarende kamre er mellem 1 ml og 8 ml.

11. Anordning ifølge krav 10, hvor det totale volumen er mellem 1,5 ml og 4 ml.

15

12. Anordning ifølge krav 1, hvor flerheden af porer har en gennemsnitlig diameter på mellem ca. 8 µm til ca. 100 µm.

13. Anordning ifølge et hvilket som helst foregående krav, hvor holderlegemet omfatter
20 mindst 12 kamre, f.eks. mindst 24 kamre.

14. Anordning ifølge et hvilket som helst foregående krav, hvor holderlegemet omfatter et udvendigt periferivægelement, og endvidere hvor det udvendige periferivægelement omfatter en indadvendende trindelt del, der er konfigureret til at gøre det muligt for en flerhed af
25 holderlegemer at blive stakket.

15. Anordning ifølge et hvilket som helst foregående krav, hvor holderlegemet består af et materiale valgt blandt Zeonex™, Zeonor™, polystyren, polycarbonat, polyethylen, polypropylen, PMMA, celluloseacetat og glas.

30

16. Anordning ifølge et hvilket som helst foregående krav, hvor hvert tilsvarende kammer af flerheden af kamre har en dybde på mellem ca. 16 mm og 19 mm.

17. Anordning ifølge et hvilket som helst foregående krav, hvor dyrkningsanordningen endvidere omfatter et lågelement, der kan aftageligt placeres over holderlegemet, hvilket lågelement tilvejebringer en i alt væsentligt plan afdækning, der strækker sig over flerheden af kamre.

5

18. Anordning ifølge et hvilket som helst foregående krav, hvor flerheden af kamre er anbragt i rækker og søjler i et tilsvarende retvinklet forhold inde i holderlegemet.

