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(54) **Title**: CELL CULTURE

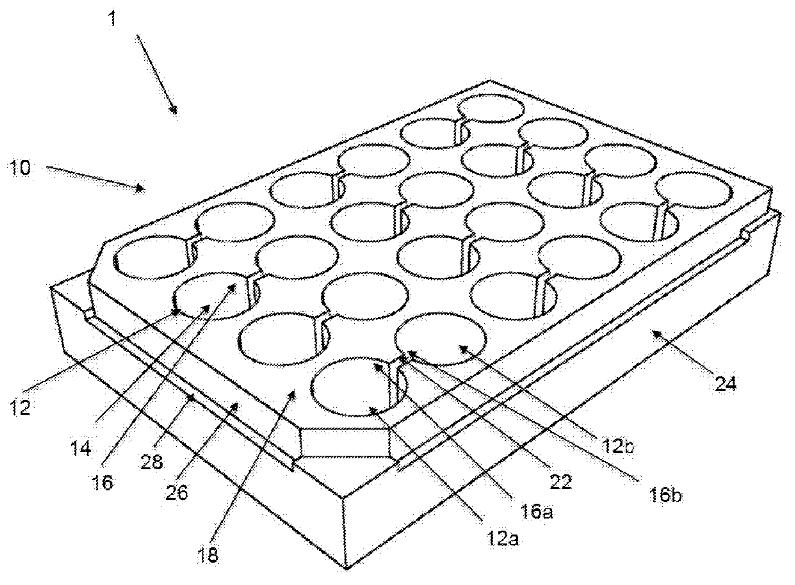

Figure 1

(57) **Abstract**: Aspects of the present invention relate to apparatus for use in cell and tissue culture techniques. Particularly, although not exclusively, embodiments of the present invention relate to apparatus which contribute to providing a dynamic cell culture environment. Also disclosed herein are methods for culturing cells and/or tissues, together with in vitro methods of testing drug efficacy as well as other subject matter.

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Cell Culture

Field of the Invention

Aspects of the present invention relate to apparatus for use in cell and tissue culture techniques. Particularly, although not exclusively, embodiments of the present invention relate to apparatus which contribute to providing a dynamic cell culture environment. Also disclosed herein are methods for culturing cells and/or tissues, together with *in vitro* methods of testing drug efficacy as well as other subject matter.

Background to the Invention

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.

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.

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.

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.

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.

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.

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.

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.

It is an aim of aspects of the present invention to at least partially mitigate the problems associated with the prior art.

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

It is an aim of certain embodiments of the present invention to provide a system and a method which more accurately reflect the *in vivo* environment of disease for example.

It is an aim of certain embodiments of the present invention to provide apparatus which utilise small volume of cell culture media.

Summary of Certain Embodiments of the Invention

In a broad aspect of the present invention, there is provided apparatus for culturing cells and/or a tissue, comprising:

a holder body comprising a plurality of chambers for containing a cell culture media, wherein the holder body comprises at least one fluid communication pathway extending between at least two respective chambers of the plurality of chambers, each fluid communication pathway permitting bi-directional fluid flow between the at least two chambers.

This apparatus may also be referred to as a “culture apparatus” or a multi-well plate. Thus, the culture apparatus described herein may include a multi-well plate. The term “multi-well plate” is known in the art and such plates typically comprise a plurality of chambers or wells.

In a first aspect of the present invention, there is provided an apparatus for providing bi-directional fluid flow, the apparatus comprising:

a culture apparatus for culturing cells and/or a tissue or portion thereof and comprising a holder body, the holder body comprising a plurality of chambers for containing a tissue culture media, and at least one fluid communication pathway extending between at least two respective chambers of the plurality of chambers, each fluid communication pathway permitting bi-directional fluid flow between said at least two chambers, and wherein the apparatus for providing bi-directional fluid flow further comprises:

a holder body support; and

at least one drive element arranged to rock a holder body supported via the support to thereby repeatedly raise and lower spaced apart ends of the holder body, wherein the apparatus is configured to repeatedly rock the holder body.

Thus, certain embodiments of the present invention provide a cost-effective, medium- or high-throughput bioreactor system. Aptly, the system introduces bi-directional flow of culture media through chambers of a plate apparatus without the need for a pump. Aptly, the volumes of media needed for the system are small, which may be advantageous for drug discovery.

Thus, in the first aspect of the present invention, the apparatus provided is configured to provide bi-directional fluid flow and which incorporates an apparatus which comprises:

a holder body support; and

at least one drive element arranged to rock a holder body supported via the support to thereby repeatedly raise and lower spaced apart ends of the holder body and which may be referred to as a “rocker apparatus”.

In certain embodiments, the holder body is separable from the holder body support and/or the at least one drive element. Aptly, the holder body is locatable adjacent to a surface of the holder body support.

Aptly, the chambers are arranged in a regular pattern. Aptly, the chambers are configured to accommodate a cell or tissue culture and a predetermined volume of cell culture media. As referred to herein, the term “tissue” may relate to a tissue portion e.g. an organ slice.

In an embodiment, each chamber of said plurality of chambers comprises a base element and at least one side wall element. Aptly, the fluid communication pathway comprises a through channel extending between a base element or side wall element of a first one of the plurality of chambers to a base element or side wall element of a further one of the plurality of chambers.

Aptly, the fluid communication pathway comprises a through slot extending between a base element or side wall element of a first one of the plurality of chambers to a base element or side wall element of a further one of the plurality of chambers. In certain embodiments, the first chamber is provided adjacent to the further chamber. In certain embodiments, the first chamber and the further chamber are connected via the fluid communication pathway to one or more additional chambers of the plurality of chambers.

In certain embodiments, the holder body comprises a plurality of fluid communication pathways, each fluid communication pathway extending between at least two chambers of the plurality of chambers.

Aptly, each fluid communication pathway of the plurality of fluid communication pathways is substantially parallel to and spaced apart from each other fluid communication pathway.

In certain embodiments, each chamber of the plurality of chambers is configured to accommodate a chamber insert element configured to support a cell scaffold element.

In certain embodiments, each chamber of the plurality of chambers comprises an opening for receiving and/or removing tissue culture media and/or a respective chamber insert element.

In certain embodiments, the apparatus further comprises at least one generally cylindrical chamber insert element which comprises a plurality of radially outwardly extending flanges for supporting the chamber insert element within a respective chamber of the plurality of chambers. In one embodiment, the culture apparatus comprises a plurality of chambers, each chamber comprising an insert element.

Aptly, each respective chamber insert element further comprises a cell scaffold element. Aptly, the cell scaffold element comprises a plurality of pores, said plurality of pores having an average diameter of between about 8µm to about 150µm. In certain embodiments, the average pore size may be for example 8µm, 10 µm, 20µm, 30µm, 40 µm, 50 µm, 60 µm, 70 µm, 80 µm, 90 µm, 100 µm or 150 µm. In certain embodiments, the pores have an average diameter of between about around 8µm to about 100µm. Aptly, the pores are provided on a lower surface of the cell scaffold element.

In certain embodiments, the cell scaffold element comprises an inner surface, wherein the inner surface is coated with a biological molecule. The biological molecule may be for example a scaffold protein such as for example collagen.

In certain embodiments, the holder body comprises at least twelve chambers, e.g. 12, 24, 96, or more. In one embodiment, the holder body comprises 24 chambers.

In certain embodiments, the holder body comprises an outer perimeter wall element. Aptly, the outer perimeter wall element comprises a recessed outer edge portion. Aptly, the outer perimeter wall element is a continuous outer perimeter wall element. Aptly, the continuous outer perimeter wall element is planar. That is to say, in certain embodiments, the holder body does not require connection with a pump or tubing and therefore comprises an outer edge region which does not comprise any inlet or outlet elements. Furthermore, the holder body does not contain elements configured for connection with tubing for example.

Aptly the holder body comprises an underside surface which comprises a recessed edge region which is configured to locate with respect to the recessed outer edge portion of a further holder body such that the holder bodies are located in a nested relationship. In certain embodiments, a plurality of holder bodies may be located in a vertical stacked e.g. nested relationship.

Aptly, the holder body consists essentially of the plurality of chambers, at least one fluid communication pathway, an outer perimeter wall element, wherein each one of said plurality of chambers is connected via a web of material.

In certain embodiments, the holder body is composed of a material selected from polystyrene, polycarbonate, polyethylene, polypropylene, PMMA, cellulose acetate, Zeonex™, Zeonor™ and glass.

In certain embodiments, each respective chamber of the plurality of chambers has a depth of between about 16mm and 19mm, e.g. 16mm, 16.5mm, 17mm, 17.5mm, 18mm, 18.5mm or 19mm.

In an embodiment, the channel has a width of approximately 1.5mm to 3.5mm e.g. approx. 2 mm. In one embodiment, the channel has a length of between about 3mm to about 5 mm. In one embodiment, the channel has a length of approx. 3.5mm to approx. 4mm.

In certain embodiments, each chamber may have a depth of between about 16mm and 19mm, e.g. 16mm, 16.5mm, 17mm, 17.5mm, 18mm, 18.5mm, or 19mm. In one embodiment, the chamber has a depth of approximately 18mm. In one embodiment, the chamber has a depth of approximately 16.5mm.

In certain embodiments, the culture apparatus further comprises a lid element which is removably positionable over the holder body, the lid element providing a substantially planar cover extending over the plurality of chambers.

In certain embodiments, the plurality of chambers are arranged in rows and columns in a respective orthogonal relationship within the holder body.

In certain embodiments, the culture apparatus may be stackable relative to at least one other culture apparatus.

Aptly, the holder body support comprises a pivot element about which the holder body is rocked. In certain embodiments, the apparatus comprises a platform element supported by the holder body support, the platform element being configured to retain the holder body in a fixed location during rocking. In certain embodiments, the platform element is configured to support and/or retain a plurality of holder bodies in a fixed location during rocking. Aptly, the plurality of holder bodies is retained in a side-by-side arrangement adjacent an upper surface of the platform element. In certain embodiments, the apparatus comprises a plurality of holder bodies in a vertically stacked arrangement.

In certain embodiments, the drive element is configured to rock the holder body at a speed of approximately 1 minute to 20 minutes per complete rocking motion.

In certain embodiments, the drive element comprises a linear actuator. Aptly, the platform element comprises a first end region and a further end region spaced apart from the first end region, and wherein the linear actuator is located at either the first end region or the further end region.

In certain embodiments, the apparatus further comprises a base element, wherein the base element comprises a groove into which the pivot element is locatable.

In certain embodiments neither the culture apparatus nor the rocker apparatus comprise a pump for forcing fluid flow between adjacent chambers.

In a further aspect of the present invention, there is provided an apparatus for repeatedly rocking a holder body, said holder body comprising a plurality of chambers for containing a cell or tissue culture media, wherein the holder body comprises at least one fluid communication pathway extending between at least two respective chambers of the plurality of chambers, each fluid communication pathway permitting bi-directional fluid flow between said at least two chambers, said apparatus comprising:

a holder body support; and

at least one drive element arranged to rock a holder body supported via the support to thereby repeatedly raise and lower spaced apart ends of the holder body.

In a further aspect of the present invention, there is provided an *in vitro* method of culturing cells and/ or a tissue or portion thereof, the method comprising:

a) providing a culture apparatus as described herein; and

b) for a predetermined period of time, applying a rocking motion to the culture apparatus.

In a further aspect of the present invention, there is provided an *in vitro* method of culturing cells and/ or a tissue or portion thereof comprising:

a) providing an apparatus according to the first aspect of the present invention; and

b) for a predetermined period of time, applying a rocking motion to the culture apparatus.

In certain embodiments, the method further comprises locating at least one cell in at least one chamber of the plurality of chambers. Aptly, the method further comprises locating a tissue portion comprising the at least one cell in at least one chamber of the plurality of chambers.

In certain embodiments, the method comprises locating the tissue portion by locating at least one chamber insert element supporting the tissue portion in the at least one chamber of the holder body. Aptly the at least one cell is provided in a cell culture.

In certain embodiments, the cell culture is supported by a cell scaffold element, and the method further comprises locating the cell culture by locating at least one chamber insert element accommodating the cell scaffold element in the at least one chamber of the holder body.

In certain embodiments, the at least one cell is selected from eukaryotic cells and prokaryotic cells, e.g. plant cells, mammalian cells, yeast cells, fungal cells and/or bacterial cells. Aptly, the at least one cell is a mammalian cell selected from epithelial cells, tumour cells, hepatocytes, fibroblast cells, stem cells, myocardiocytes, kidney cells, lung cells, neuronal cells, adipocytes, intestinal cells, skin cells, and immune cells, either alone or in combination.

Aptly the at least one cell is comprised within a tissue portion e.g. an organ slice. Aptly, the tissue portion is sourced from a tissue selected from a lung, a liver, a kidney, a heart, a brain, skin, a pancreas. In certain embodiments, the cell culture and/or tissue portion comprises a plurality of cell types.

In certain embodiments, the at least one chamber is a first one of the plurality of chambers and further wherein the first chamber is connected to at least one further chamber of the plurality of chambers via a fluid communication pathway extending there between, the fluid communication pathway permitting bi-directional fluid flow between the first chamber and further chamber.

Aptly, the chamber insert element comprises a base surface comprising a plurality of pores. Aptly, the plurality of pores are configured to allow bidirectional flow of fluid cell culture media therethrough.

In certain embodiments, the method comprises:

providing a fluid cell culture media to the first and/or further chamber and;

applying the rocking motion for a predetermined period of time such that the fluid cell culture media repeatedly flows from the at least one chamber to a further one of the plurality of the chambers and back again.

Aptly, the method comprises providing between about 0.5ml to 5 ml e.g. 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml, 1.75ml, 2ml, 2.25ml, 2.5ml, 3ml, 3.25ml, 3.5ml, 3.75ml, 4ml, 4.25ml, 4.5ml, 4.75ml or 5ml of the fluid cell culture media per chamber to the first and/or further chamber of the plurality of chambers.

Aptly, the method comprises providing between about 1.5ml to 4ml of the fluid cell culture media per chamber to the first and/or further chamber of the plurality of chambers. In certain embodiments, the amount of fluid cell culture media added to each chamber will be dependent of the size of the chamber and/ or the size of pore provided by a cell scaffold insert.

In certain embodiments, the method further comprises locating a cell culture, wherein the cell culture is seeded with at least one cell, and a cell scaffold element in at least two chambers of the plurality of chambers, and wherein the cell culture located in a first respective chamber is seeded with cells of the same or different cell type to the cell culture located in a further respective chamber.

In certain embodiments, the method comprises;

locating a culture apparatus as described herein to be supported by a platform element of an apparatus according to a first aspect of the present invention;

providing a driving force to the drive element; and

repeatedly raising and lowering spaced apart ends of the holder body so as to apply a rocking motion to the holder body.

In certain embodiments, the method comprises applying a rocking motion for at least 24 hours, e.g. 48 hours, 72 hours, or more.

Aptly, the method further comprises determining viability or other measurable parameter of the cell(s) in the cell culture. Aptly, the or each cell culture comprises hepatocytes, and further wherein the step of determining cell viability or other measurable parameter comprises measuring production of albumin by the cell(s) of the cell culture(s).

In certain embodiments, the method comprises locating a plurality of culture apparatus to be supported by the platform element of the rocker apparatus. Aptly, the method comprises locating the plurality of culture apparatus to be supported in a side-by-side arrangement on an upper surface of the platform element.

In a still yet further aspect of the present invention, there is provided a method of *in vitro* testing liver toxicity of an agent comprising:

a) providing a culture apparatus according to an aspect of the invention;

b) locating a cell culture or a tissue or portion thereof comprising at least one hepatocyte, and a cell scaffold element to at least one chamber of the plurality of chambers;

c) adding at least one agent to be tested to said at least one chamber;

d) for a predetermined period of time, applying a rocking motion to the apparatus; and

e) monitoring at least one effect of the agent on the hepatocyte.

In a still yet further aspect of the present invention, there is provided a method of *in vitro* testing liver toxicity of an agent comprising:

a) providing an apparatus according to the first aspect of the invention;

b) locating a cell culture or a tissue or portion thereof comprising at least one hepatocyte, and a cell scaffold element to at least one chamber of the plurality of chambers;

c) adding at least one agent to be tested to said at least one chamber;

d) for a predetermined period of time, applying a rocking motion to the apparatus; and

e) monitoring at least one effect of the agent on the hepatocyte.

In certain embodiments, monitoring at least one effect of the agent comprises monitoring the effect of the agent on the proliferation and/or differentiation and/or function of the hepatocyte as a measure of toxicity of the agent.

Aptly, the hepatocyte is comprised in a liver slice. The hepatocyte cells may be human or non-human. The hepatocytes cells e.g. the liver slice may comprise primary hepatocyte cells. Alternatively or in addition, the hepatocyte cells e.g. the liver slice may comprise non-primary hepatocyte cells e.g. stem cell derived hepatocytes or progenitor cell derived hepatocytes. The hepatocyte cells may be freshly-derived from a patient or donor or multiple donors or from a cryopreserved source.

The method may comprise adding cell culture media to the chamber. The method may also comprise adding the agent to the hepatocyte positioned within a chamber. A combination of agents may be added. The agent may be for example a drug candidate.

In a further aspect of the present invention, there is provided a method of *in vitro* modelling of tissue disease comprising:

a) providing a culture apparatus according to an aspect of the invention;

b) locating a tissue portion, the tissue portion comprising at least one cell, and a cell scaffold element to at least one chamber of the plurality of chambers;

c) for a predetermined period of time, applying a rocking motion to the culture apparatus; and

d) monitoring at least one characteristic of the tissue portion.