DRAWINGS

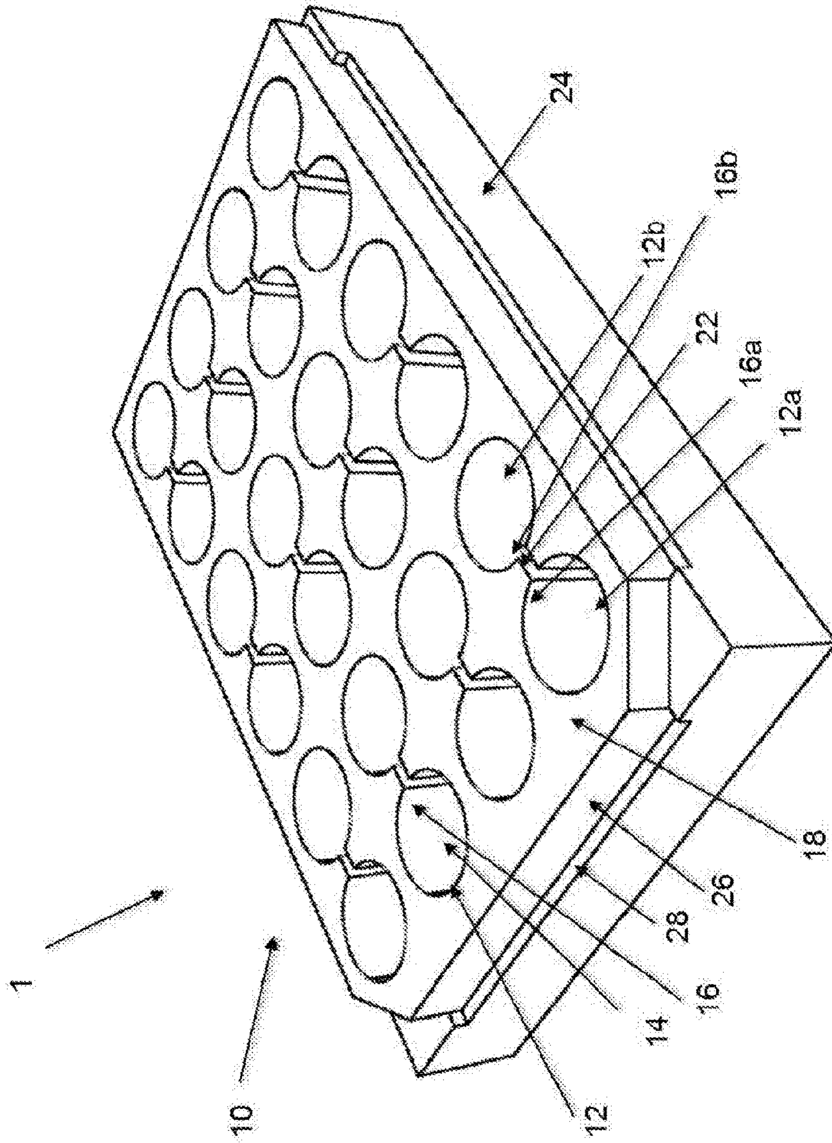

Figure 1

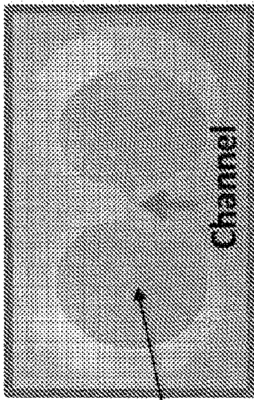

Figure 2

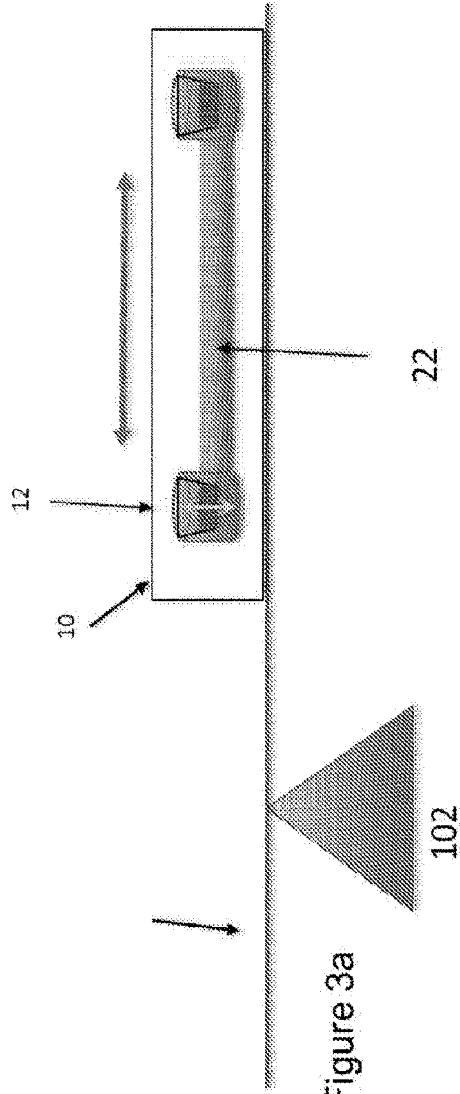

Figure 3a

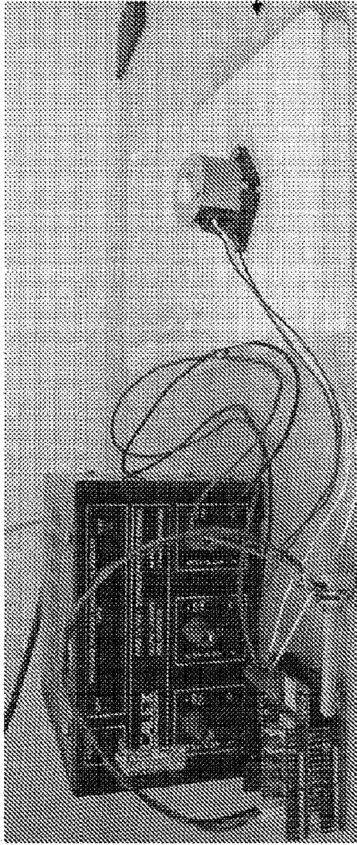

Figure 3b

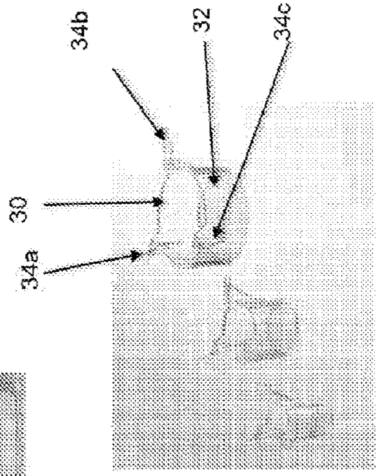

Figure 4b

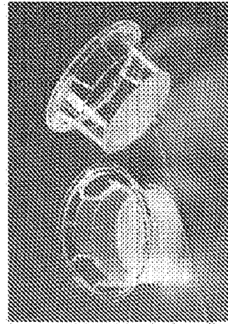

Figure 4a

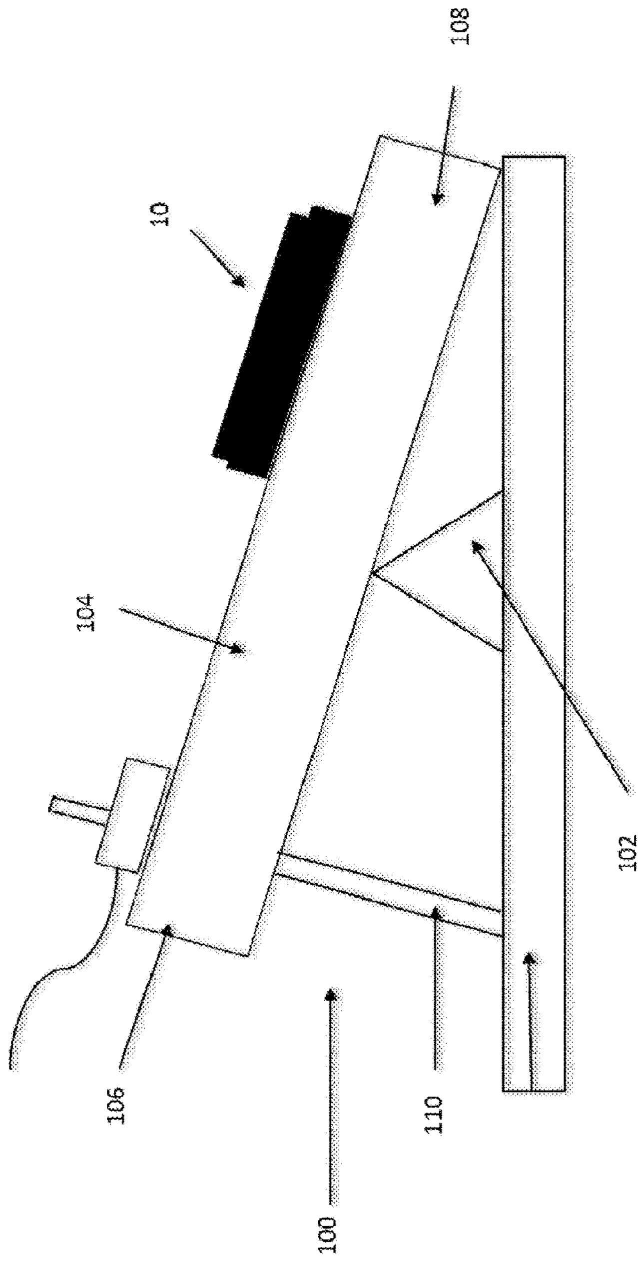

Figure 5

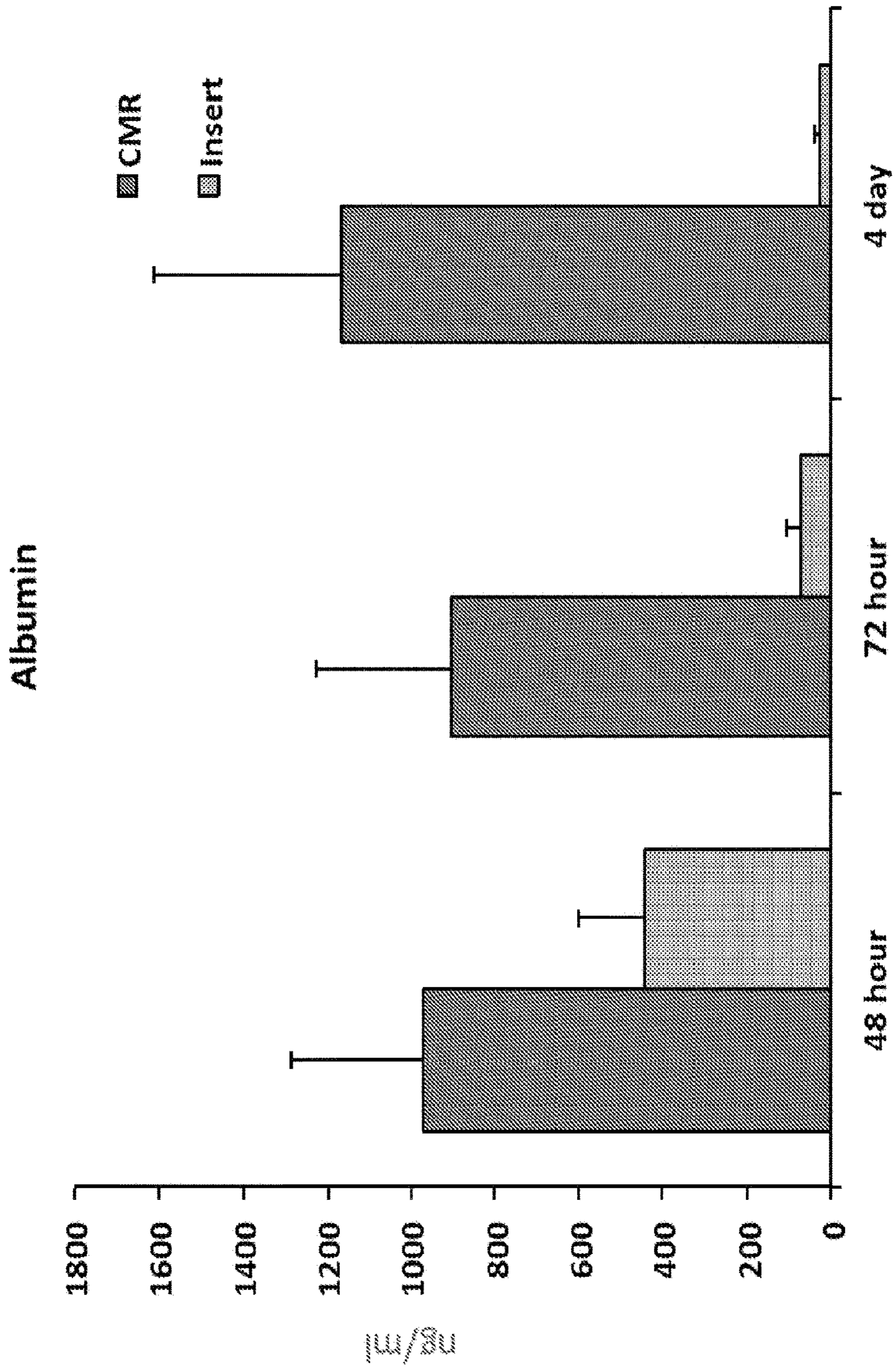

Figure 6

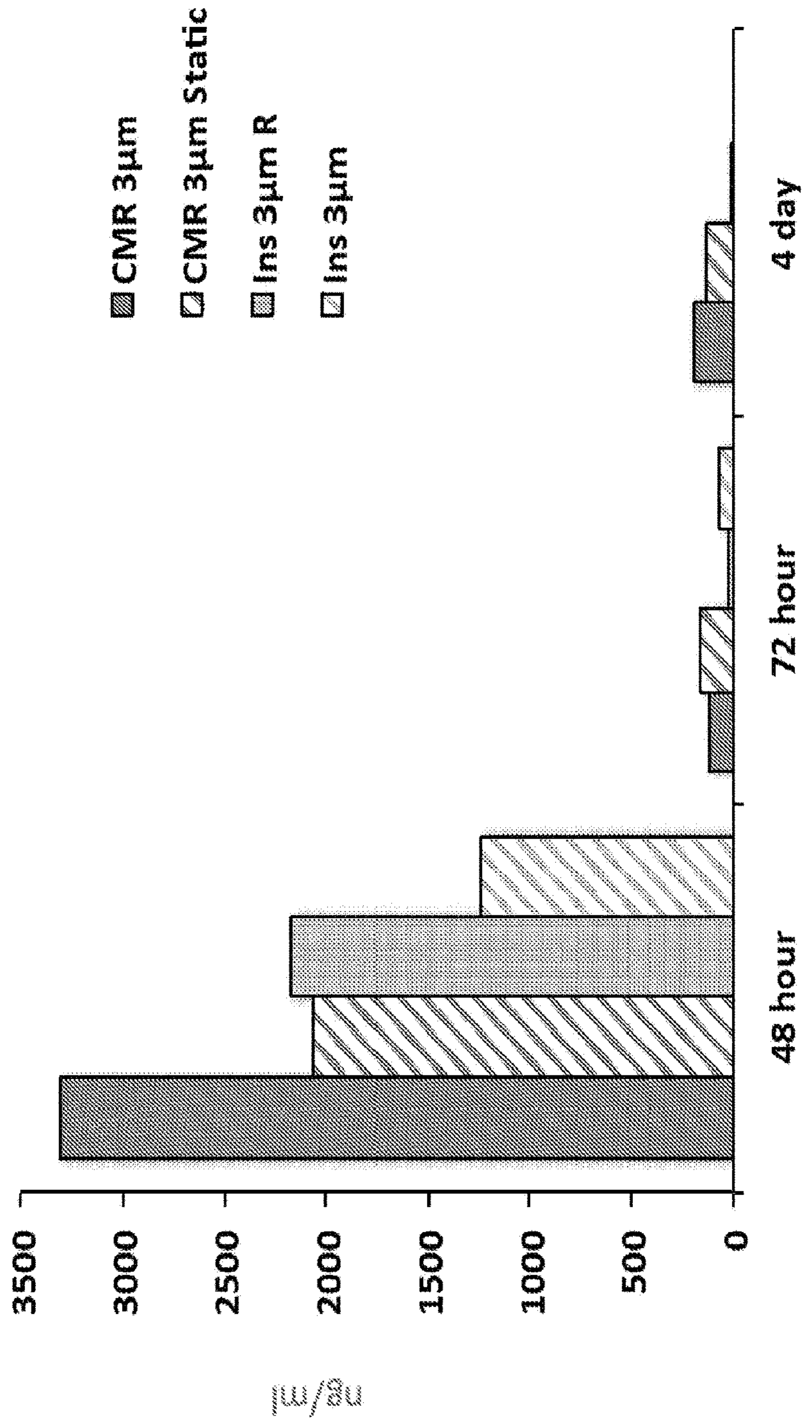

Figure 7

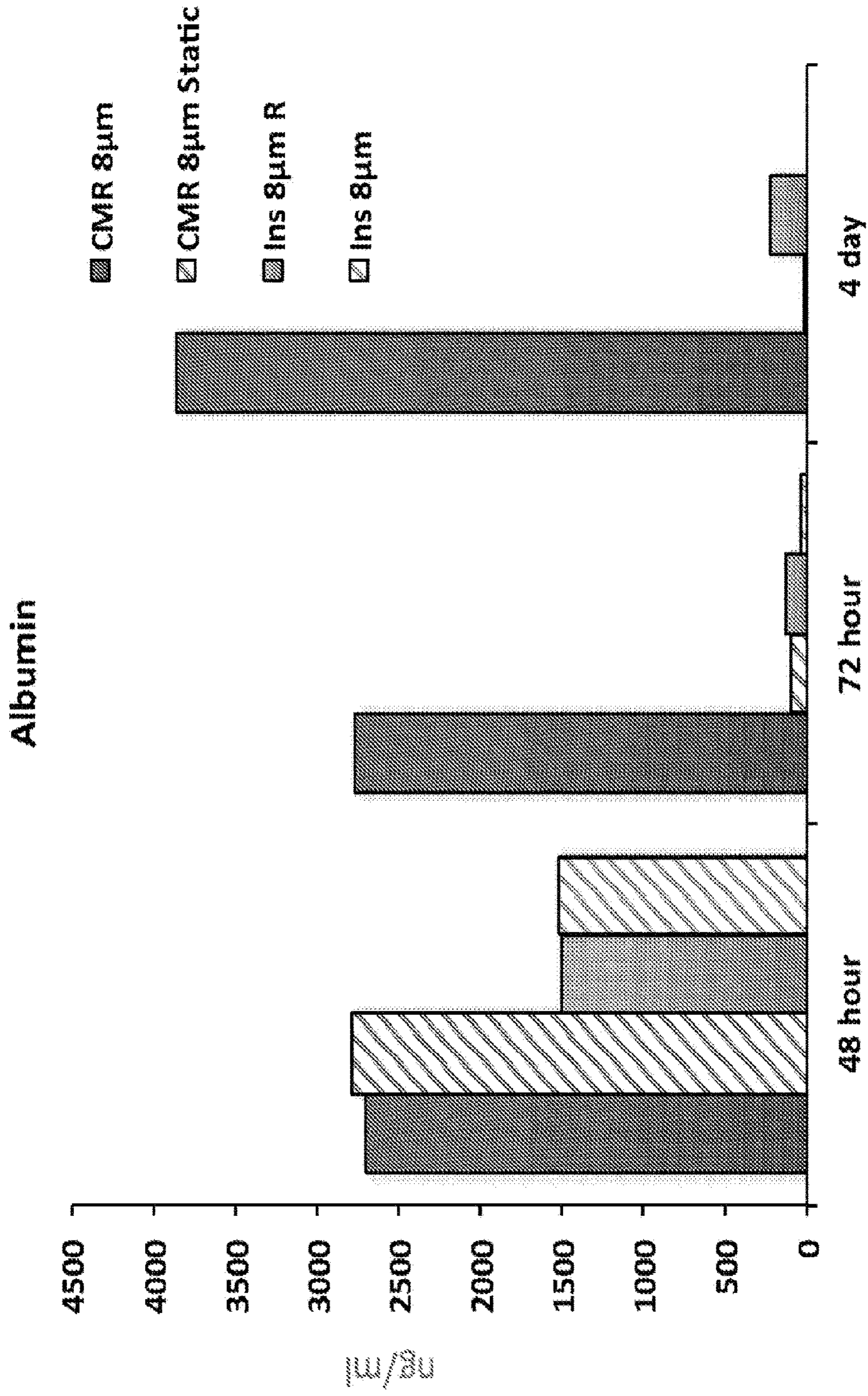

Figure 8