In a further aspect of the present invention, there is provided a method of *in vitro* modelling of tissue disease comprising:

a) providing an apparatus according to a first aspect of the invention;

b) locating a tissue portion, the tissue portion comprising at least one cell, and a cell scaffold element to at least one chamber of the plurality of chambers;

c) for a predetermined period of time, applying a rocking motion to the culture apparatus; and

d) monitoring at least one characteristic of the tissue portion.

Aptly, the disease is a cancer. Aptly, the disease is fibrosis. Aptly, the tissue disease is a fibrotic disease e.g. lung fibrosis, kidney fibrosis or liver fibrosis. Aptly, the tissue disease is a liver disease.

Aptly, the tissue portion is a liver slice, a kidney slice and/or a lung slice. In certain embodiments, the method comprises determining activation of a cell type within the tissue portion. Aptly, the method comprises determining activation of hepatic myofibroblasts in a tissue portion. Aptly, the method comprises identifying a marker of hepatic myofibroblasts. Aptly, the marker is α-smooth muscle actin.

In certain embodiments, the method comprises determining expansion of a cell type which is associated with a disease state. Aptly, the tissue slice is a liver portion and the method comprises determining expansion of ductular cells. Aptly, the method comprises a step of histological analysis of the tissue portion. In certain embodiments, the method comprises determining presence or absence of an immune cell in the tissue portion. Aptly, the immune cell is a Kupffer cell.

In certain embodiments, the method comprises locating the tissue portion and the cell culture scaffold element by locating at least one chamber insert element supporting a cell culture scaffold element holding a tissue portion in said at least one chamber of the holder body.

In certain embodiments, the method further comprises adding one or more cells to the chamber. Aptly, the one or more cells are immune cells. In certain embodiments, the method comprises adding one or more cancer cells to the chamber.

In certain embodiments, the method comprises locating the cell culture and the cell culture scaffold element by locating at least one chamber insert element supporting a cell culture scaffold element holding a cell culture in said at least one chamber of the holder body.

In certain embodiments, the method further comprises:

adding a fluid cell culture media to said at least one chamber of the plurality of chambers and;

applying the rocking motion for a predetermined period of time such that the fluid cell culture media repeatedly flows from said chamber to a further one of the plurality of the chambers and back again.

Aptly, the method further comprises;

locating a culture apparatus on a platform element of the apparatus according to of the first aspect of the invention;

providing a driving force to the drive element; and

repeatedly raising and lowering spaced apart ends of the holder body so as to apply a rocking motion to the holder body.

In a further aspect of the present invention, there is provided an insert element for supporting a cell and/or tissue portion, wherein the insert element is generally cylindrical and comprises a plurality of radially outwardly extending flanges for supporting the chamber insert element within a respective chamber of an apparatus as described herein, and further wherein respective chamber insert element further comprises a cell scaffold element comprising a plurality of pores, said plurality of pores having an average diameter of between about around 8µm to about 150µm.

Aptly, the plurality of pores has an average diameter of from about around 8µm to about 100µm.

Certain embodiments of the present invention may provide an *in vitro* model of liver. *In vitro* models of liver are an important tool in pharmaceutical drug development and in understanding liver pathophysiology. Certain embodiments of the present invention provide a system which more accurately models organ fibrosis and which may be used to test anti-fibrotic candidate molecules.

In certain embodiments, the apparatus and method is for modelling a liver related disease. Many people with cirrhosis and other liver related disease conditions experience no symptoms in the early stages of the disease. However, as scar tissue replaces healthy tissue, liver functions begin to fail and a person may experience fatigue, exhaustion, loss of appetite, nausea, weakness and loss of weight. As the disease progresses, complications may develop as a result of the loss of liver functions.

As used herein, the term “liver related disease” may refer to one or more diseases, conditions or symptoms or susceptibility to diseases, conditions or symptoms that involve directly or indirectly, the liver, the biliary ducts, the hepatic ducts, the cystic ducts or the gallbladder including the following: acute liver failure, Alagille syndrome, Alcoholic Liver disease, Alpha 1-antitrypsin deficiency, autoimmune hepatitis, biliary atresia, chronic hepatitis, cirrhosis, cholestatic liver disease, cystic disease of the liver, fatty liver, galactosemia, gallstones, Gilbert's syndrome, hemochromatosis, hepatitis A, hepatitis B, hepatitis C, liver cancer, neonatal hepatitis, Non-Alcohol Related Fatty Liver disease, non-alcoholic steatohepatitis, porphyria, primary biliary cirrhosis, primary sclerosing cholangitis, Reye's syndrome, sarcoidosis, steatohepatitis, tyrosinemia, type I glycogen storage disease, viral hepatitis and/or Wilson's disease.

In certain embodiments, the method is a method of modelling fatty liver disease. Fatty liver disease may be Non-Alcohol Related Fatty Liver Disease (NAFLD) or Alcoholic Liver Disease and may be characterised by an inappropriate build up of fat in a subject’s liver. Over time, the build up of fat may result in inflammation of the liver and fibrosis.

NAFLD may lead to Non-Alcohol Steatohepatitis (NASH). Thus, in certain embodiments, the method is a method of modelling NASH and/or a progression from NAFLD to NASH.

In certain embodiments, the method of modelling fatty liver disease comprises adding one or more lipids to the tissue portion. Aptly, the one or more lipid is selected from palmitic acid, oleic acid and linoleic acid and combinations thereof. Aptly, the lipid is conjugated to Bovine Serum Albumin (BSA). Aptly, the method comprises culturing the tissue portion with the one or more lipids for up to four days, e.g. 1, 2, 3 or 4 days.

In certain embodiments, the method comprises culturing the tissue portion with a fibrosis-stimulating factor such as for example transforming growth factor-β (tgfb), platelet derived growth factor-bb (pdgf-bb). Aptly, the method comprises the step of culturing the tissue portion with the fibrosis-stimulating factor for up to 4 days e.g. 1, 2, 3 or 4 days.

In certain embodiments, the method comprises culturing the tissue portion with an inflammatory mediator. Aptly, the inflammatory mediator is selected from a pathogen-associated molecular patterns (PAMPs) e.g. lipopolysaccharide (LPS) or poly IC and damage associated molecular (DAMPs) e.g. apoptotic or damaged cells. Aptly, the step of culturing the tissue portion with the inflammatory mediator for up to 4 days e.g. 1, 2, 3 or 4 days.

In certain embodiments, the method comprises culturing the tissue portion with a hepatotoxic agent e.g. e.g. acetaminophen (which causes liver failure) or bile acids (which cause biliary disease) for up to but not limited to 4 days.

In certain embodiments, one or more stimulating agents may be added to the tissue portion. For example, the method may comprise adding one or more of a lipid, an inflammatory agent and a fibrotic stimulating agent. The one or more stimulating agents may be as described herein.

In certain embodiments, the method comprises maintaining the tissue portions standard normoxic tissue culture conditions (21% oxygen, 5% carbon dioxide and 74% nitrogen). In certain embodiments, e.g. to mimic hypoxic injury that occurs during tissue fibrosis and cancer, the tissue portions may be maintained as low as 0.1% oxygen (hypoxia).

Existing *in vitro* models which utilise human organ slices in culture have limited value due to a rapid loss of organ function in culture. For example, liver slices culture on Transwells® have a lifespan of 2 days to 3 days due to hypoxia and degradation of the tissue. An example of a dynamic incubation system for testing cytotoxicity of certain compounds is described in Leeman *et al*, Toxic. In Vitro, Vol. 9, No. 3, pp. 291-298. However, this system does not provide bi-directional flow between adjacent chambers. Results reported by Leeman et al suggests that cell viability decreases rapidly after 72 hours. Leeman et al also used a high (40%) oxygen concentration during culture.

As a result, liver slices currently have limited value in drug discovery. In contrast to the prior art, certain embodiments of the present invention may be suitable to extend the lifespan of liver slices in culture and therefore improve their usefulness in the testing of candidate drug molecules and modelling of disease e.g. liver-related disorders as detailed above, including for example fibrotic disease of the liver.

In addition, certain embodiments of the present invention may provide a cost-effective, small or medium throughput bioreactor system which more accurately represents *in vivo* environments including for example disease states.

Furthermore, certain embodiments of the present invention may provide a 3D dynamic model of human or animal disease which can be used to test candidate molecules. Certain embodiments of the present invention provide apparatus and bioreactor systems which enable the viability of cells and/or tissues in culture to be improved.

Particularly, certain embodiments enable the length of cell viability e.g. hepatocyte viability to be extended to 72 hours or more. As a result, the apparatus, systems and methods described herein can be used to maintain cell and tissues for a sufficient length of time to test candidate compound efficacy and the like.

Detailed Description of Embodiments of the Invention

Embodiments of the present invention 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 embodiments of the present invention.

“CMR2” refers to a bidirectional system according to certain embodiments of the present invention.

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

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

Figure 3a is a schematic representation of the rocket apparatus holding a multi-well plate apparatus according to certain embodiments of the present invention. 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 embodiments of the present invention;

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

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 embodiments of the invention. N=8. The left hand bars represent the use of the apparatus of embodiments of the present invention whilst the right hand bars illustrate the static Transwell® insert. The data shows that the apparatus of certain embodiments 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 embodiments of the present invention, 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.

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.

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 embodiments (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”).

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.

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.

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.

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.

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.

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

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.

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 embodiments of the present invention and those cultured in a unidirectional flow system (uniflow). Conclusion: Liver function is improved in CMR cultured slices compared to static conditions.

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.

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;

Figure 18: is a graph illustrating the tissue area stained with α-smooth muscle actin (α-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.

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 (α-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.

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.

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.

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.

Figure 23 is a graph illustrating the production of albumin (ng/ml) using a static cell culture plate and apparatus according to certain embodiments of the invention. Conclusion: CMR plate version comprising through holes between chambers (CMR) and an embodiment 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.

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.

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.

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.

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.

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

Figures 29A, 29B and 29C are perspective views of an apparatus according to certain embodiments of the present invention (referred to herein as CMR2).

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

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.

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

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 embodiment. 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.

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 embodiment the organ slice may be for example a liver slice, a kidney slice or a lung slice.

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.

Certain embodiments of the present invention 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.

The tissue slice may be of any suitable size. In certain embodiments, 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.

In certain embodiments, 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.

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.

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.

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.

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.

Aptly, the methods of certain embodiments 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.

In certain embodiments, 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).

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 embodiments, 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.

In certain embodiments, the cells may be sourced from established cells lines. In certain embodiments, the cells may be genetically modified. In certain embodiments, 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.

In certain embodiments of the present invention, 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 embodiment 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 embodiments, the tissues may be for example, skeletal tissue, muscle tissue, connective tissue, nervous tissue, epithelial tissue and/or mineralised tissue. In certain embodiments, 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.

In certain embodiments of the present invention, 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.

In one embodiment, the candidate molecule may be an anti-fibrotic compound as described herein.

Turning now to the Figures, certain embodiments of the apparatus according to the present invention are illustrated in Figure 1 for example.

As shown in Figure 1, an apparatus 1 according to certain embodiments 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).

In one embodiment, 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.

The chambers may also be referred to as “wells”. In the illustrated embodiment, each chamber is cylindrical. Other shapes of chamber e.g. cuboidal, are envisaged and within the scope of certain embodiments of the invention. 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.

Each chamber 12 comprises a lower planar base 20 as shown in Figure 2. The lower planar base in certain embodiments 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.

In certain embodiments, 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 embodiments.

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.

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.

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.

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.

In an alternative embodiment, 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.

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

In a yet further embodiment, 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.

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.

In the illustrated embodiment, a through passageway is provided between two adjacent chambers. It will be understood that in certain embodiments 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 embodiments, the through passageway is provided in a linear non-angled direction between the plurality of chambers.

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 embodiments, a plurality of holder bodies may be located in a vertical nested relationship.

In certain embodiments, the apparatus also comprises one or more insert elements 30 which are configured to support one or more cells. In certain embodiments, 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.

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 embodiments, the insert element may contact the base of the chamber for example. The insert element may comprise a lower surface membrane 40 with pores.

Aptly, the pores having an average diameter of between about around 8µm to about 150µm. in one embodiment, 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.

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.

Turning to Figures 3 and 5, a rocker apparatus 100 according to certain embodiments of the present invention is illustrated.

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 embodiments of the present invention. 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.

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.

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.

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 embodiment, the rocker apparatus comprises a drive element comprising a linear actuator 110 provided at the first end region 106. In one particular embodiment, 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.

In some embodiments, 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.

In one particular embodiment, 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.

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

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 or 20 degrees.

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 embodiments, 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 embodiments, 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.

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.

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.

Figure 29 and 30 depict an apparatus 1000 according to certain embodiments of the invention. 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 embodiments, a plurality of multi-well plates may be stacked as shown in Figure 29.

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.

Certain embodiments of the present invention relate to a method of culturing cells and/or tissues as described herein below:

Examples

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 embodiments of the present invention 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 embodiments of the present invention.

Precision cut slice isolation and culture

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.

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.

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 embodiments of the invention 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

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)

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)

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)

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)

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.

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

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.

Features, integers, characteristics or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or 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. The invention is not restricted to any details of any foregoing embodiments. The invention extends to any novel one, or novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

CLAIMS:

1. Apparatus for providing bi-directional fluid flow, the apparatus comprising:

a culture apparatus for culturing cells and/or a tissue or portion thereof and comprising a holder body, the holder body comprising a plurality of chambers for containing a tissue culture media and at least one fluid communication pathway extending between at least two respective chambers of the plurality of chambers, each fluid communication pathway permitting bi-directional fluid flow between said at least two chambers, and wherein said apparatus for providing bi-directional fluid flow further comprises:

a holder body support; and

at least one drive element arranged to rock a holder body supported via the support to thereby repeatedly raise and lower spaced apart ends of the holder body, wherein the apparatus is configured to repeatedly rock the holder body.

2. Apparatus according to claim 1, wherein each chamber of said plurality of chambers comprises a base element and at least one side wall element.

3. Apparatus according to claim 2, wherein the fluid communication pathway comprises a through channel extending between a base element or side wall element of a first one of the plurality of chambers to a base element or side wall element of a further one of the plurality of chambers.

4. Apparatus according to claim 2, wherein the fluid communication pathway comprises a through slot extending between a base element or side wall element of a first one of the plurality of chambers to a base element or side wall element of a further one of the plurality of chambers.

5. Apparatus according to claim 3 or claim 4, wherein the first chamber is provided adjacent to the further chamber.

6. Apparatus according to any preceding claim, wherein the holder body comprises a plurality of fluid communication pathways, each fluid communication pathway extending between at least two chambers of the plurality of chambers.

7. Apparatus according to claim 6, wherein each fluid communication pathway of the plurality of fluid communication pathways is substantially parallel to and spaced apart from each other fluid communication pathway.

8. Apparatus according to any preceding claim, wherein each chamber of the plurality of chambers comprises an opening for receiving and/or removing tissue culture media and/or a respective chamber insert element.

9. Apparatus according to any preceding claim, wherein each chamber of the plurality of chambers is configured to accommodate a chamber insert element configured to support a cell scaffold element.

10. Apparatus according to claim 9, further comprising at least one generally cylindrical chamber insert element which comprises a plurality of radially outwardly extending flanges for supporting the chamber insert element within a respective chamber of the plurality of chambers.

11. Apparatus according to claim 10, wherein respective chamber insert element further comprises a cell scaffold element.

12. Apparatus according to claim 10 or claim 11, wherein the cell scaffold element comprises a plurality of pores, said plurality of pores having an average diameter of between about around 8µm to about 150µm.

13. Apparatus according to claim 12, wherein the plurality of pores have an average diameter of between about around 8µm to about 100µm.

14. Apparatus according to any preceding claim, wherein the holder body comprises at least twelve chambers, e.g. at least twenty-four chambers.

15. Apparatus according to any preceding claim, wherein the culture apparatus is separable from the holder body support and from the at least one drive element.

16. Apparatus according to any preceding claim, wherein the holder body comprises an outer perimeter wall element, and further wherein the outer perimeter wall element

comprises an inwardly stepped portion configured to allow a plurality of holder bodies to be vertically stacked.

17. Apparatus according to claim 16, wherein the holder body consists essentially of the plurality of chambers, at least one fluid communication pathway, an outer perimeter wall element, wherein each one of said plurality of chambers is connected via a web of material.

18. Apparatus according to any preceding claim, wherein the holder body is composed of a material selected from Zeonex™, Zeonor™, polystyrene, polycarbonate, polyethylene, polypropylene, PMMA, cellulose acetate and glass.

19. Apparatus according to any preceding claim, wherein each respective chamber of the plurality of chambers has a depth of between about 16mm and 19mm.

20. Apparatus according to any preceding claim, wherein the culture apparatus further comprises a lid element which is removably positionable over the holder body, the lid element providing a substantially planar cover extending over the plurality of chambers.

21. Apparatus according to any preceding claim, wherein the plurality of chambers are arranged in rows and columns in a respective orthogonal relationship within the holder body.

22. Apparatus according to any preceding claim, wherein the holder body support comprises a pivot element about which the holder body is rocked.

23. Apparatus according to any preceding claim, further comprising a platform element supported by the holder body support, said platform element being configured to retain the holder body in a fixed location during rocking.

24. Apparatus according to any preceding claim, wherein the platform element is configured to retain a plurality of holder bodies, each in a fixed location during rocking.