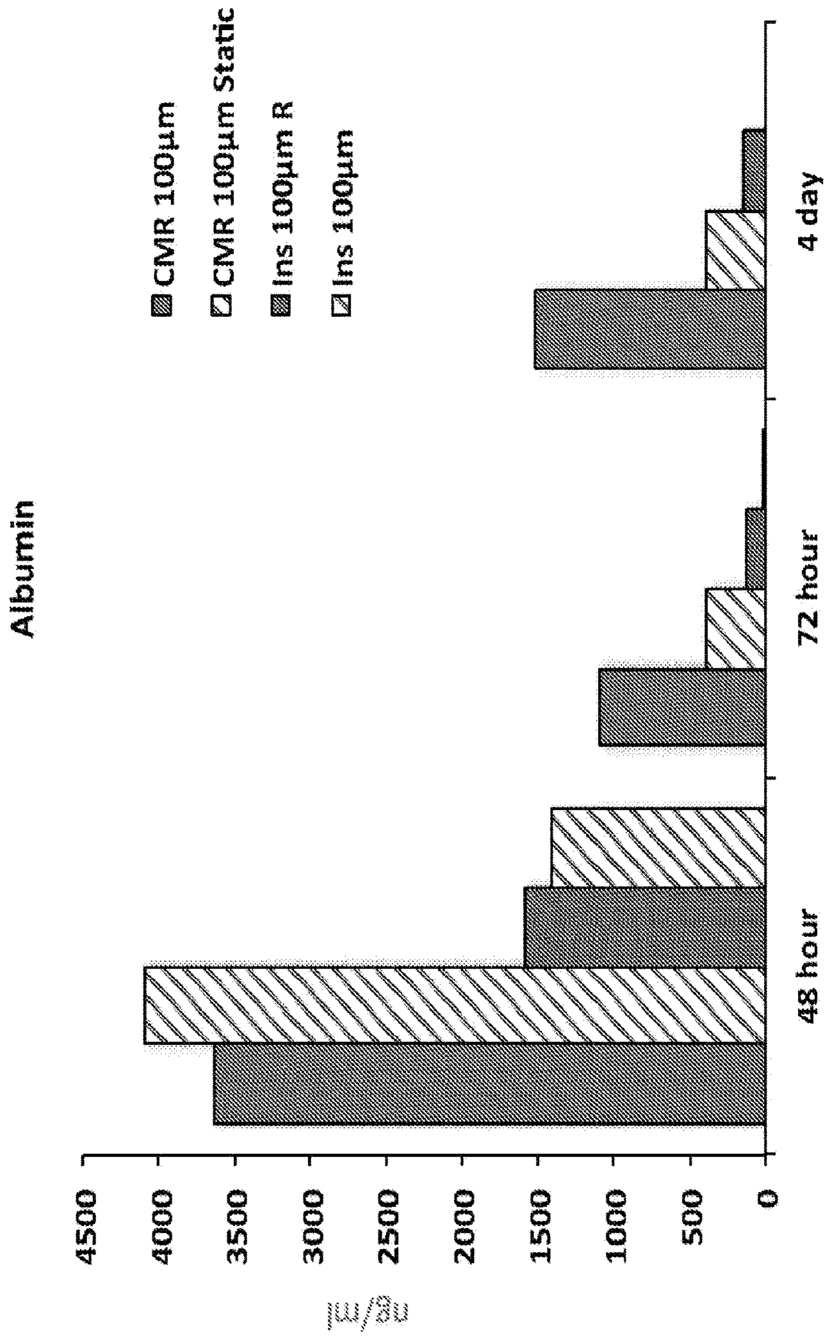

Figure 9

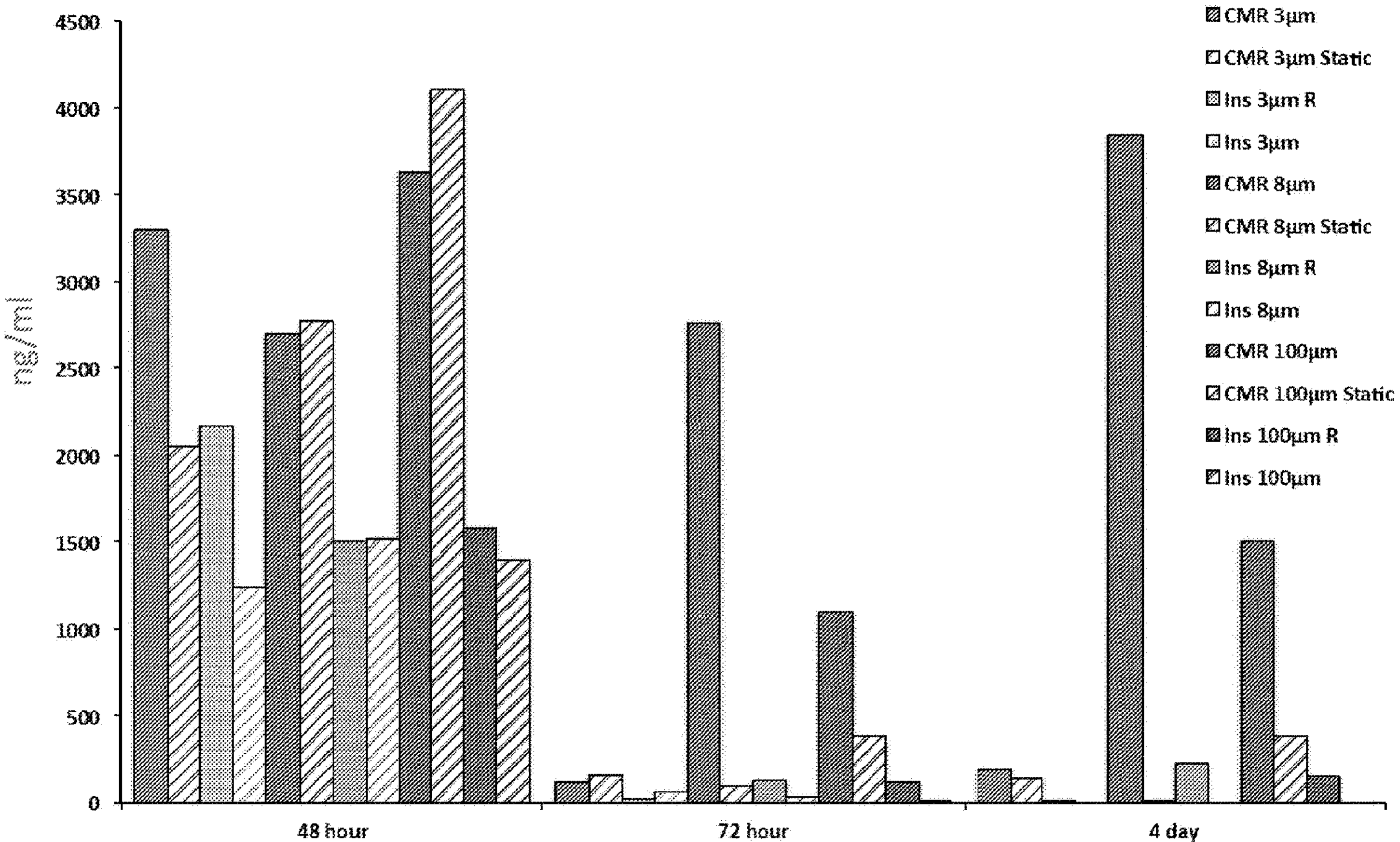

Figure 10

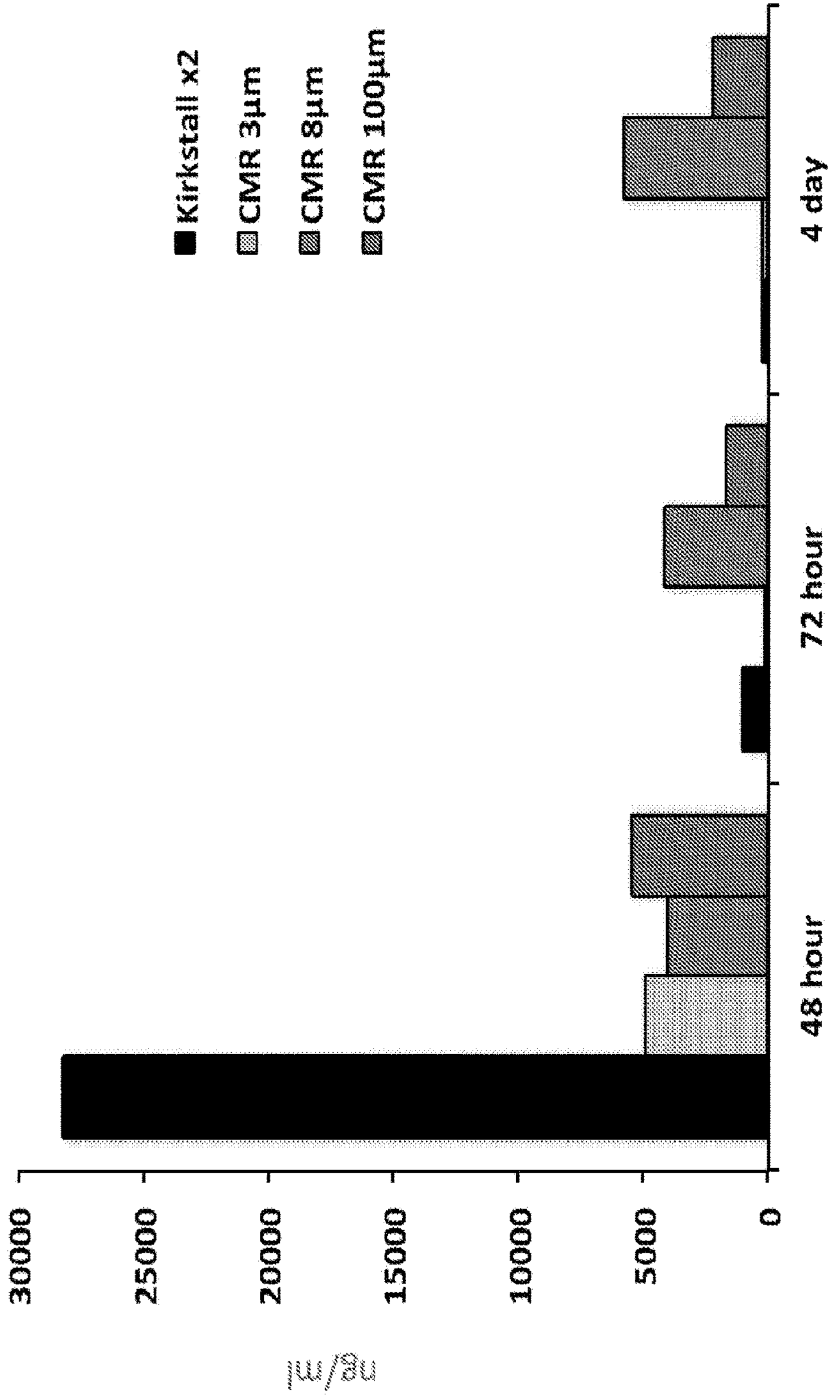

Figure 11

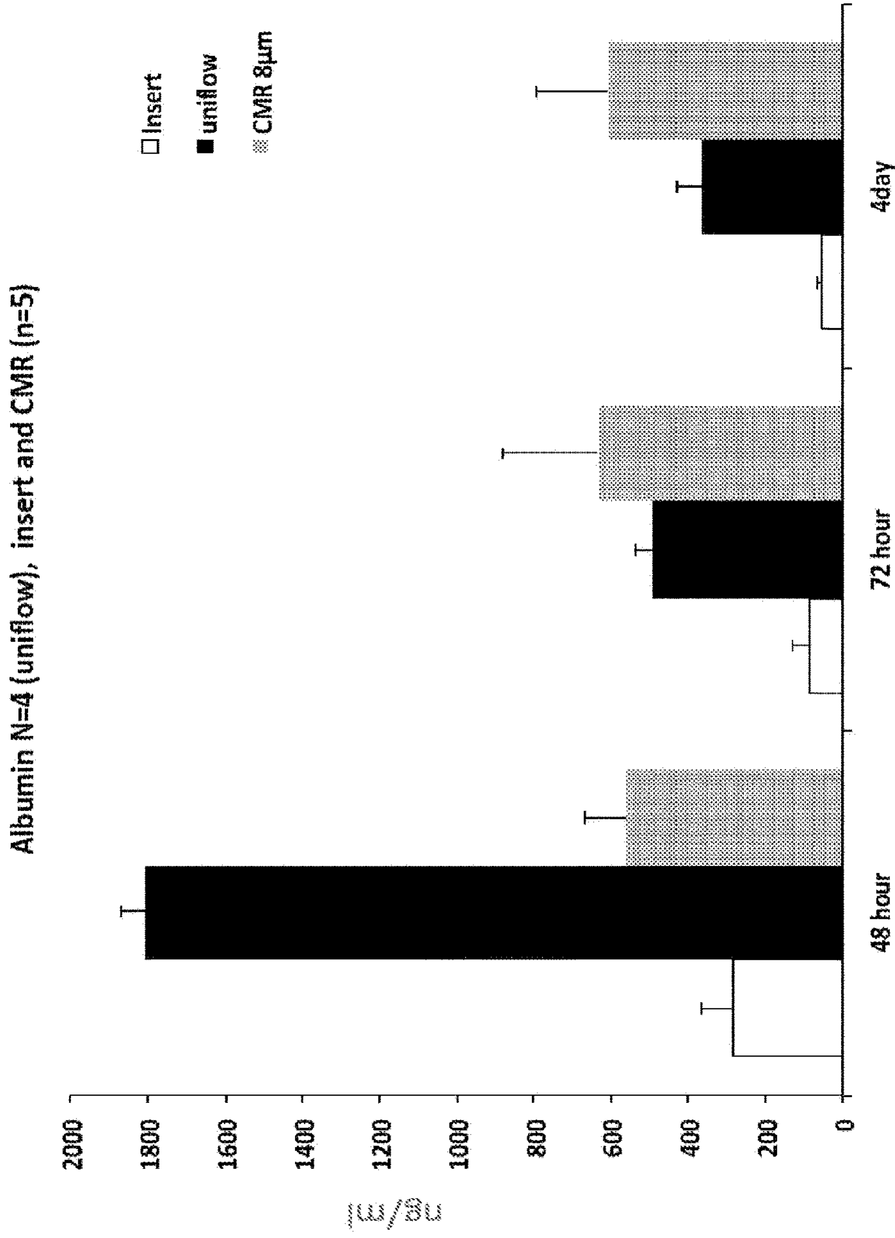

Figure 12

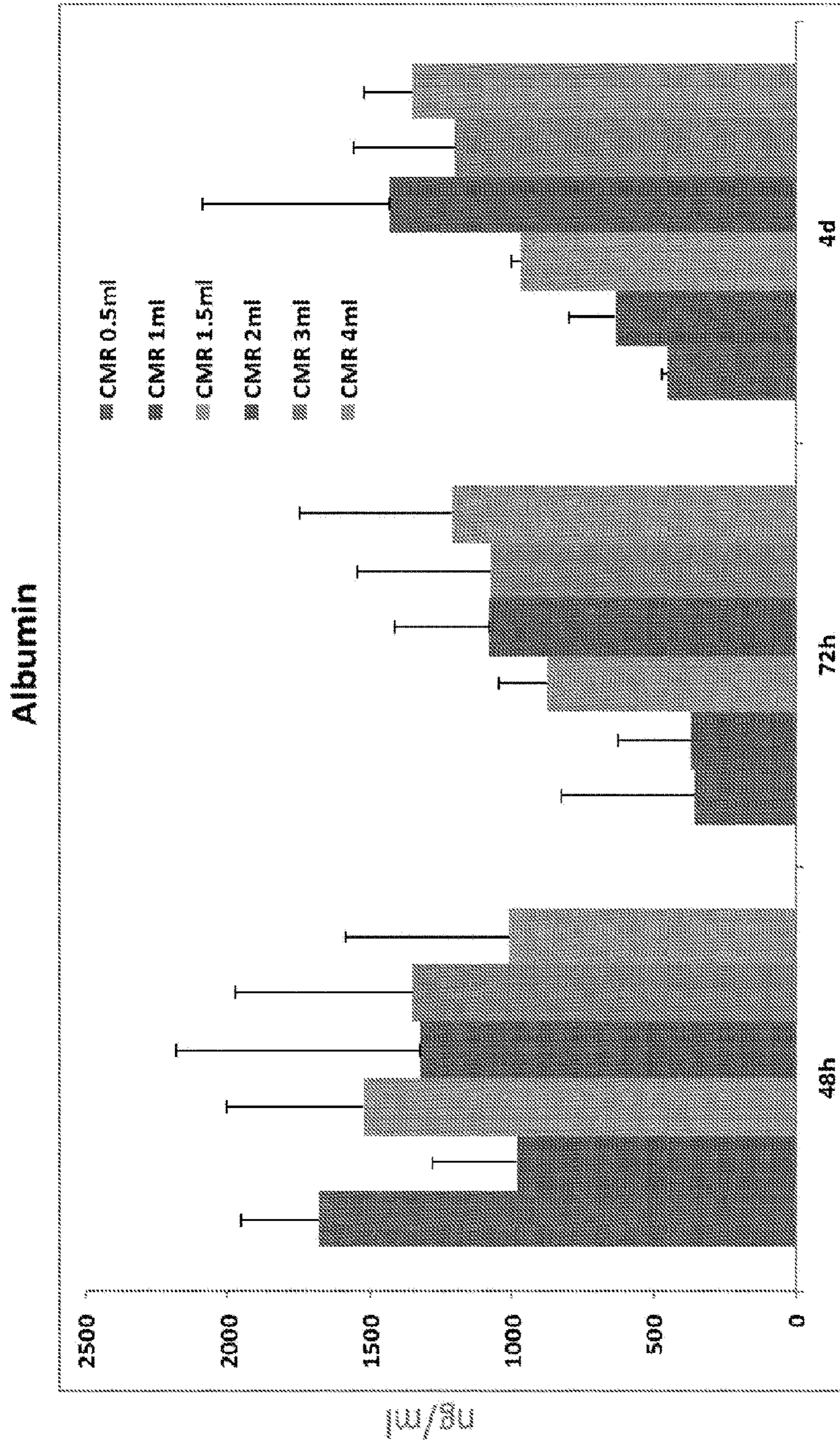

Figure 13