25. Apparatus according to any preceding claim, wherein the drive element comprises a linear actuator.

26. Apparatus according to claim 25, wherein the platform element comprises a first end region and a further end region spaced apart from the first end region, and wherein the linear actuator is located at either the first end region or the further end region.

27. Apparatus according to any preceding claim, wherein the drive element is configured to rock the holder body at a speed of approximately about around 1 to 20 minutes per complete rocking motion.

28. Apparatus according to any preceding claim, wherein the holder body support is composed of an autoclavable material.

29. An *in vitro* method of culturing cells and/ or a tissue or portion thereof comprising:

a) providing an apparatus according to any preceding claim; and

b) for a predetermined period of time, applying a rocking motion to the culture apparatus.

30. A method according to claim 29, which further comprises:

locating at least one cell in at least one chamber of the plurality of chambers.

31. A method according to claim 30, wherein the at least one cell is provided in a cell culture.

32. A method according to claim 31, wherein the cell culture is supported by a cell scaffold element, and the method further comprises locating the cell culture by locating at least one chamber insert element accommodating the cell scaffold element in said at least one chamber of the holder body.

33. A method according to any of claims 29 to 32, wherein said at least one cell is selected from eukaryotic cells and prokaryotic cells, e.g. plant cells, mammalian cells, yeast cells, fungal cells and/or bacterial cells.

34. A method according to claim 33, wherein said at least one cell is a mammalian cell 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.

35. An *in vitro* method of modelling a disease, the method comprising:

a) providing an apparatus according to any of claims 1 to 28; and

b) for a predetermined period of time, applying a rocking motion to the culture apparatus.

36. A method according to any of claims 29 to 35, which further comprises:

locating a tissue portion comprising said at least one cell in at least one chamber of the plurality of chambers.

37. A method according to claim 36, which comprises locating the tissue portion by locating at least one chamber insert element supporting the tissue portion in said at least one chamber of the holder body.

38. A method according to claim 36 or claim 37, wherein the tissue portion is sourced from a tissue selected from a lung, a liver, a heart, a brain, a pancreas and optionally wherein the tissue portion comprises multiple cell types.

39. A method according to any of claims 29 to 38, wherein said at least one chamber is a first one of the plurality of chambers and further wherein the first chamber is connected to at least one further chamber of the plurality of chambers via a fluid communication pathway extending therebetween, the fluid communication pathway permitting bi-directional fluid flow between said first chamber and further chamber.

40. A method according to any of claims 29 to 39, which further comprises:

providing a fluid cell culture media to said first and/or further chamber and;

applying the rocking motion for a predetermined period of time such that the fluid cell culture media repeatedly flows from said at least one chamber to a further one of the plurality of the chambers and back again.

41. A method according to claim 40, wherein the method comprises providing between about 0.5 to 5 ml of the fluid cell culture media per chamber to said first and/or further chamber of the plurality of chambers.

42. A method according to claim 41, wherein the method comprises providing between about 1.5 to 4 ml of the fluid cell culture media per chamber to said first and/or further chamber of the plurality of chambers.

43. A method according to any of claims 29 to 42, which further comprises locating at least one cell in at least two chambers of the plurality of chambers, wherein the at least one cell located in a first respective chamber is the same cell type or a different cell type to the at least one cell located in a further respective chamber.

44. A method according to claim 43, which comprises:

locating a tissue portion comprising said at least one cell in at least two chambers of the plurality of chambers.

45. A method according to any of claims 29 to 44, which comprises;

providing a driving force to the drive element of an apparatus according to any of claims 1 to 28; and

repeatedly raising and lowering spaced apart ends of the holder body so as to apply a rocking motion to the holder body.

46. A method according to any of claims 29 to 45, which comprises applying a rocking motion for at least 24 hours, e.g. 48 hours, 72 hours, or more.

47. A method according to any of claims 29 to 46, which further comprises determining viability or other measurable parameter of the at least one cell(s).

48. A method according to claim 47, wherein the at least one cell comprises a hepatocyte, and further wherein the step of determining cell viability or other measurable parameter comprises measuring production of albumin by the cell(s), wherein optionally the cell culture is a liver slice.

49. A method according to any of claims 29 to 48, which is a method of modelling a liver disease.

50. A method according to claim 49, which is a method of modelling fatty liver disease and further comprises the steps of:

culturing the liver slice with one or more lipids for up to about four days.

51. A method according to claim 50, wherein the one or more lipid is selected from palmitic acid, oleic acid and linoleic acid conjugated to Bovine Serum Albumin (BSA) and combinations thereof.

52. A method according to claim 50 or claim 51, which further comprises the steps of:

culturing the liver slice with one or more fibrosis stimulating factors for up to about four days, wherein optionally the one or more fibrosis stimulating factors is selected from transforming growth factor-β (tgfb), platelet derived growth factor-bb (pdgf-bb) and combinations thereof.

53. A method according to any of claims 50 or claim 52, which further comprises the steps of:

culturing the liver slice with one or more inflammatory mediators for up to about four days wherein optionally, the inflammatory mediator is selected from pathogen-associated molecular patterns (PAMPs) e.g. lipopolysaccharide (LPS) or poly IC and damage associated molecular (DAMPs) e.g. apoptotic or damaged cells.

54. A method according to any of claims 51 to 53, which further comprises the steps of:

culturing the liver slice with one or more hepatotoxic agents for up to around 4 days, wherein optionally the hepatotoxic agent is selected from acetaminophen, bile acids and a combination thereof.

55. A method of *in vitro* testing liver toxicity of an agent comprising:

a) providing an apparatus according to any of claims 1 to 28;

b) locating at least one hepatocyte, and a cell scaffold element to at least one chamber of the plurality of chambers;

c) adding at least one agent to be tested to said at least one chamber;

d) for a predetermined period of time, applying a rocking motion to the culture apparatus; and

e) monitoring at least one effect of the agent on the hepatocyte.

56. A method according to claim 55, wherein the at least one hepatocyte is comprised in a liver slice, the method comprising locating the liver slice in the at least one chamber, and wherein optionally the liver slice further comprises a plurality of cell types.

57. A method according to claim 55 or claim 56, wherein monitoring at least one effect of the agent comprises monitoring the effect of the agent on the proliferation and/or differentiation and/or function of the hepatocyte as a measure of toxicity of the agent.

58. A method according to any of claims 55 to 57, which comprises locating the at least one cell and the cell culture scaffold element by locating at least one chamber insert element supporting a cell culture scaffold element holding the at least one cell in said at least one chamber of the holder body.

59. A method according to any of claims 55 to 58, which further comprises:

adding a fluid cell culture media to said at least one chamber of the plurality of chambers and;

applying the rocking motion for a predetermined period of time such that the fluid cell culture media repeatedly flows from said chamber to a further one of the plurality of the chambers and back again.

60. A method according to any of claims 55 to 59, which comprises;

providing a driving force to the drive element; and

repeatedly raising and lowering spaced apart ends of the holder body so as to apply a rocking motion to the holder body.

61. Apparatus according to claim 1, hereinbefore described with reference to the accompanying drawings.

62. Method according to claim 29 or claim 55 hereinbefore described with reference to the accompanying drawings.

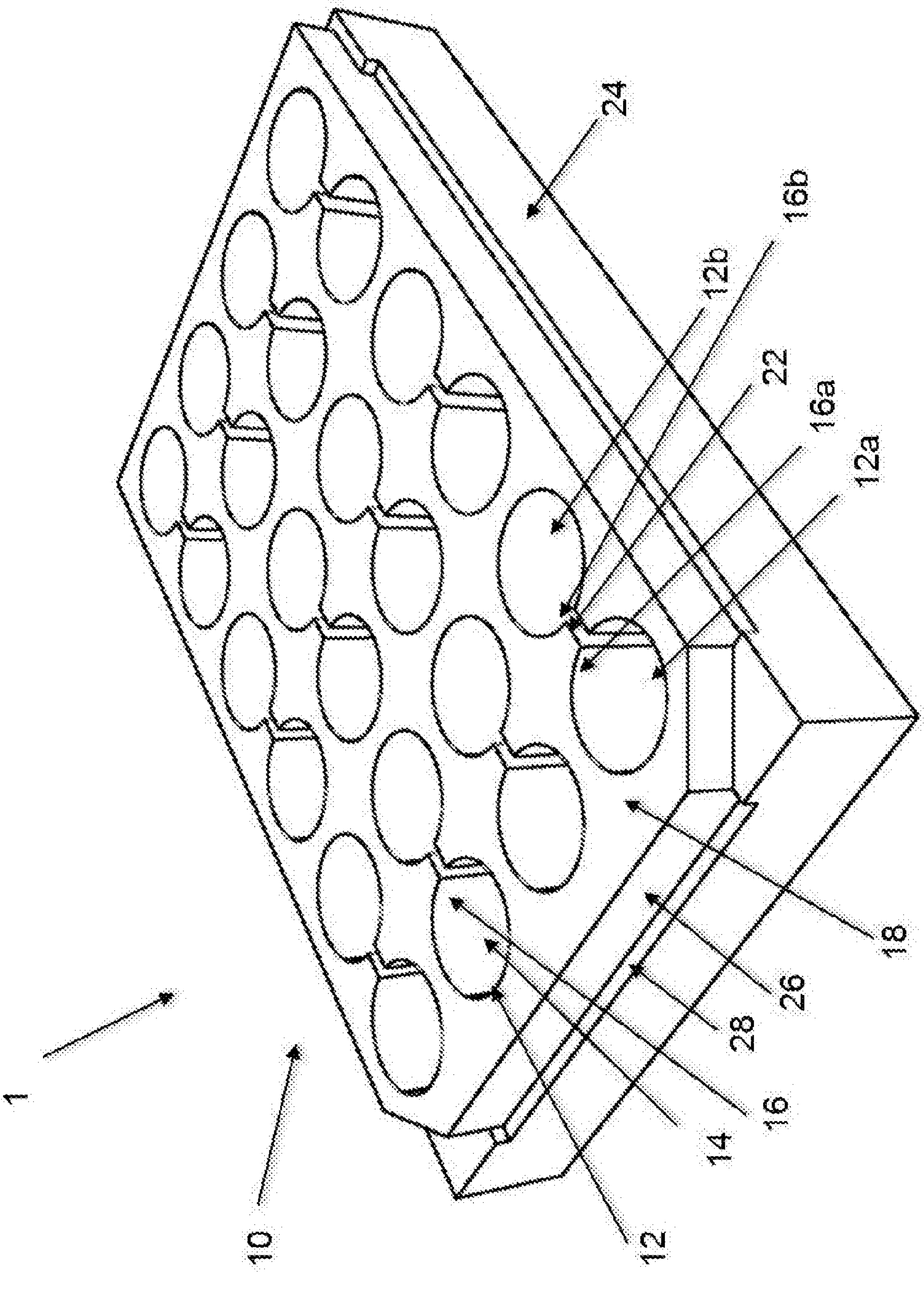


Figure 1

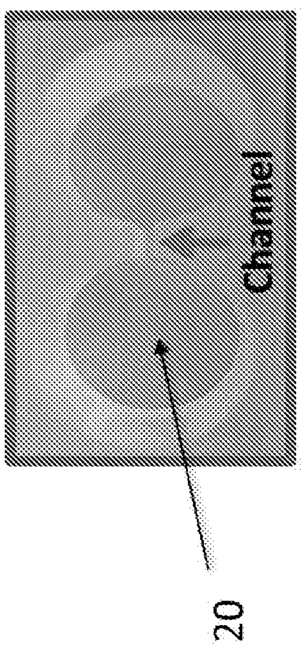


Figure 2

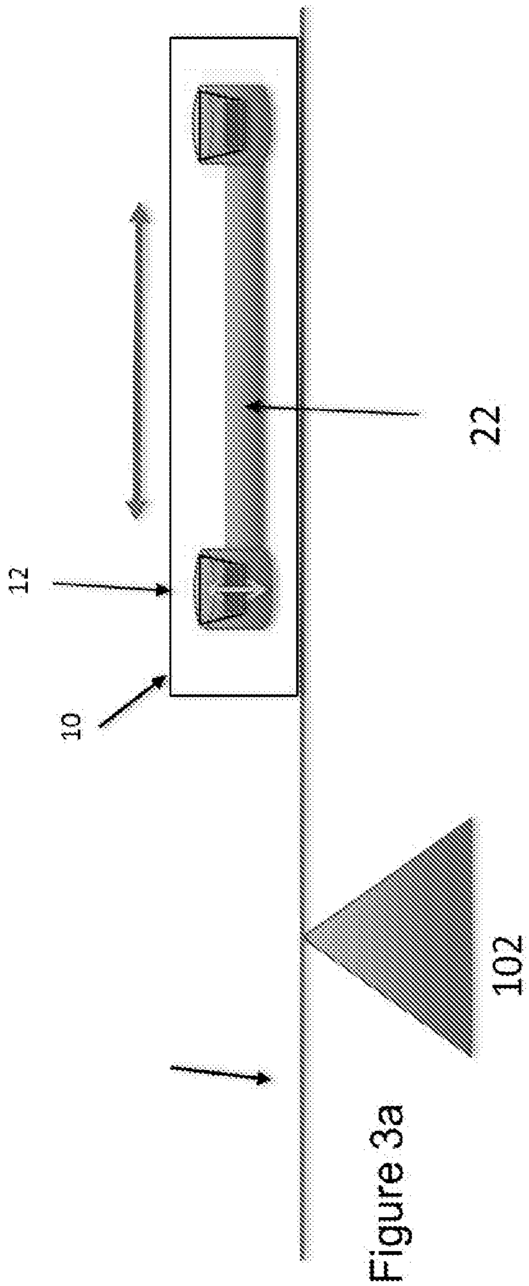


Figure 3a

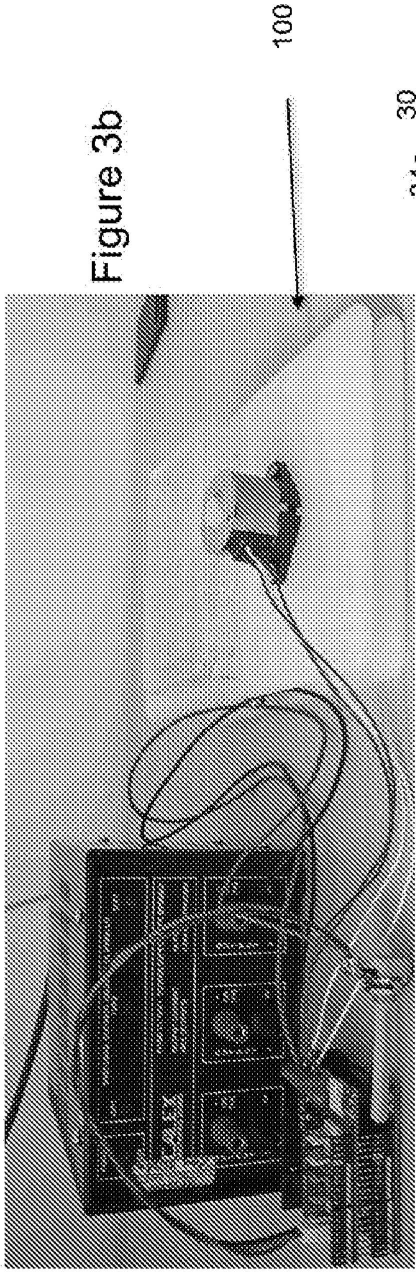


Figure 3b

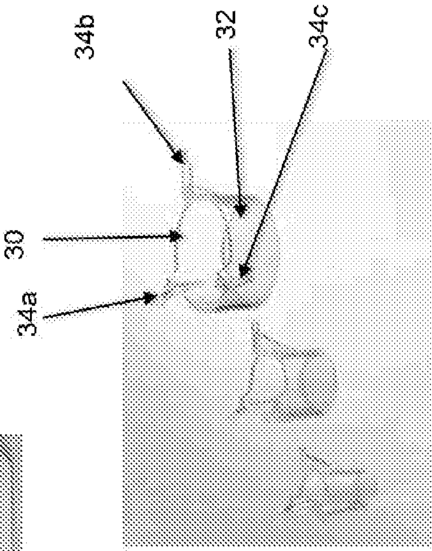


Figure 4b

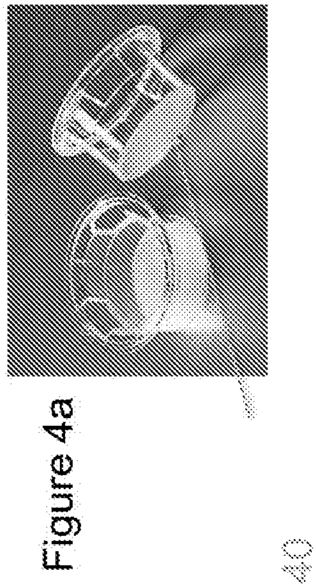


Figure 4a

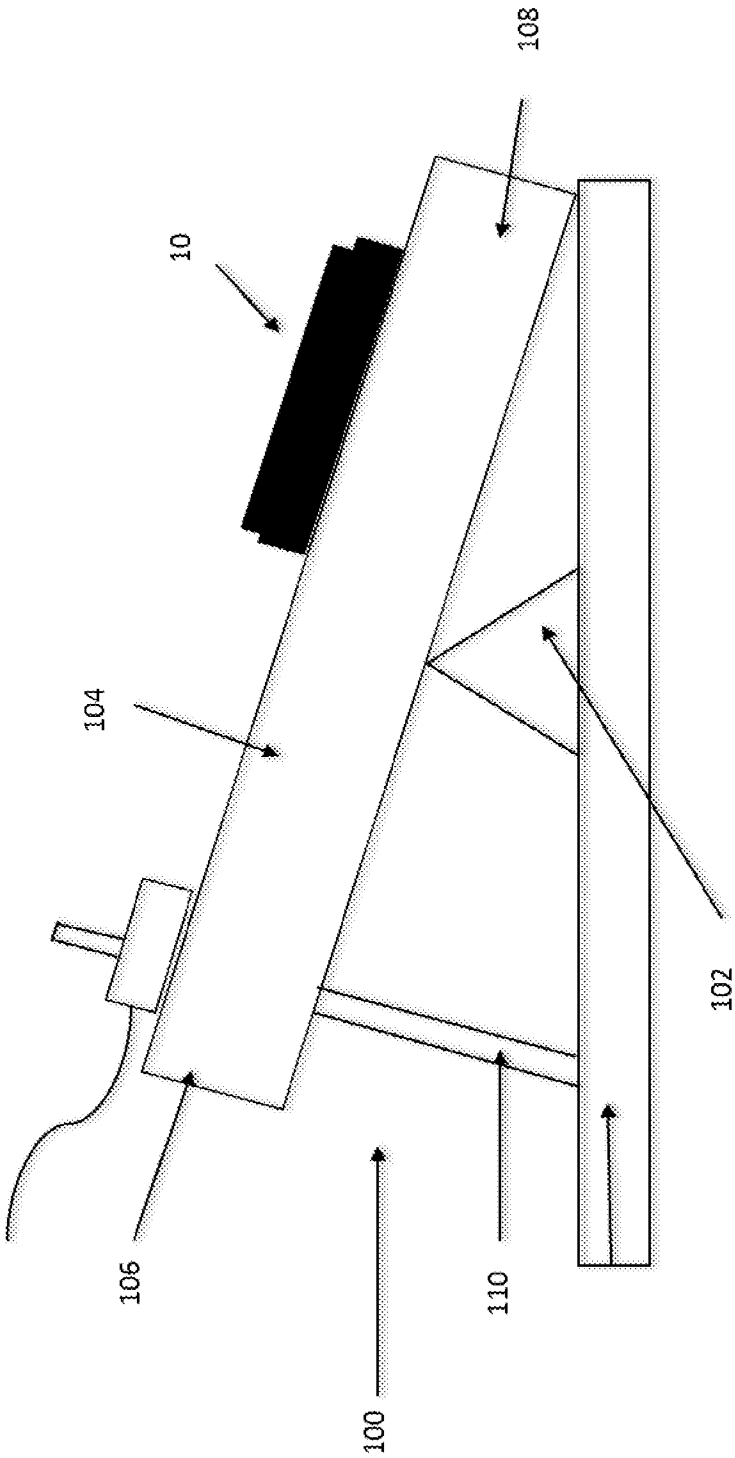


Figure 5

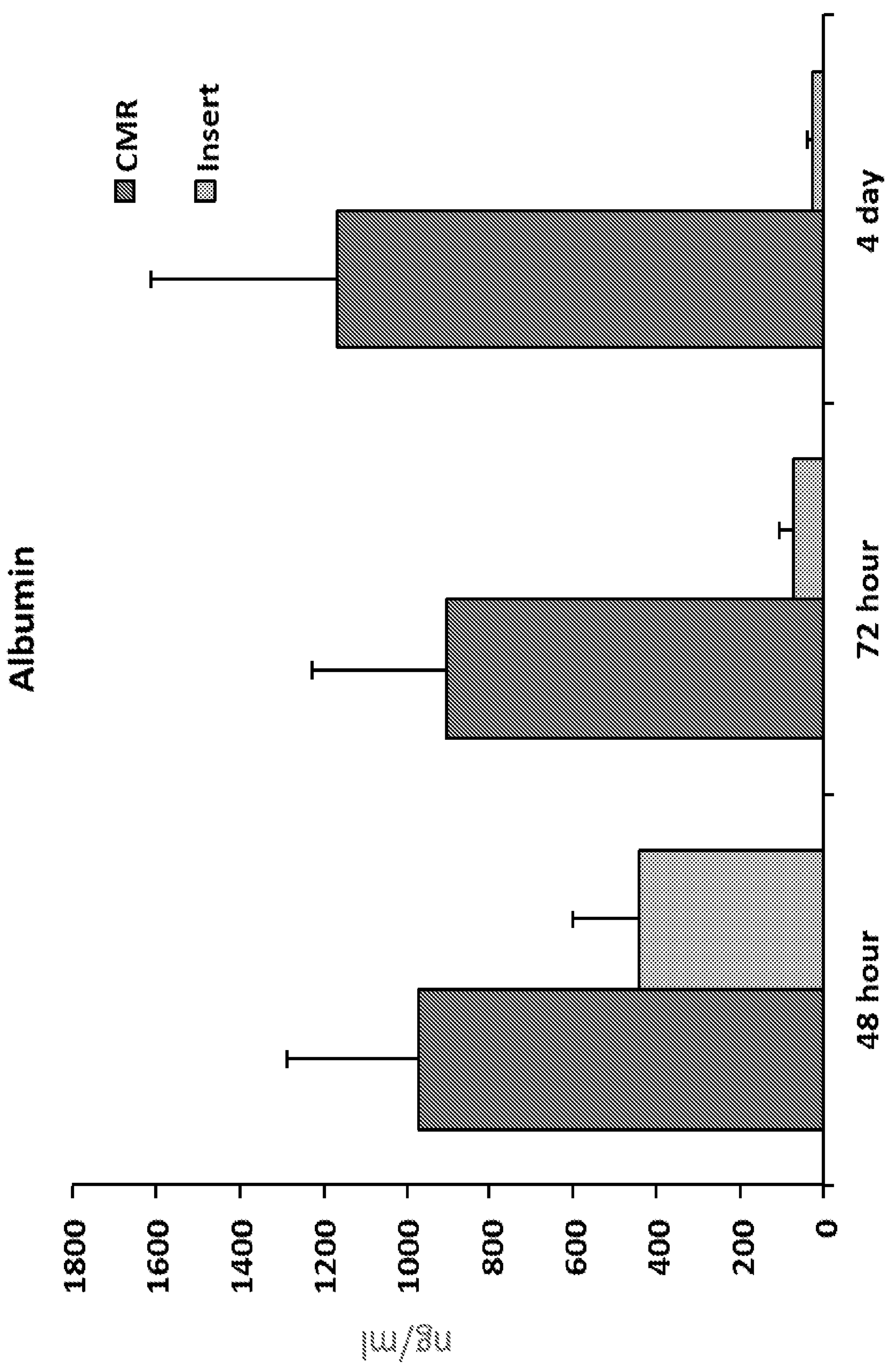

Albumin
CMR
Insert
ng/ml
1800
1600
1400
1200
1000
800
600
400
200
0
48 hour
72 hour
4 day

Figure 6

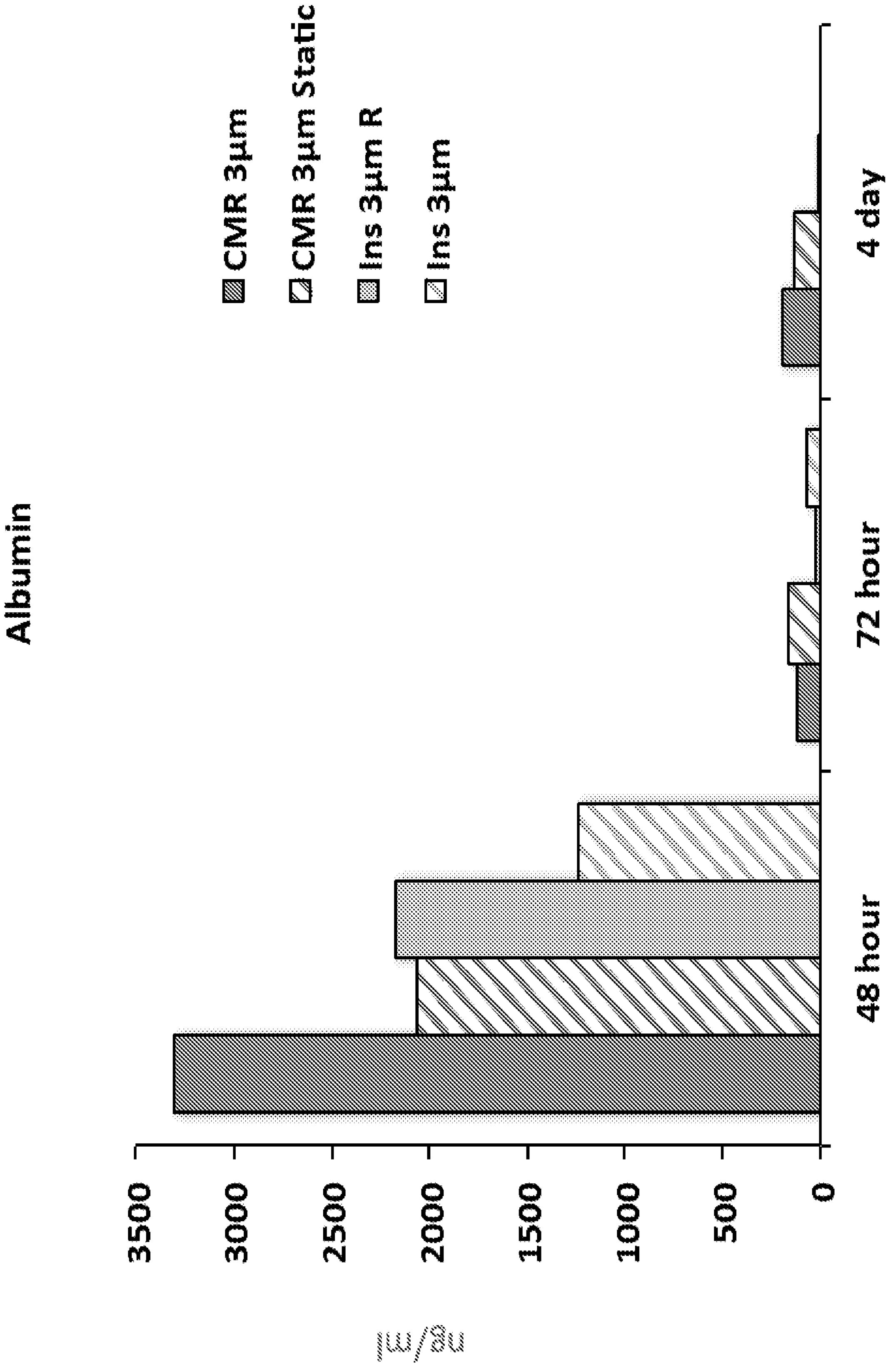

Albumin
CMR 3µm
CMR 3µm Static
Ins 3µm R
Ins 3µm
ng/ml
3500
3000
2500
2000
1500
1000
500
0
48 hour
72 hour
4 day

Figure 7

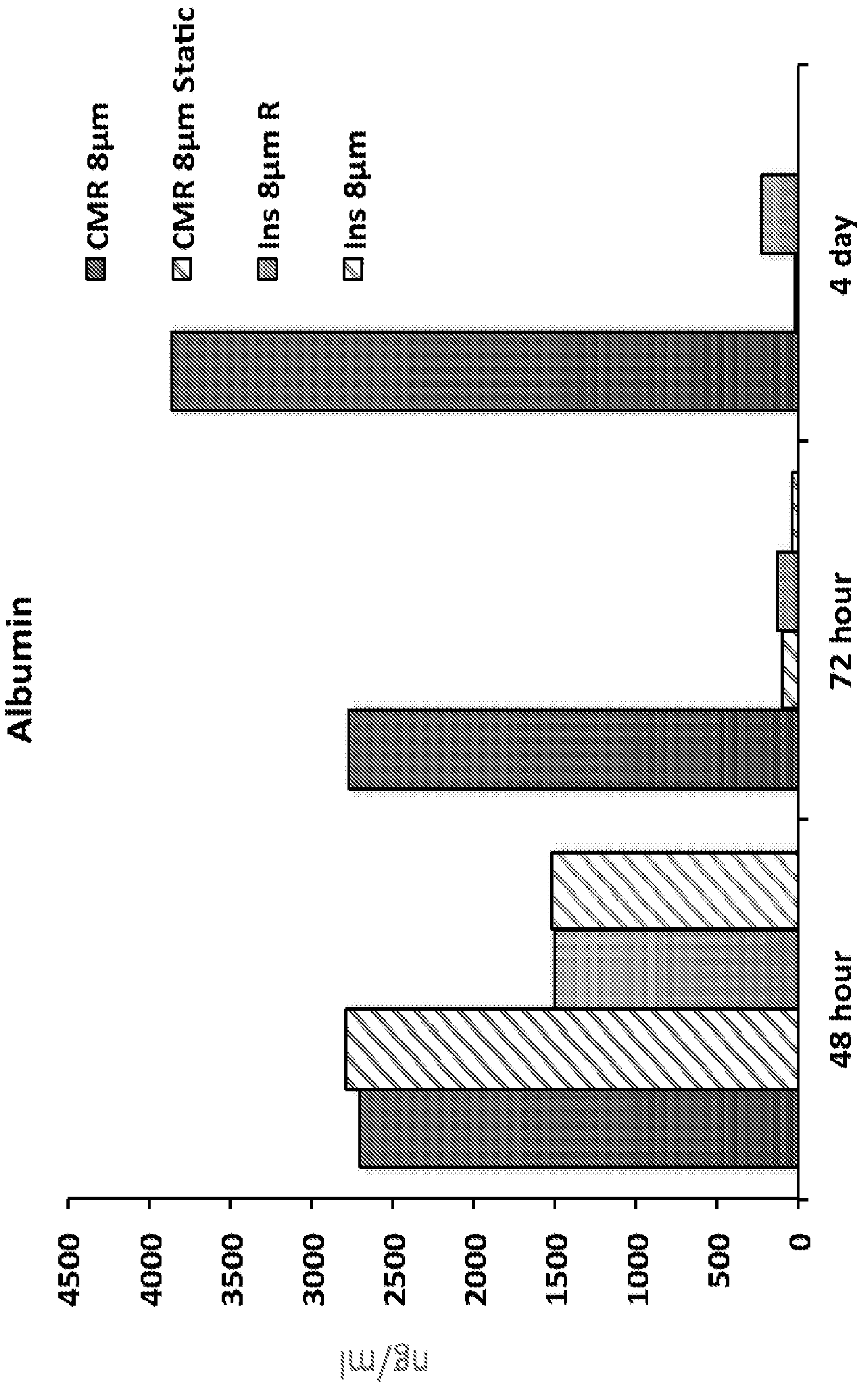

Albumin
CMR 8μm
CMR 8μm Static
Ins 8μm R
Ins 8μm
ng/ml
4500
4000
3500
3000
2500
2000
1500
1000
500
0
48 hour
72 hour
4 day

Figure 8

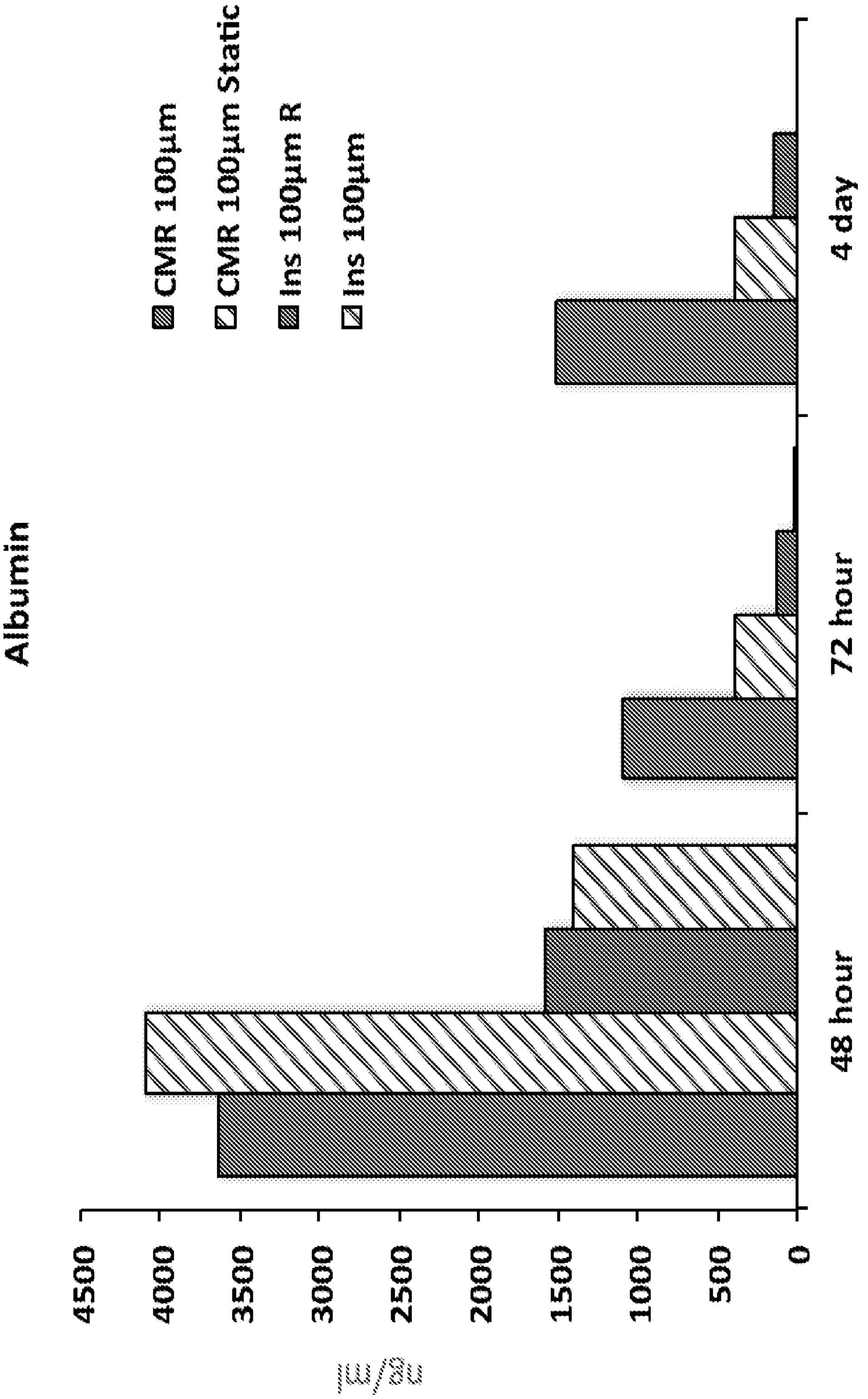

Albumin
CMR 100µm
CMR 100µm Static
Ins 100µm R
Ins 100µm
ng/ml
4500
4000
3500
3000
2500
2000
1500
1000
500
0
48 hour
72 hour
4 day