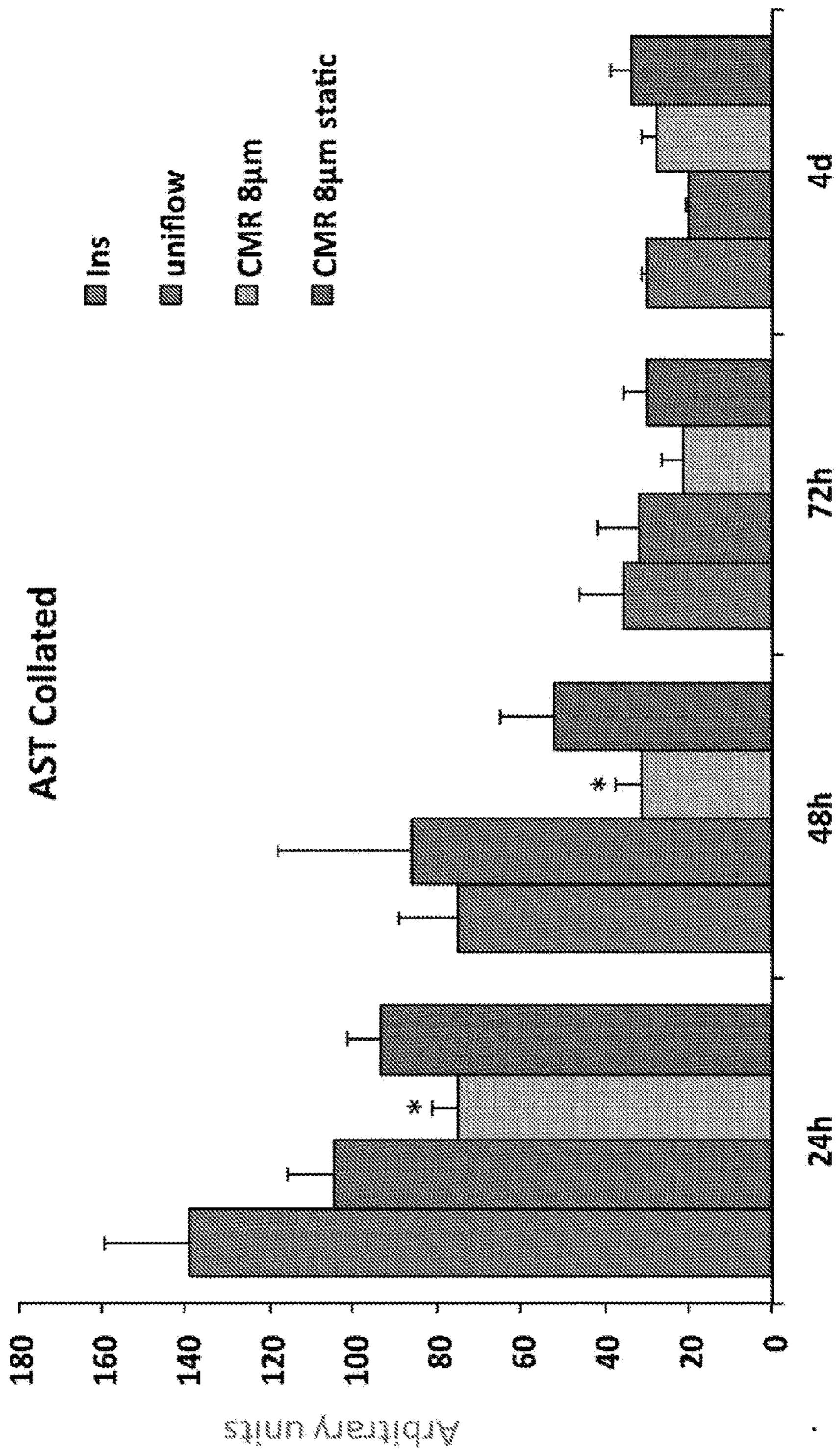

Figure 14

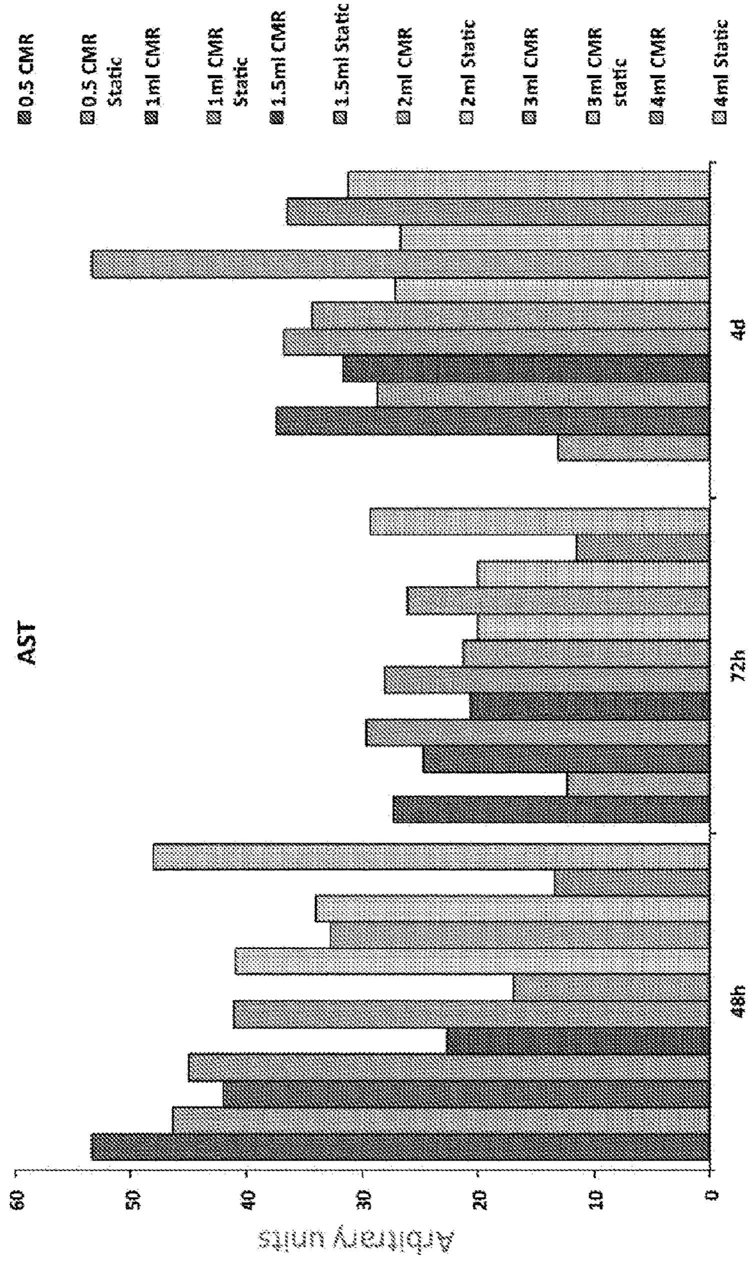

Figure 15

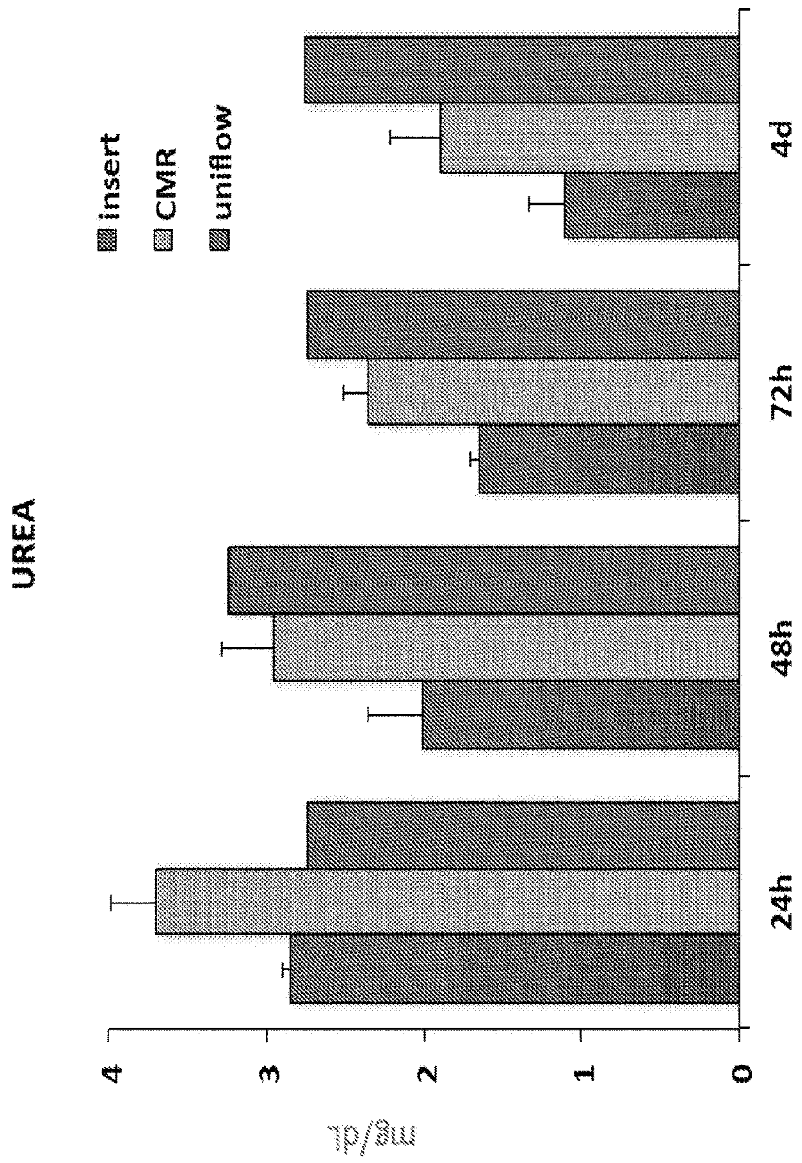

Figure 16

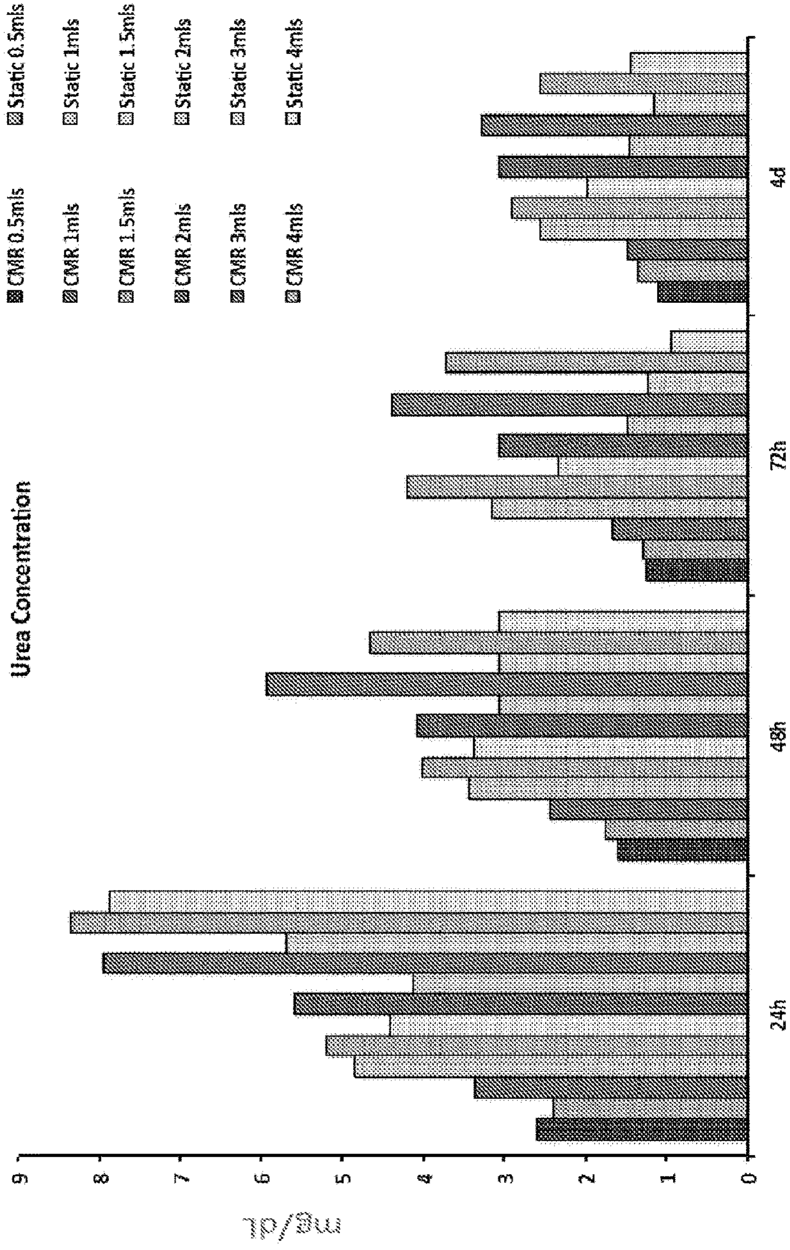

Figure 17

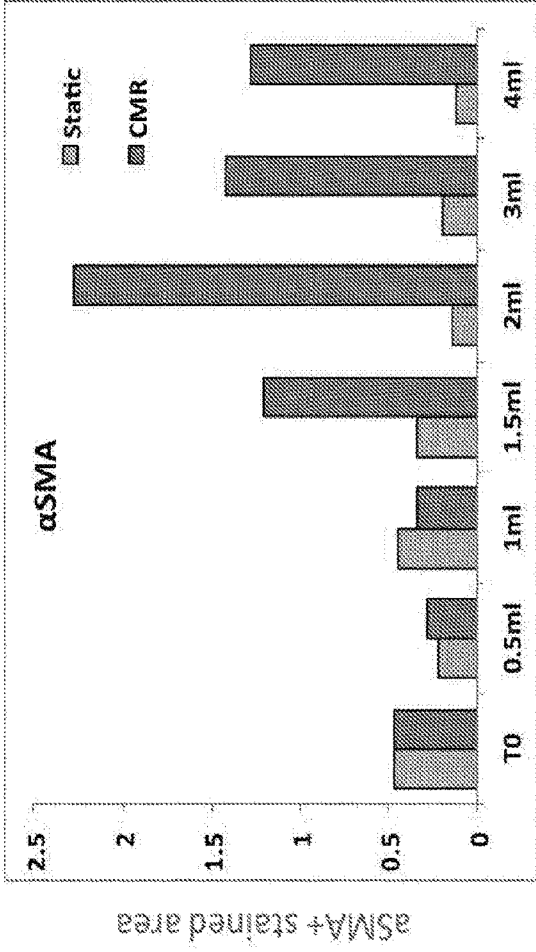

Figure 18