Figure 9

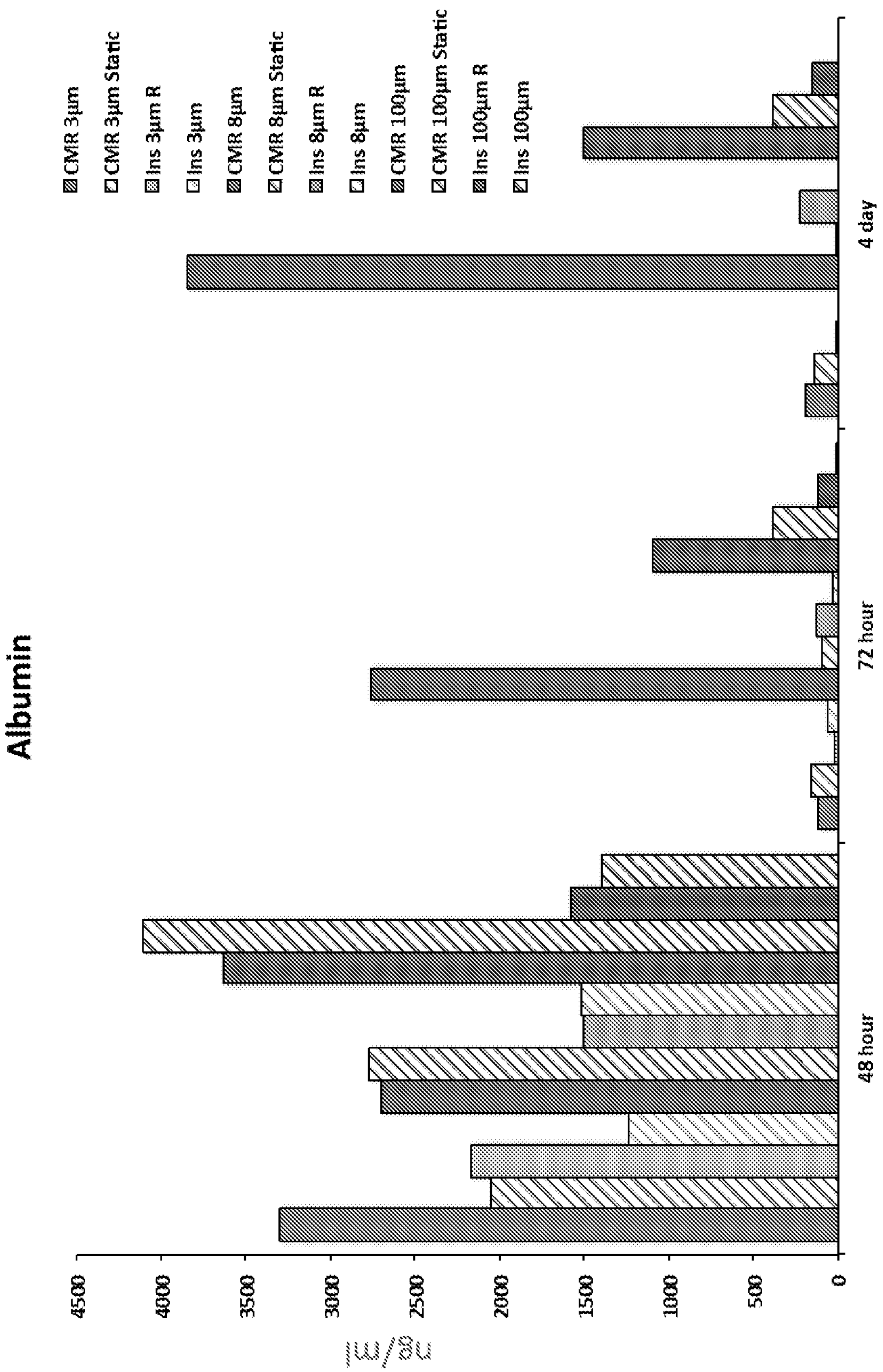

Albumin
ng/ml
0
500
1000
1500
2000
2500
3000
3500
4000
4500
48 hour
72 hour
4 day
CMR 3µm
CMR 3µm Static
Ins 3µm R
Ins 3µm
CMR 8µm
CMR 8µm Static
Ins 8µm R
Ins 8µm
CMR 100µm
CMR 100µm Static
Ins 100µm R
Ins 100µm

Figure 10

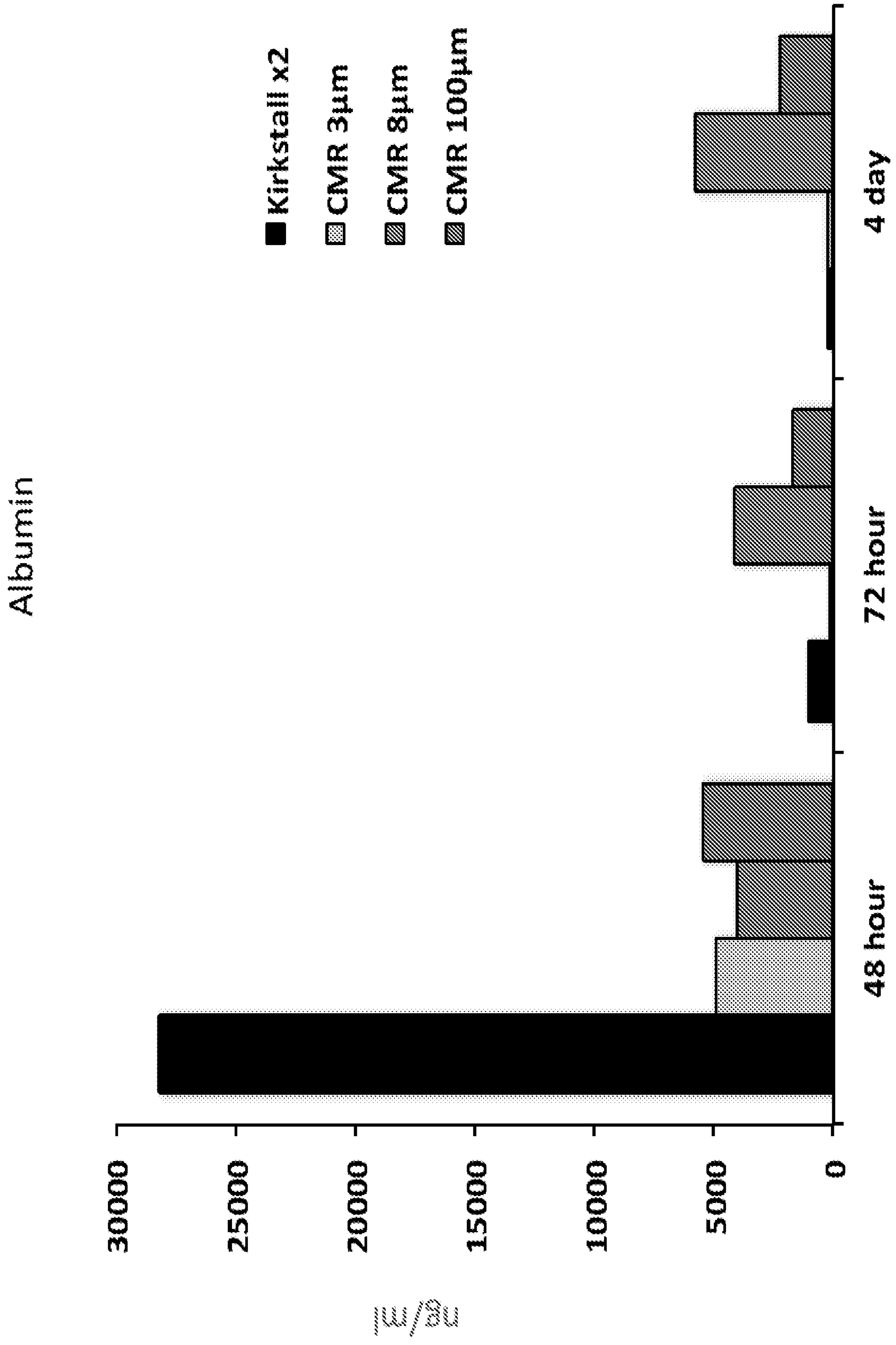

Albumin
ng/ml
30000
25000
20000
15000
10000
5000
0
48 hour
72 hour
4 day
Kirkstall x2
CMR 3µm
CMR 8µm
CMR 100µm

Figure 11

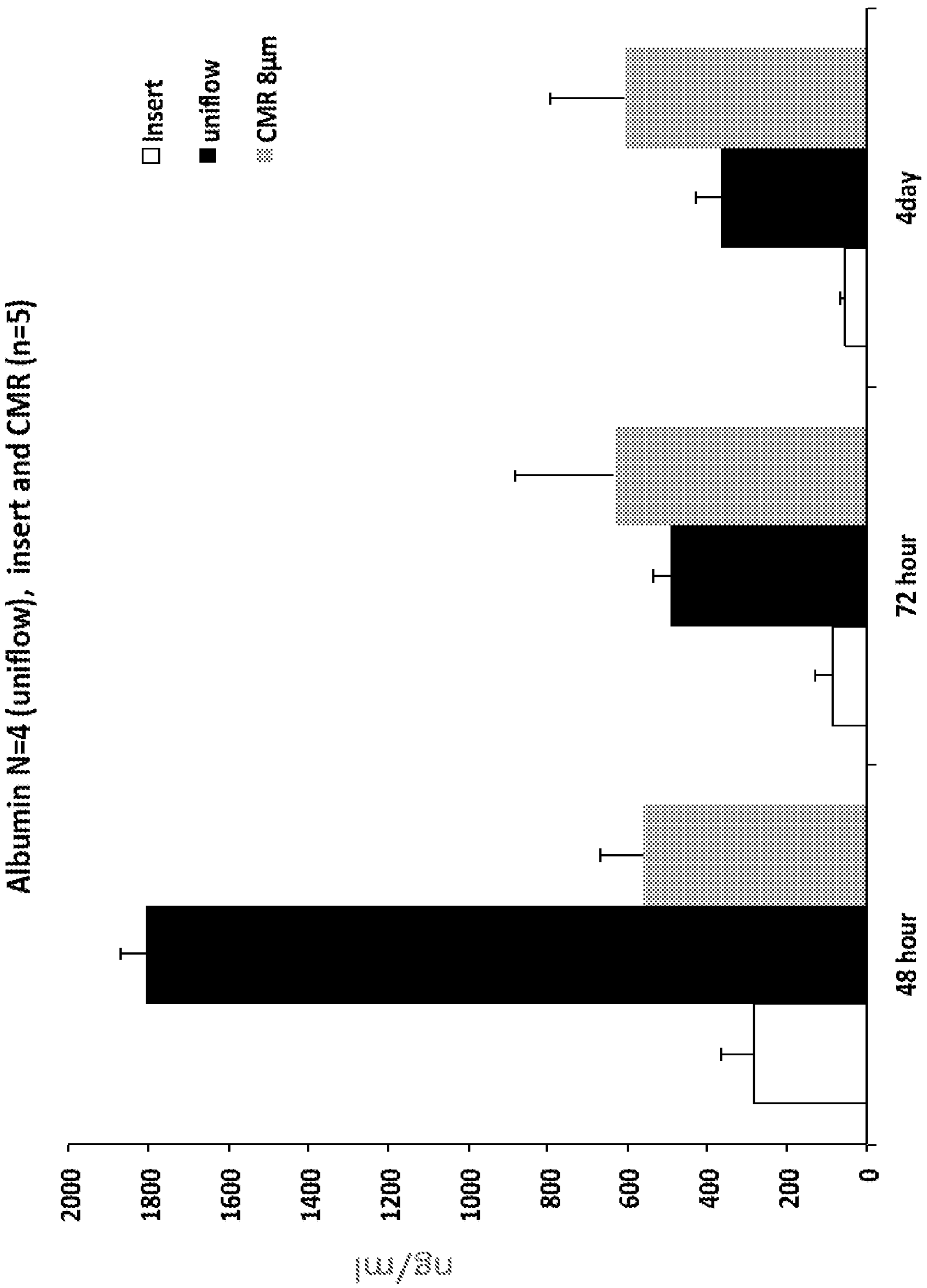

Albumin N=4 (uniflow), insert and CMR (n=5)
Insert
uniflow
CMR 8µm
ng/ml
2000
1800
1600
1400
1200
1000
800
600
400
200
0
48 hour
72 hour
4day

Figure 12

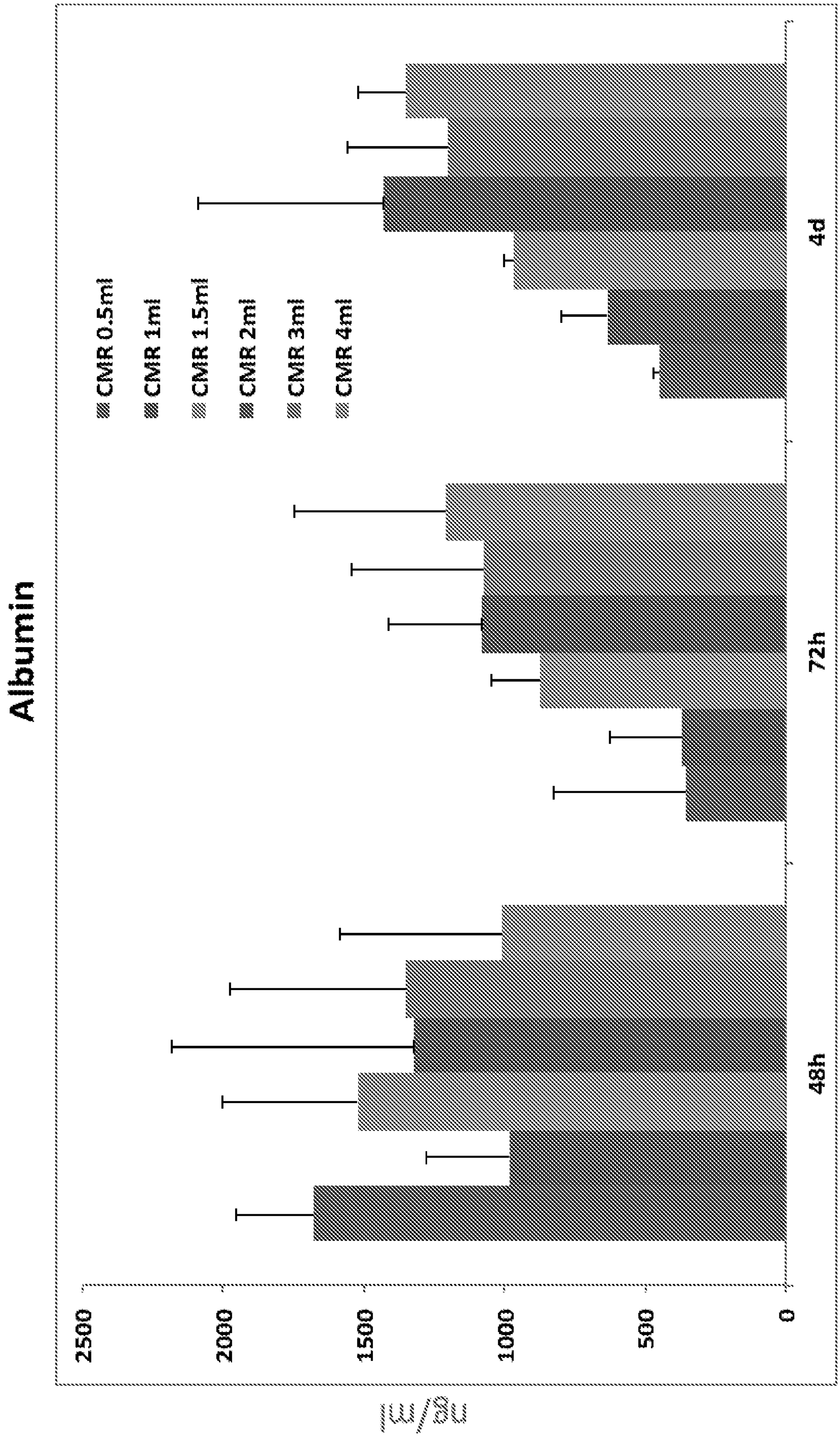

Albumin
CMR 0.5ml
CMR 1ml
CMR 1.5ml
CMR 2ml
CMR 3ml
CMR 4ml
ng/ml
0
500
1000
1500
2000
2500
48h
72h
4d