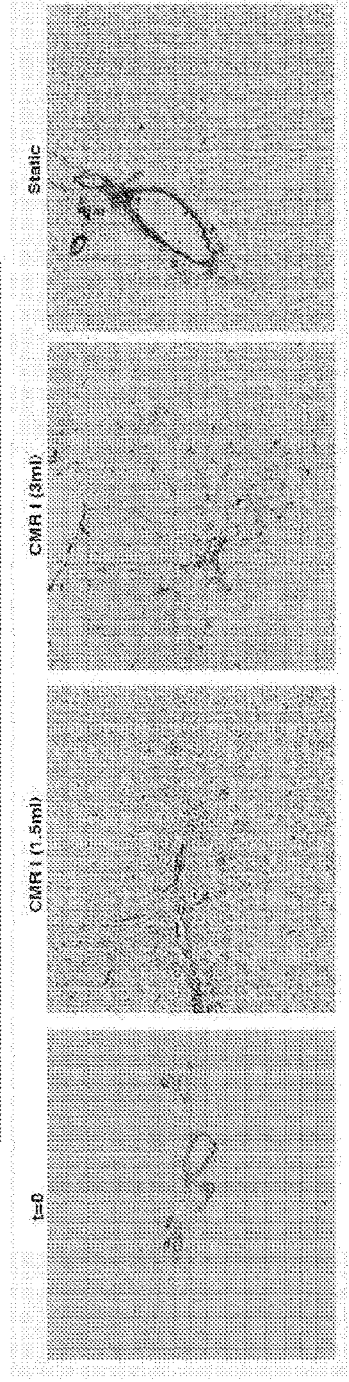

Figure 19

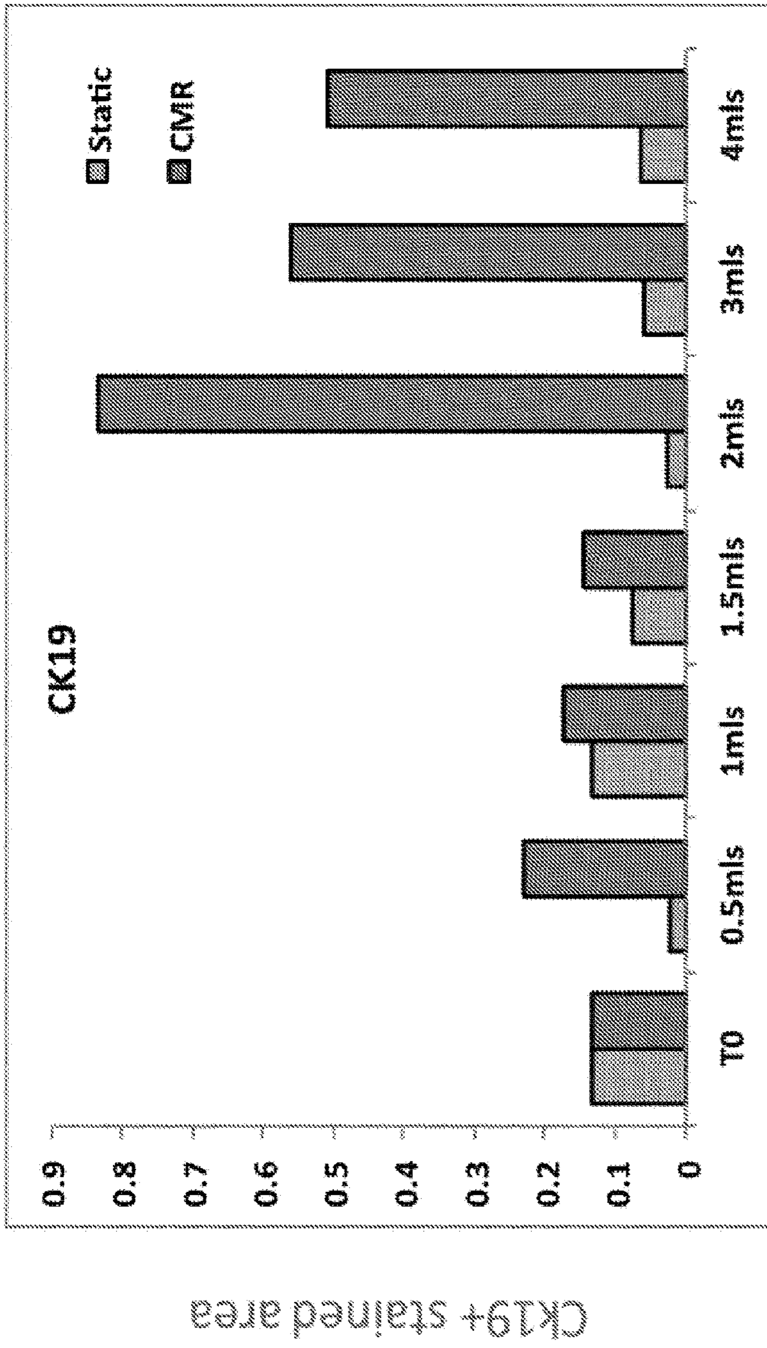

Figure 20

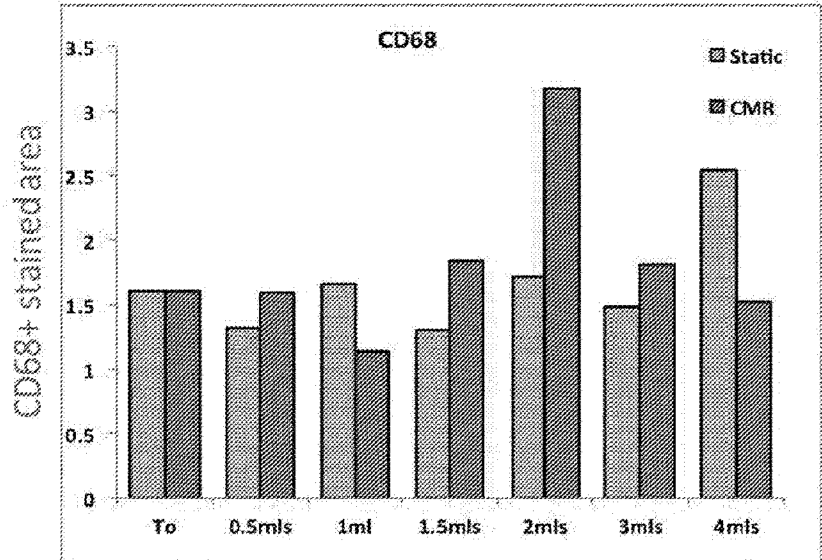

Figure 21

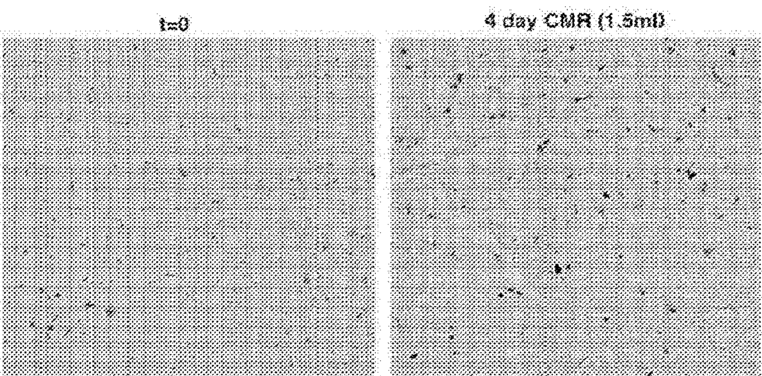

Figure 22

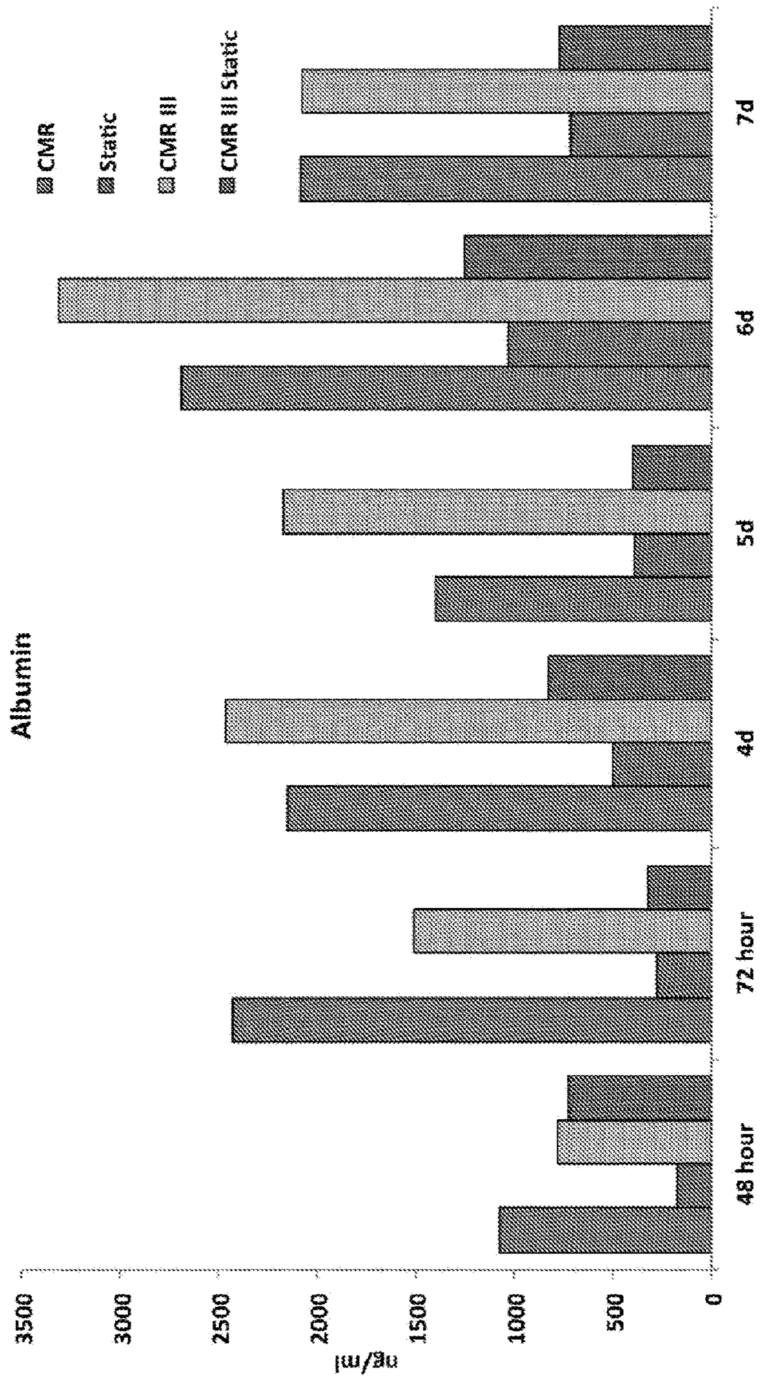

Figure 23

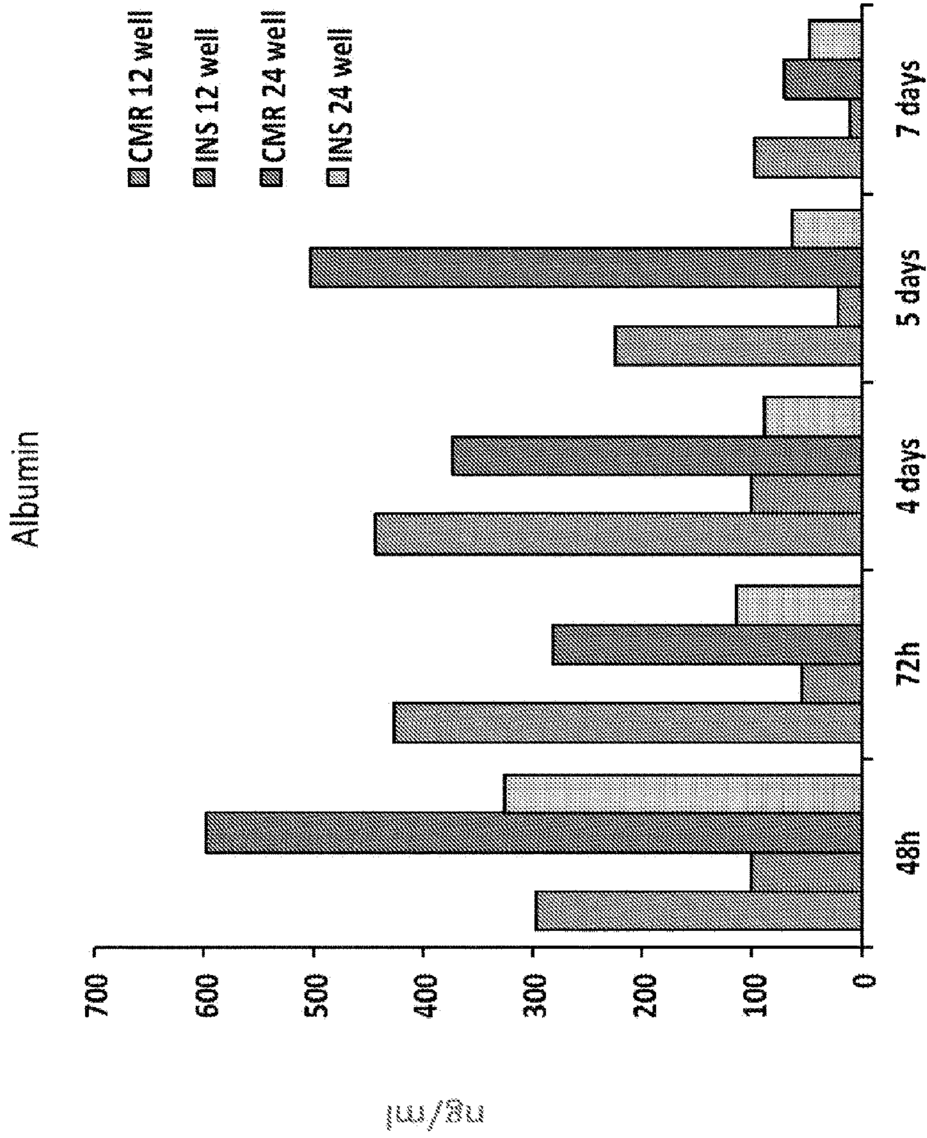

Figure 24

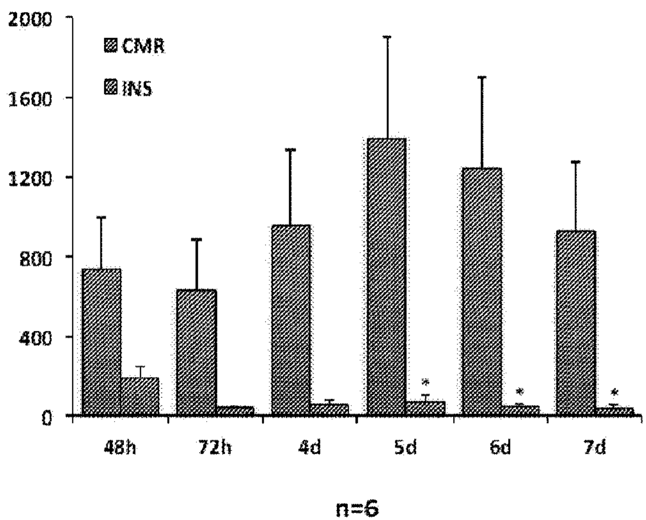

Figure 25a

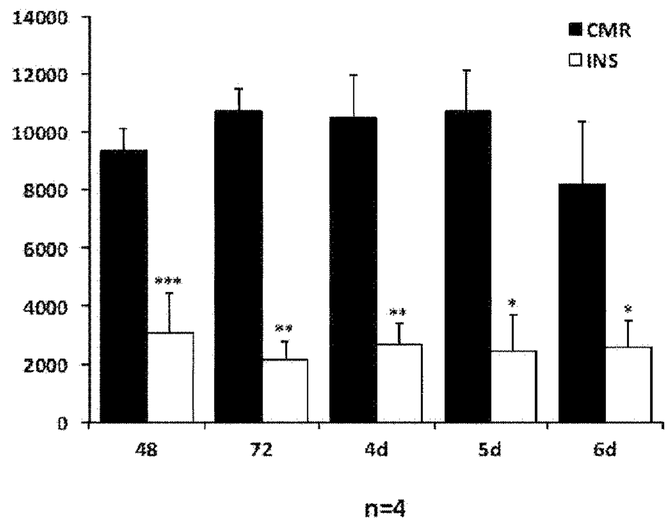

Figure 25b

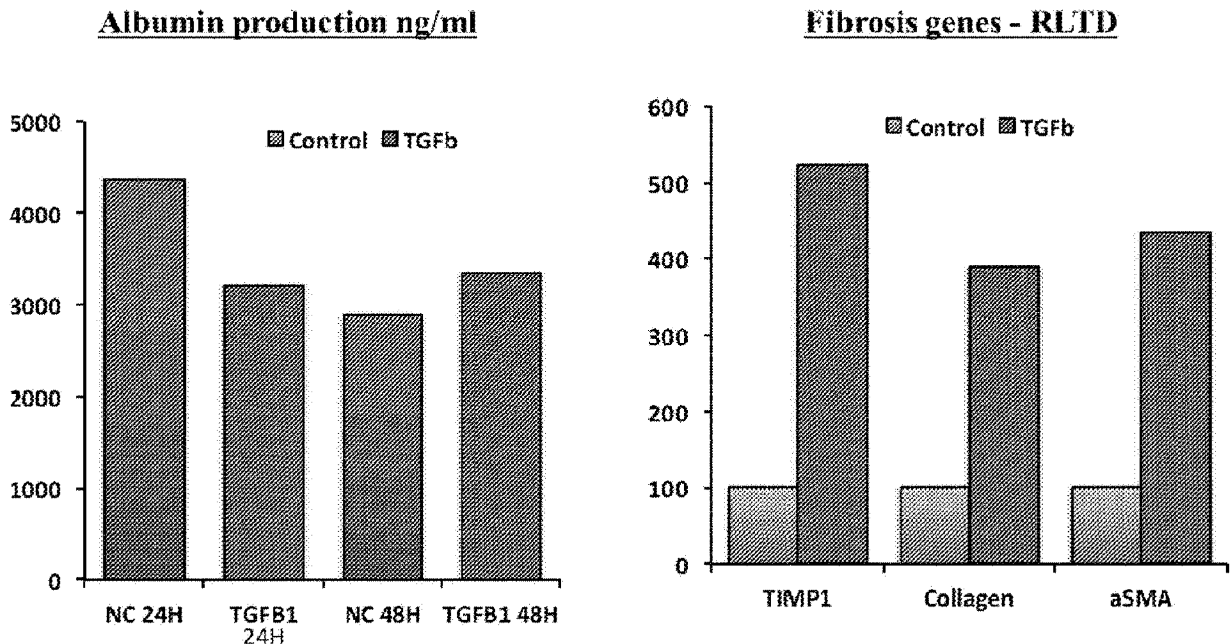

Figure 26A

Figure 26B

FAT loading

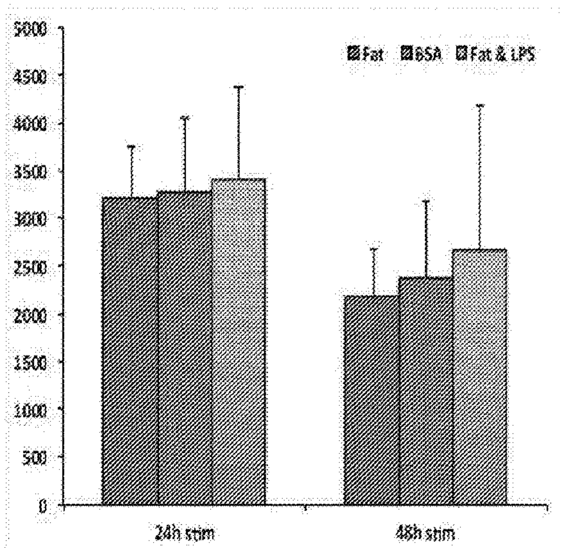