Figure 13

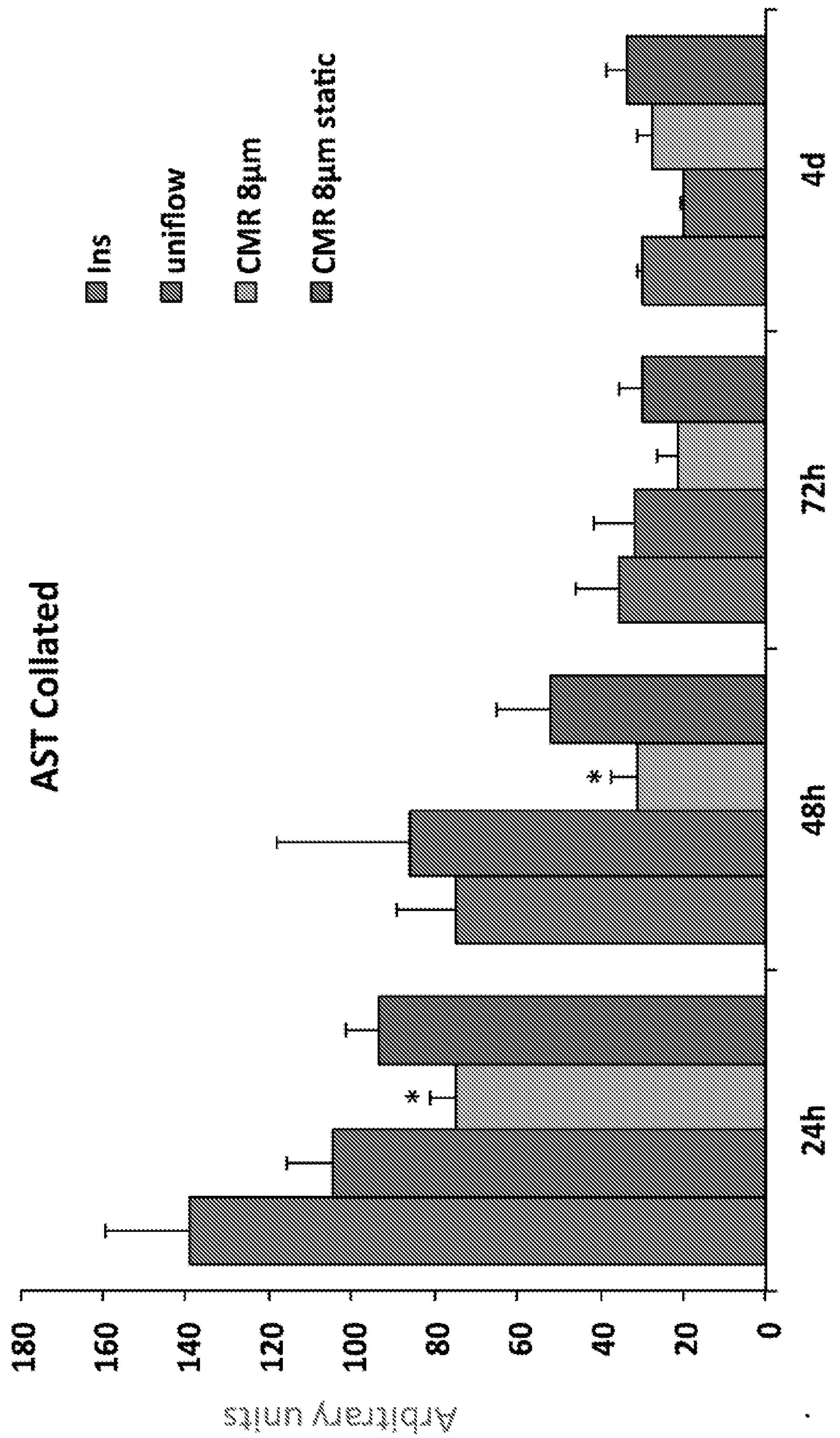

AST Collated
Ins
uniflow
CMR 8µm
CMR 8µm static
Arbitrary units
0
20
40
60
80
100
120
140
160
180
24h
48h
72h
4d
*
*

Figure 14

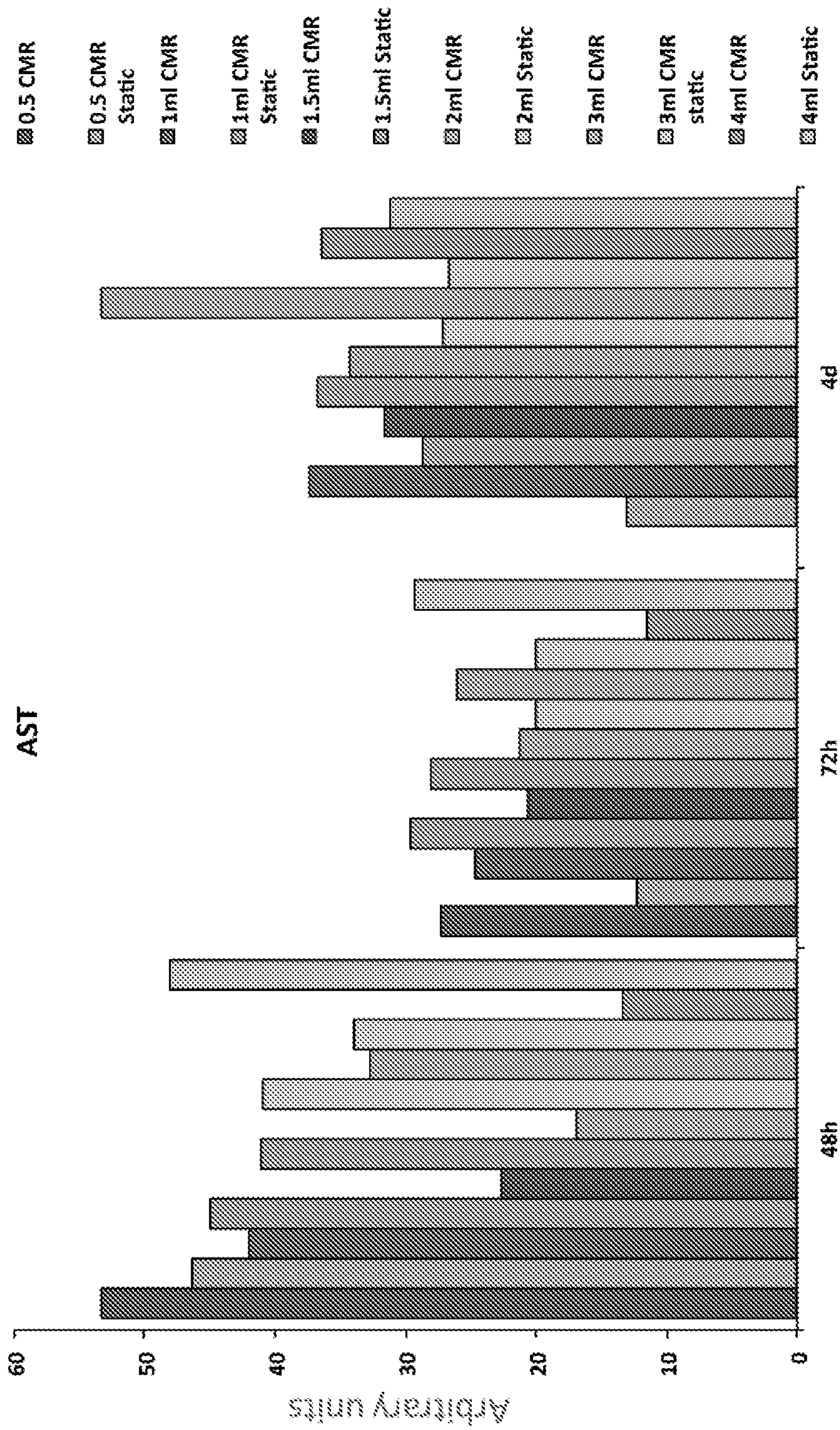

AST
0.5 CMR
0.5 CMR Static
1ml CMR
1ml CMR Static
1.5ml CMR
1.5ml Static
2ml CMR
2ml Static
3ml CMR
3ml CMR static
4ml CMR
4ml Static
Arbitrary units
0
10
20
30
40
50
60
48h
72h
4d

Figure 15

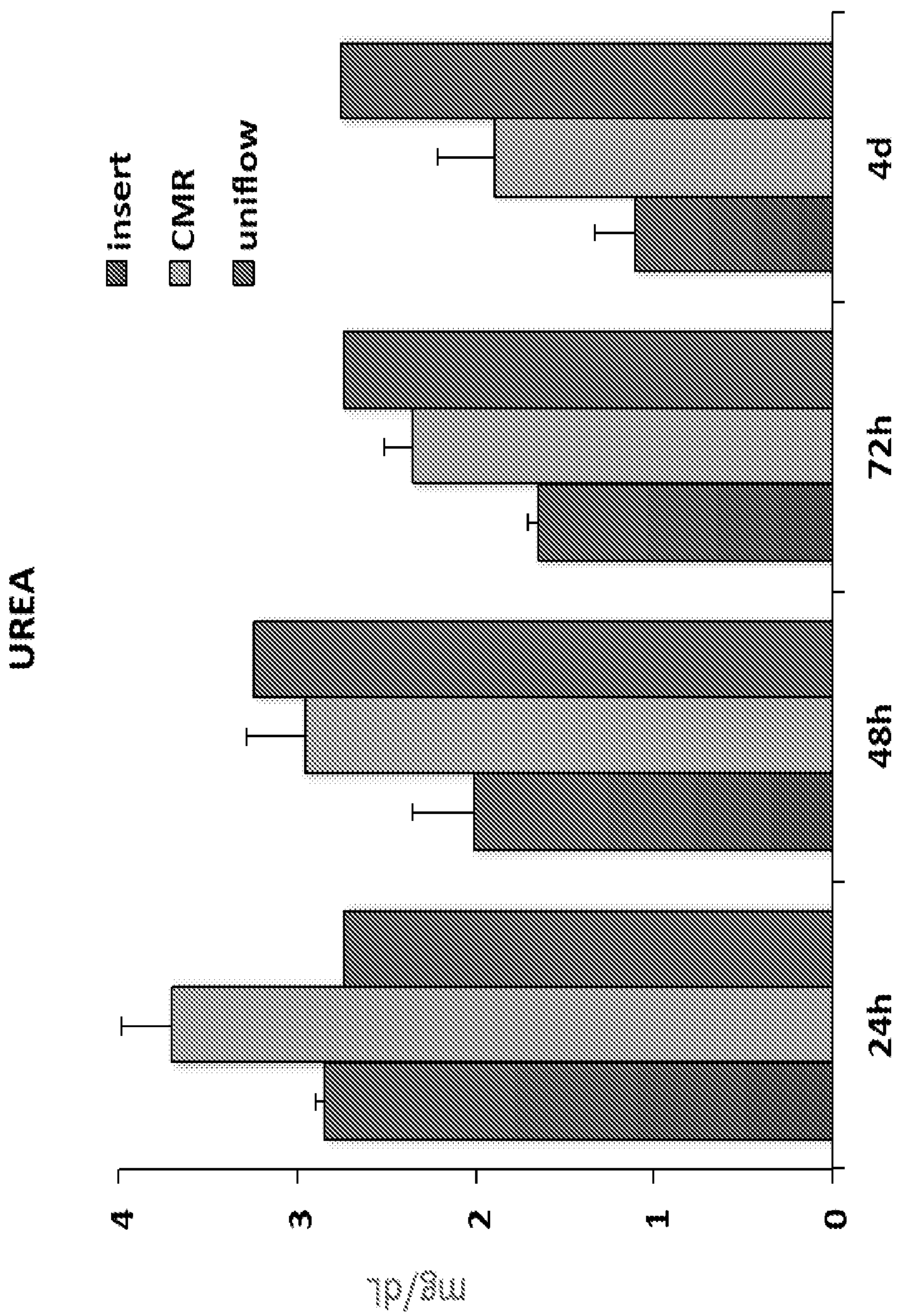


Figure 16

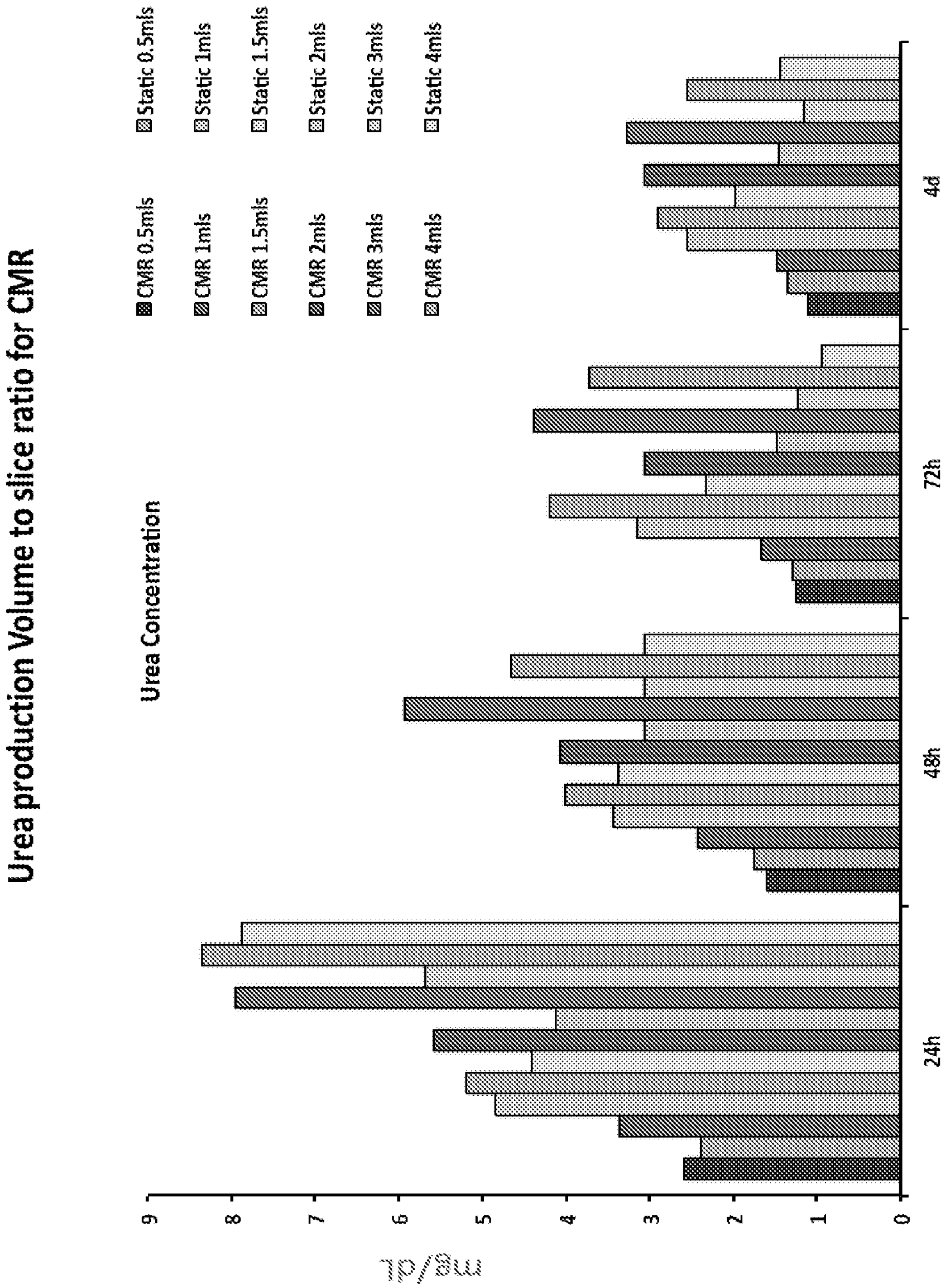

Urea production Volume to slice ratio for CMR
Urea Concentration
CMR 0.5mls
CMR 1mls
CMR 1.5mls
CMR 2mls
CMR 3mls
CMR 4mls
Static 0.5mls
Static 1mls
Static 1.5mls
Static 2mls
Static 3mls
Static 4mls
mg/dL
0
1
2
3
4
5
6
7
8
9
24h
48h
72h
4d