Figure 27

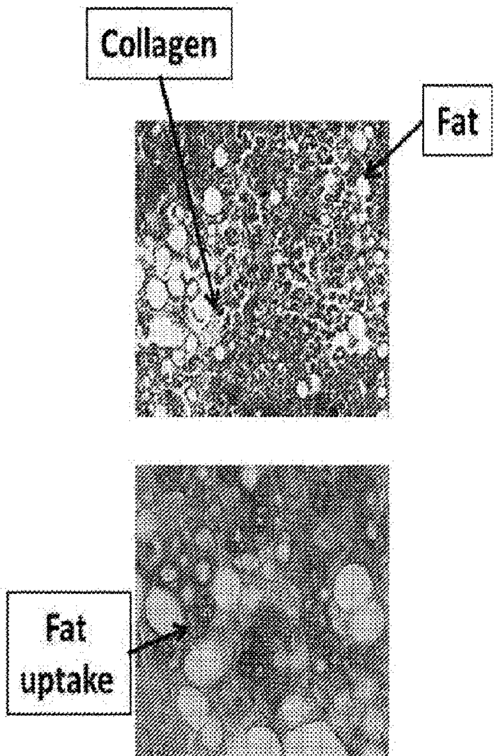

Figure 28

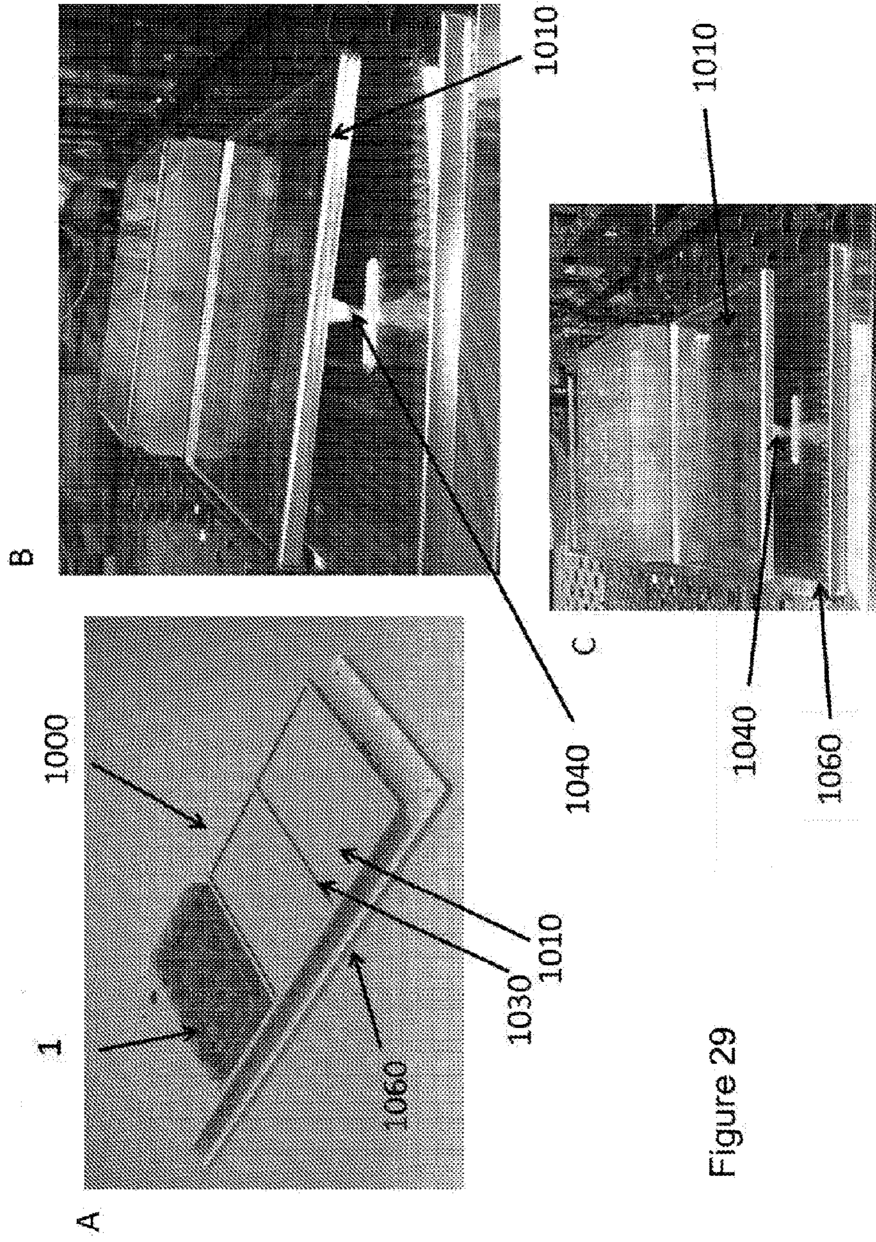

Figure 29

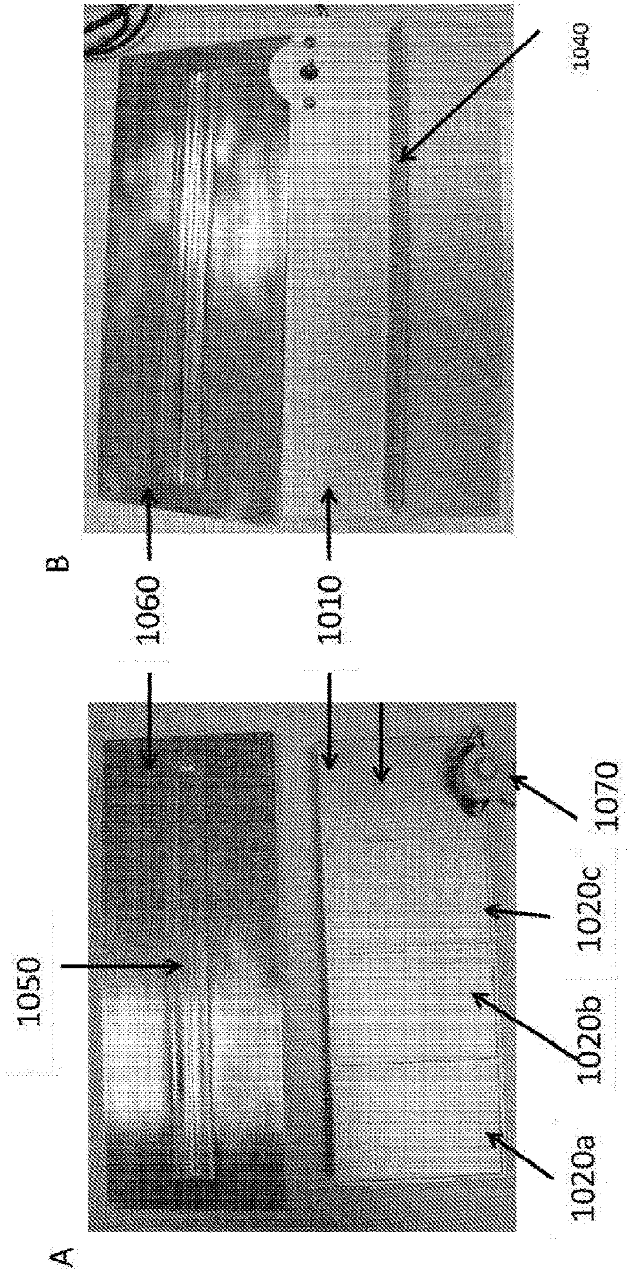

Figure 30

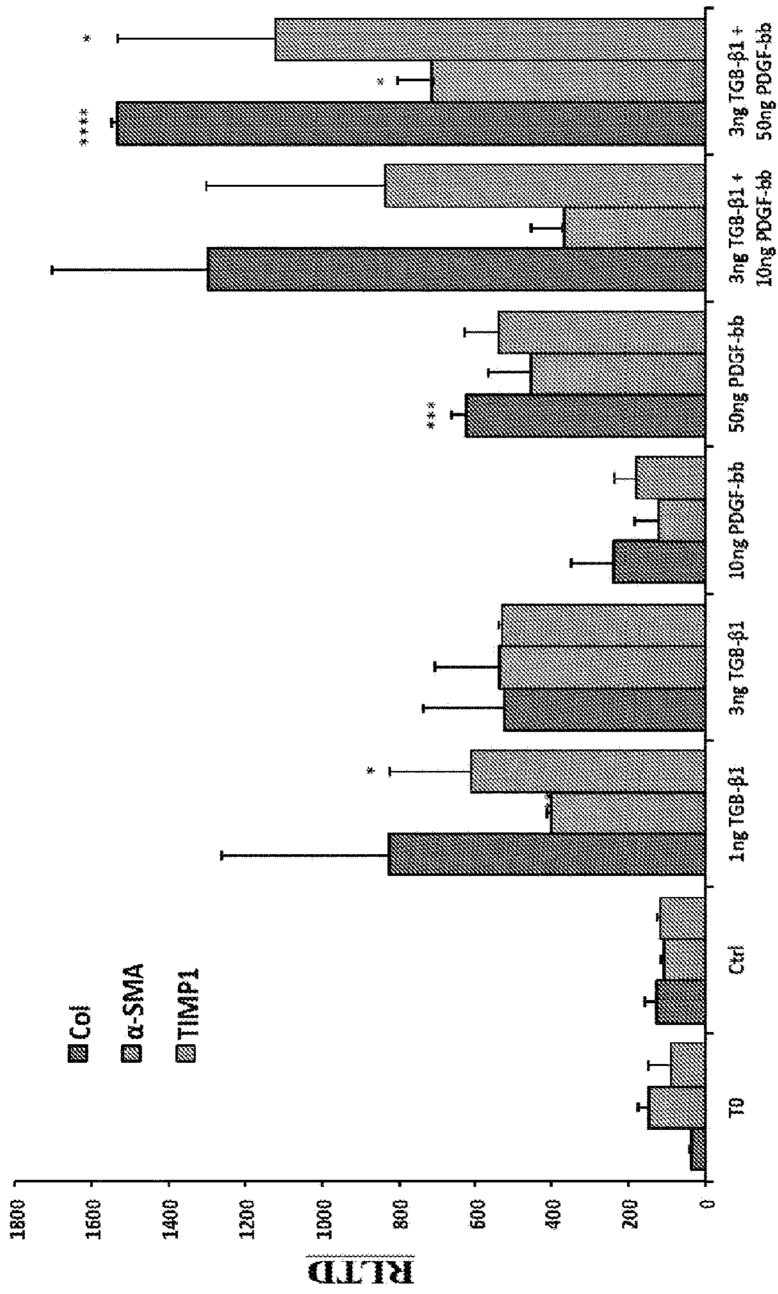

Figure 31

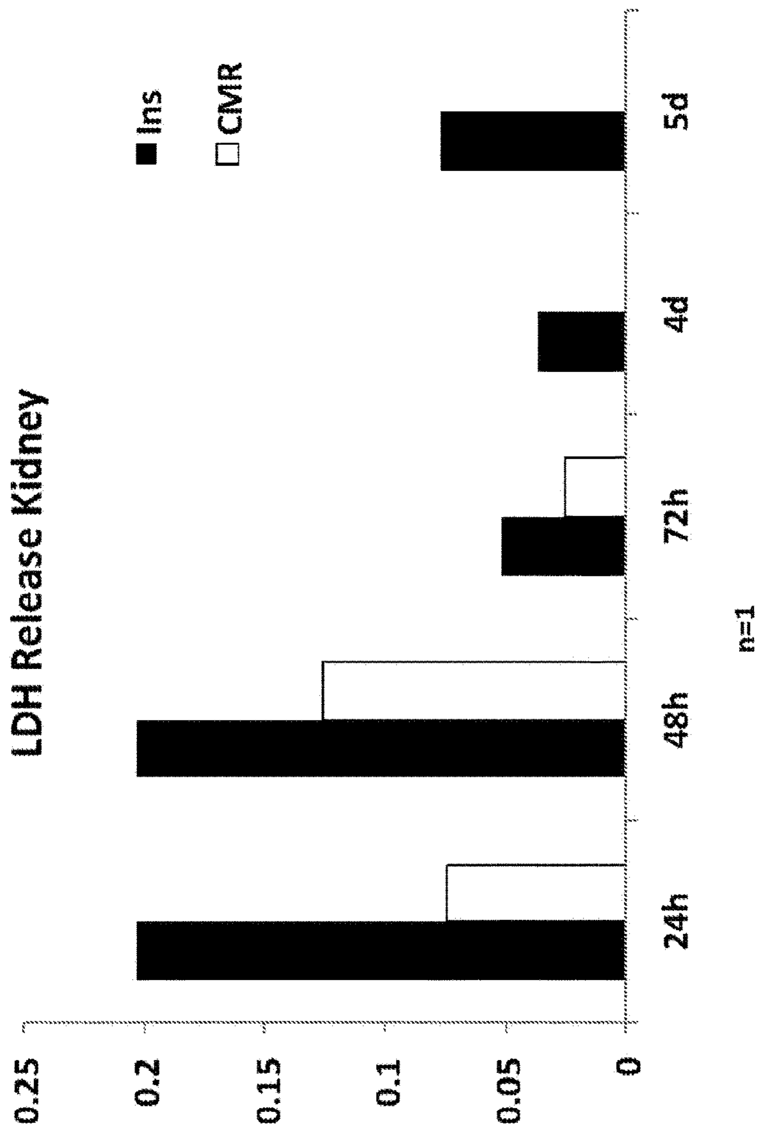

Figure 32