Figure 17

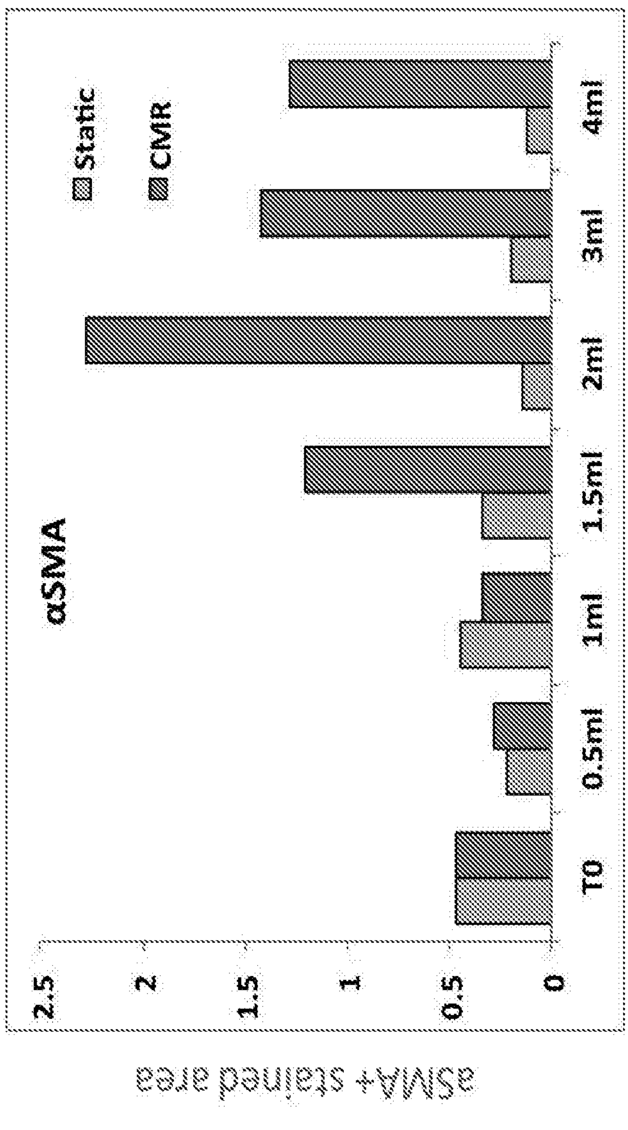


Figure 18

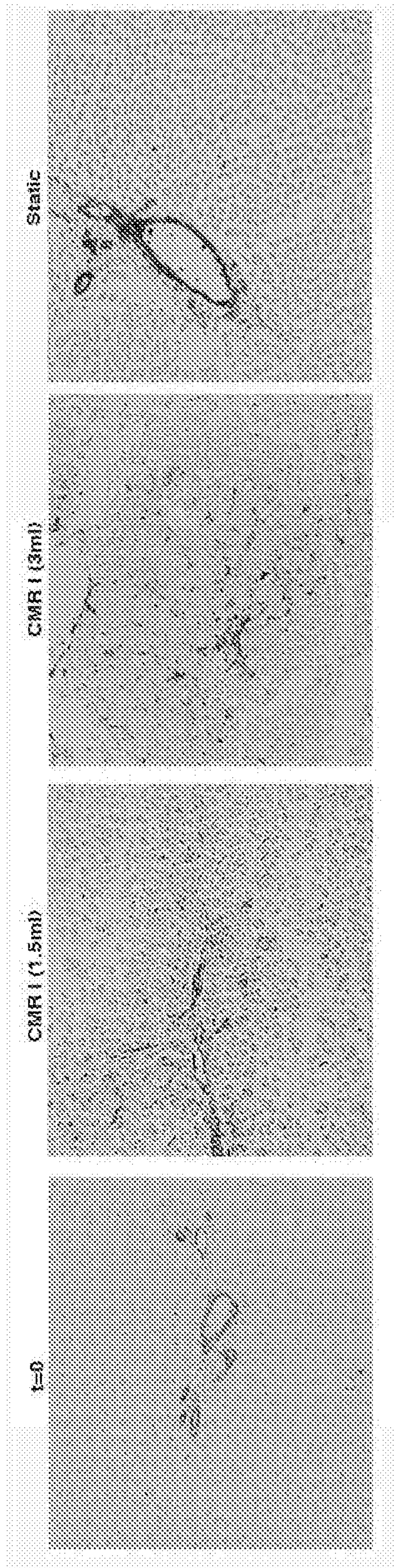


Figure 19

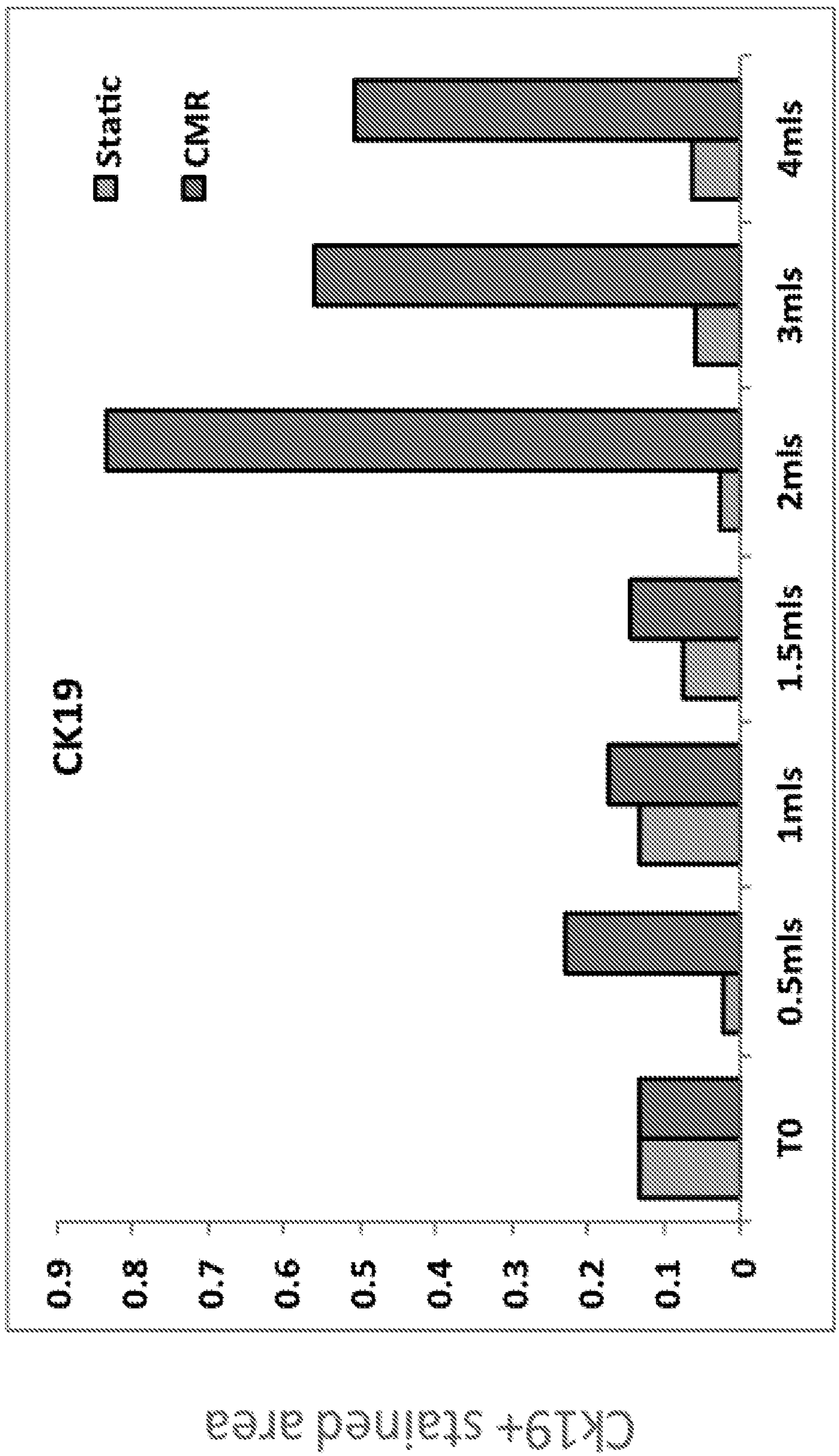

CK19
Static
CMR
Ck19+ stained area
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
T0
0.5mls
1mls
1.5mls
2mls
3mls
4mls

Figure 20

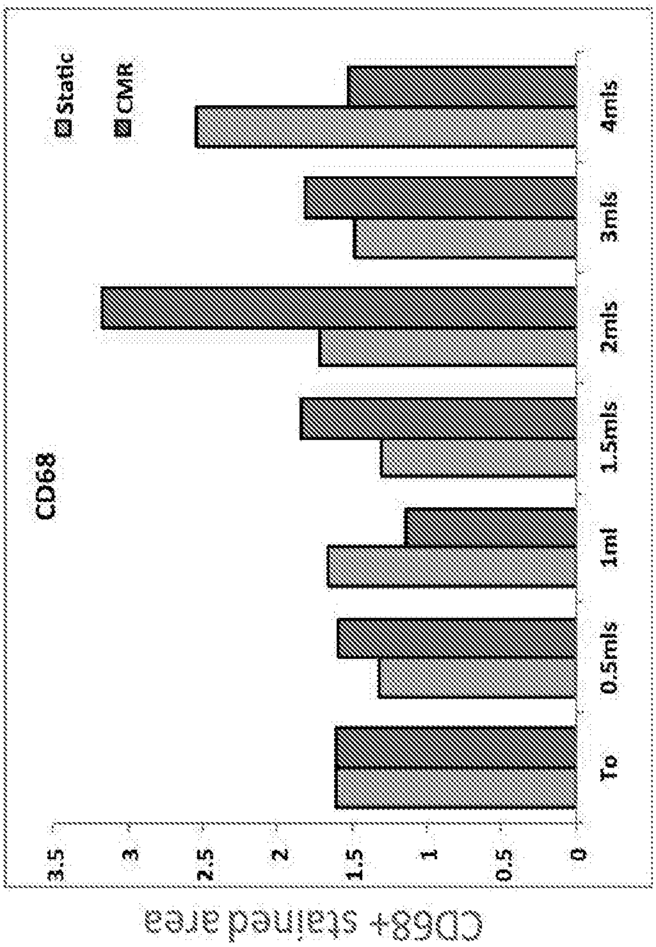


Figure 21

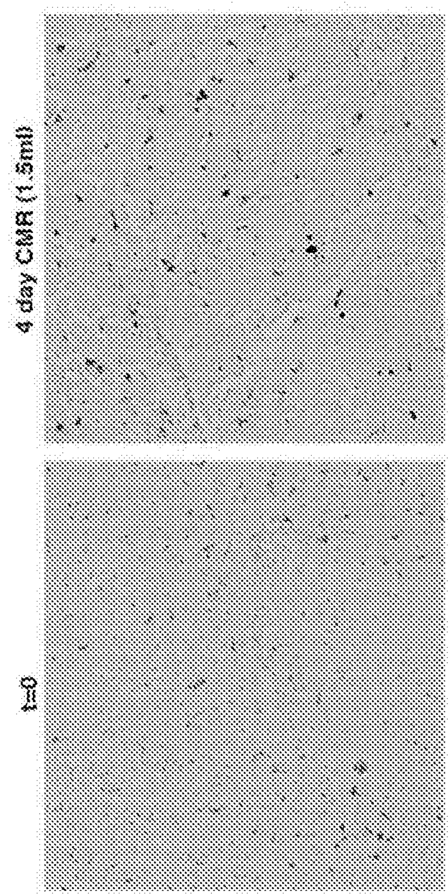


Figure 22

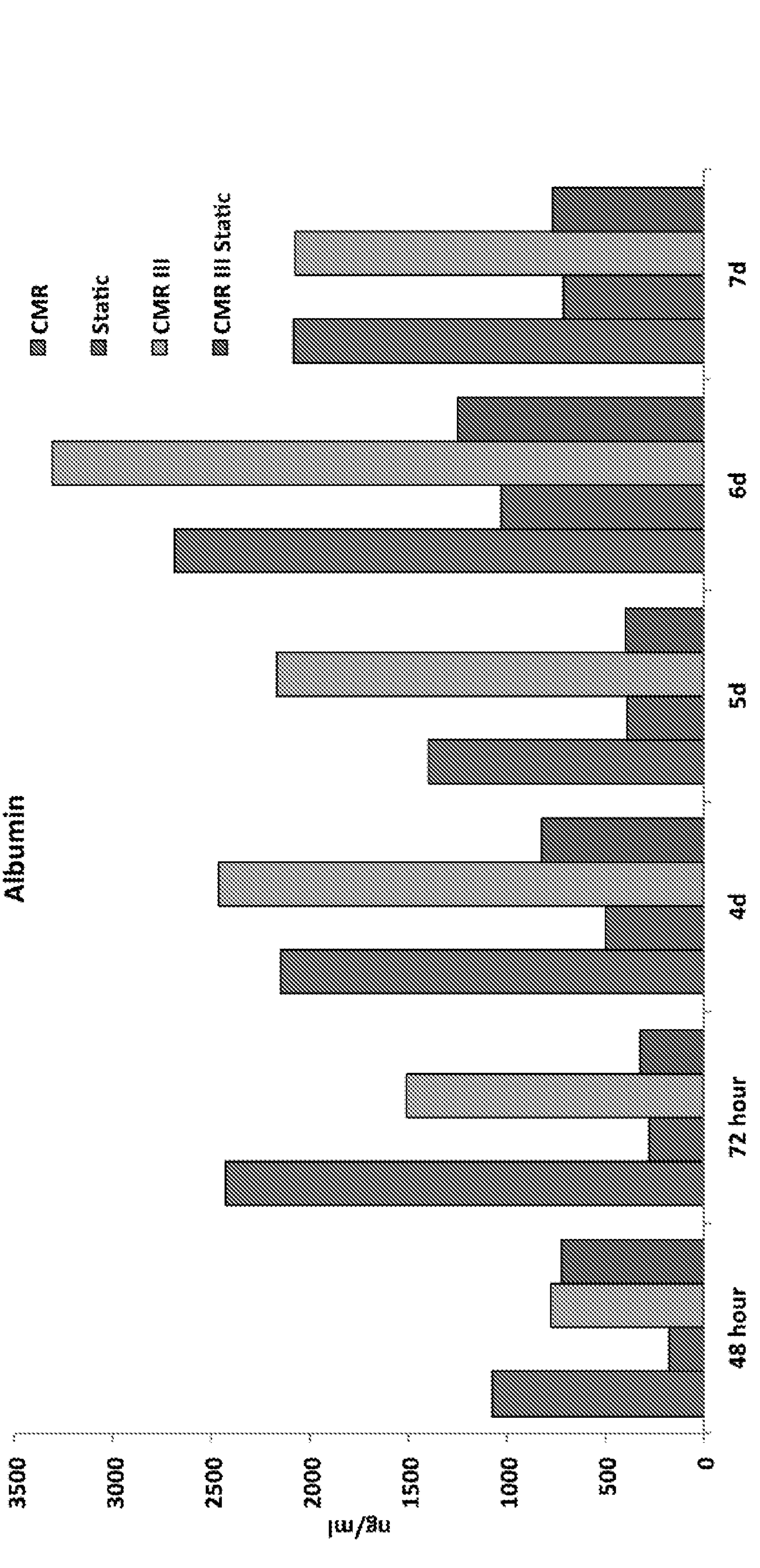

Albumin
CMR
Static
CMR III
CMR III Static
ng/ml
3500
3000
2500
2000
1500
1000
500
0
48 hour
72 hour
4d
5d
6d
7d

Figure 23

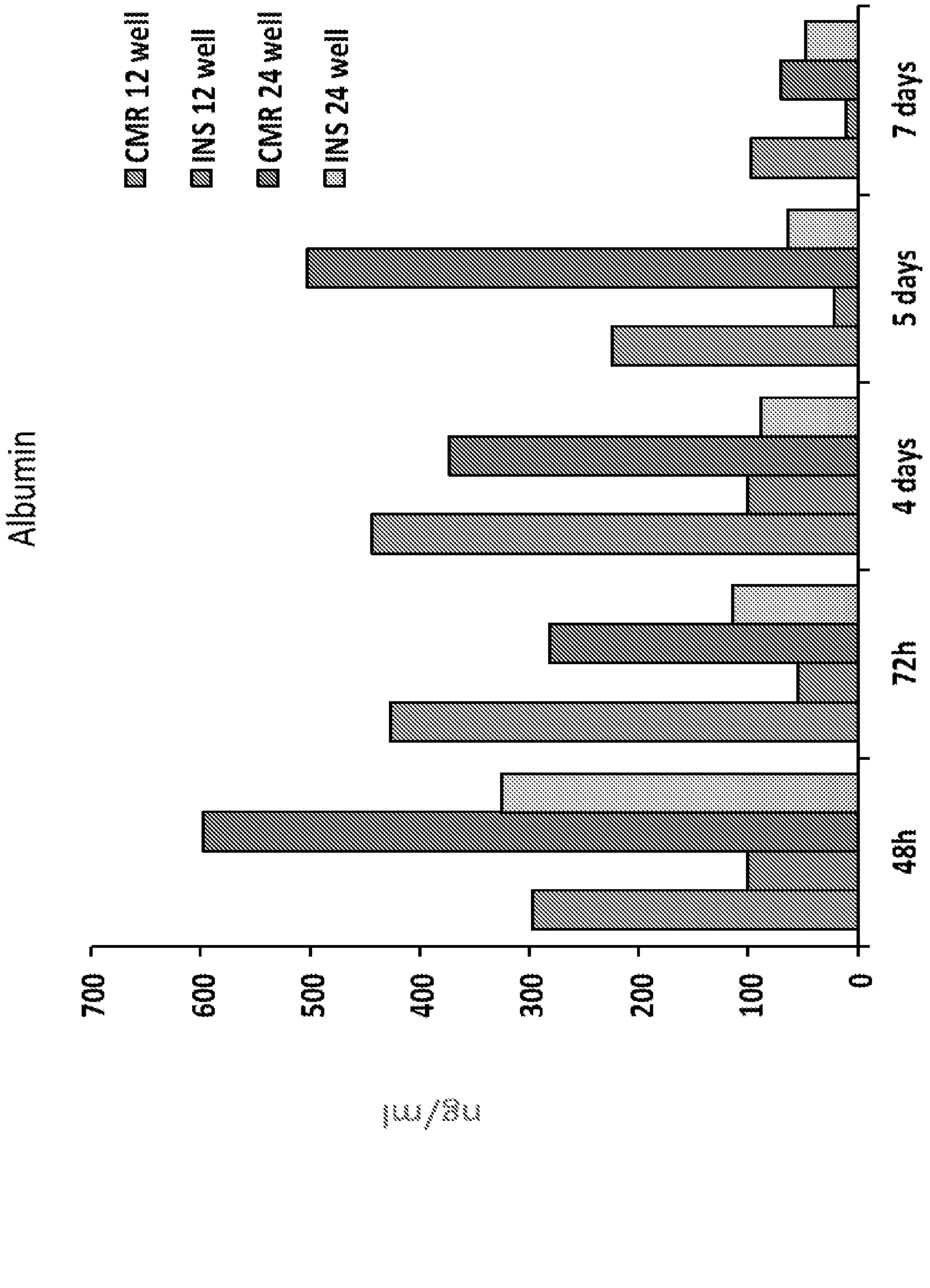
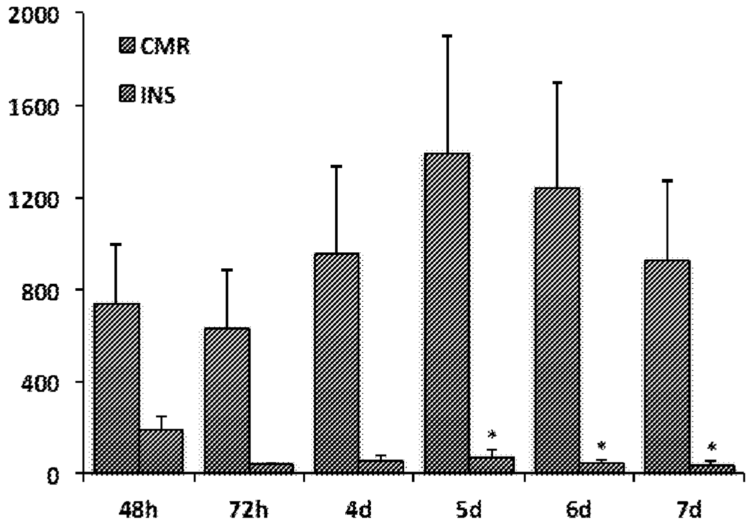

Albumin
CMR 12 well
INS 12 well
CMR 24 well
INS 24 well
ng/ml
700
600
500
400
300
200
100
0
48h
72h
4 days
5 days
7 days

Figure 24

CMR system 1

Albumin production ng/ml

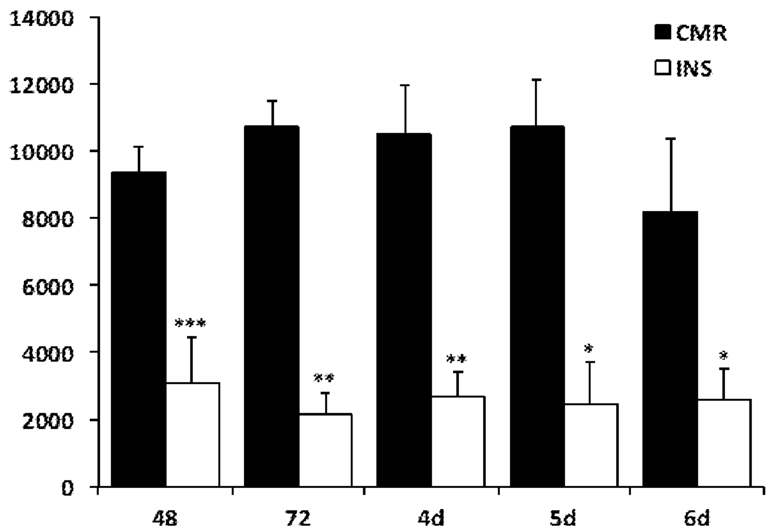


n=6

Figure 25a

CMR system 2

Albumin production ng/ml



n=4

Figure 25b

Fibrosis genes

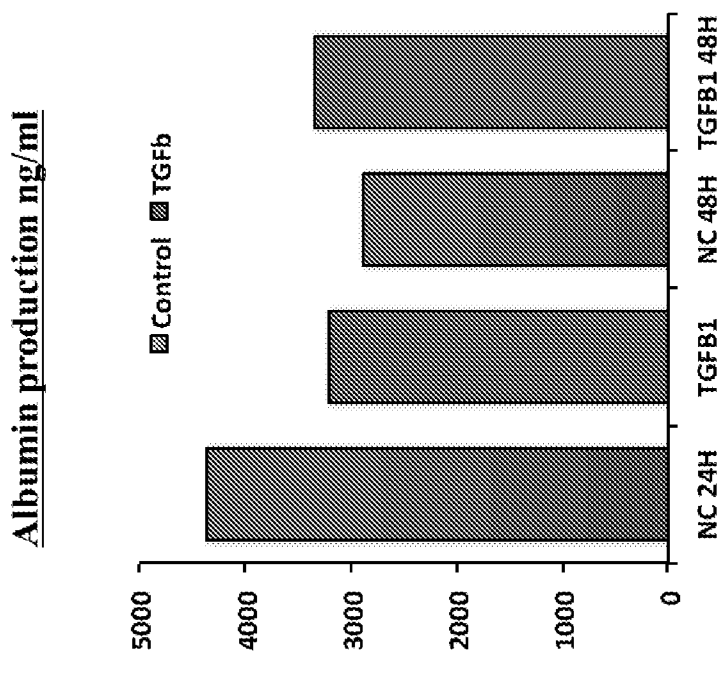


Figure 26A

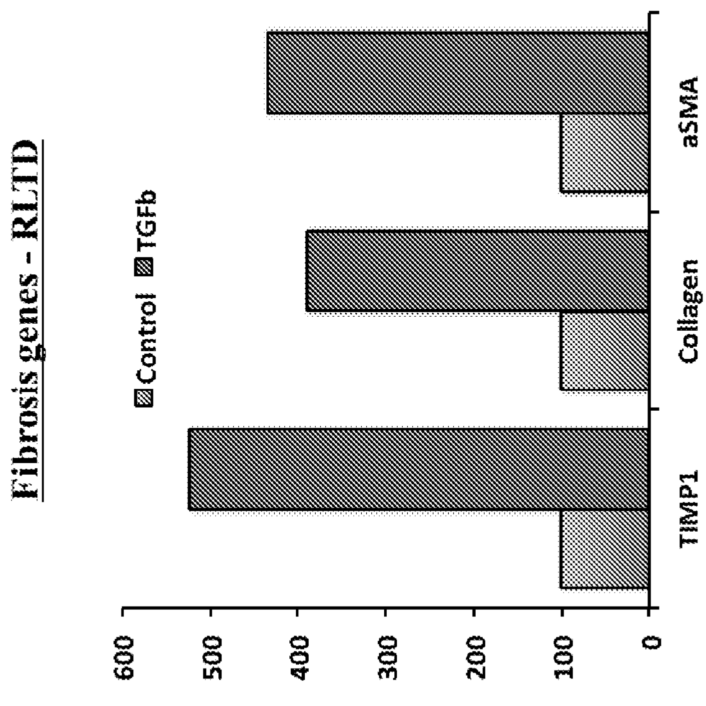


Figure 26B

FAT loading

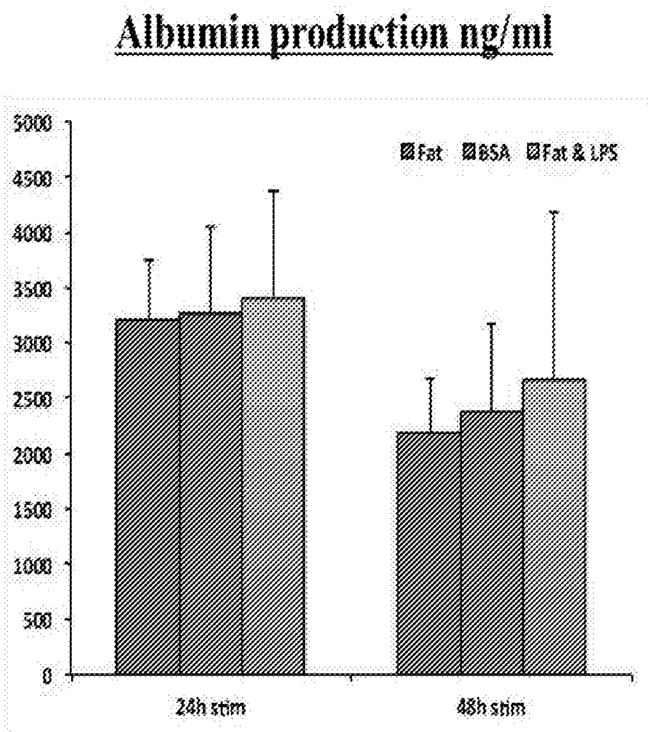


Figure 27

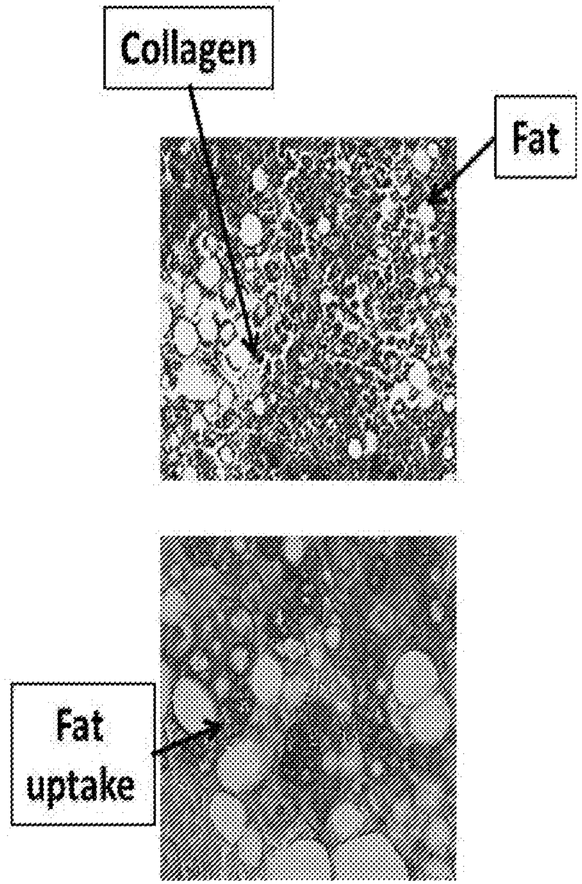


Figure 28

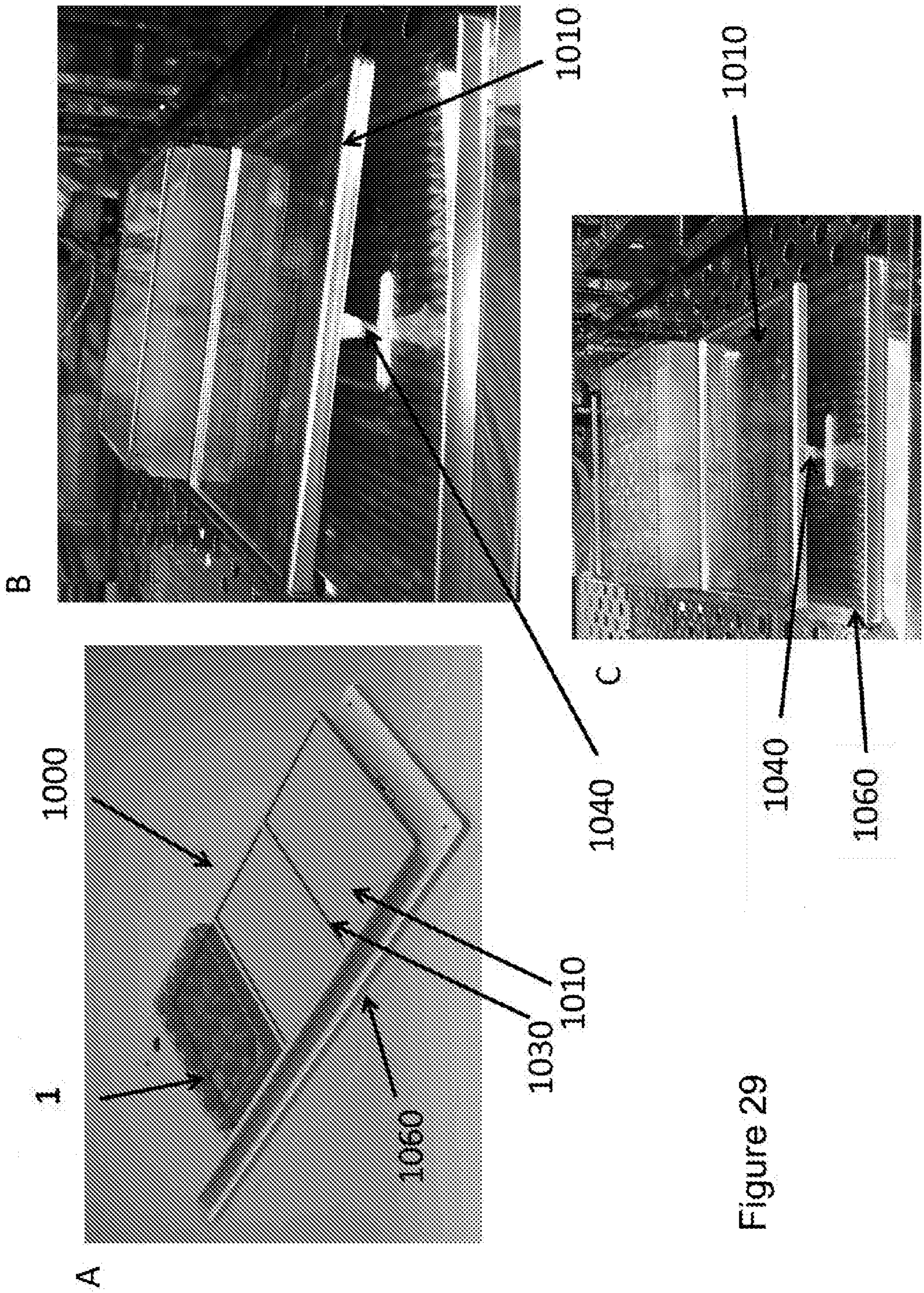


Figure 29

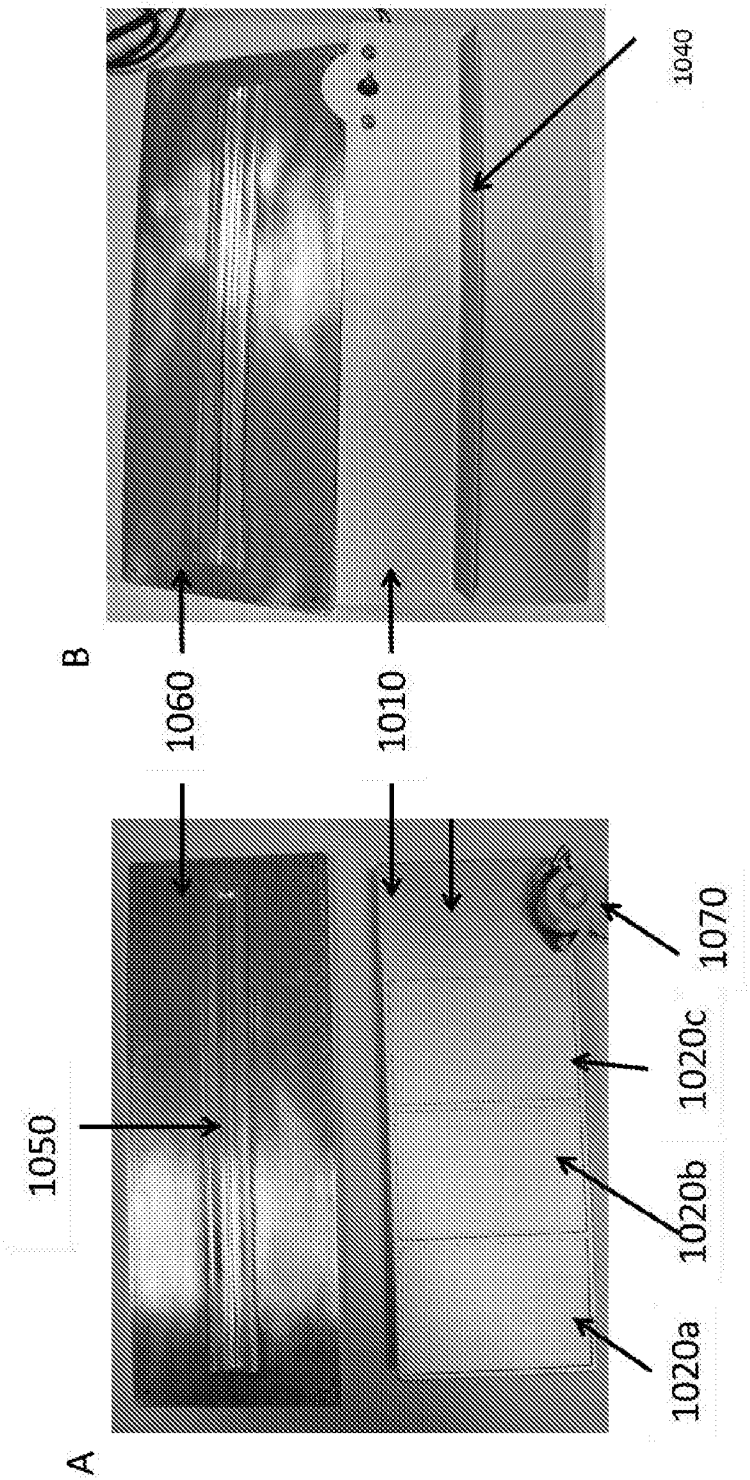
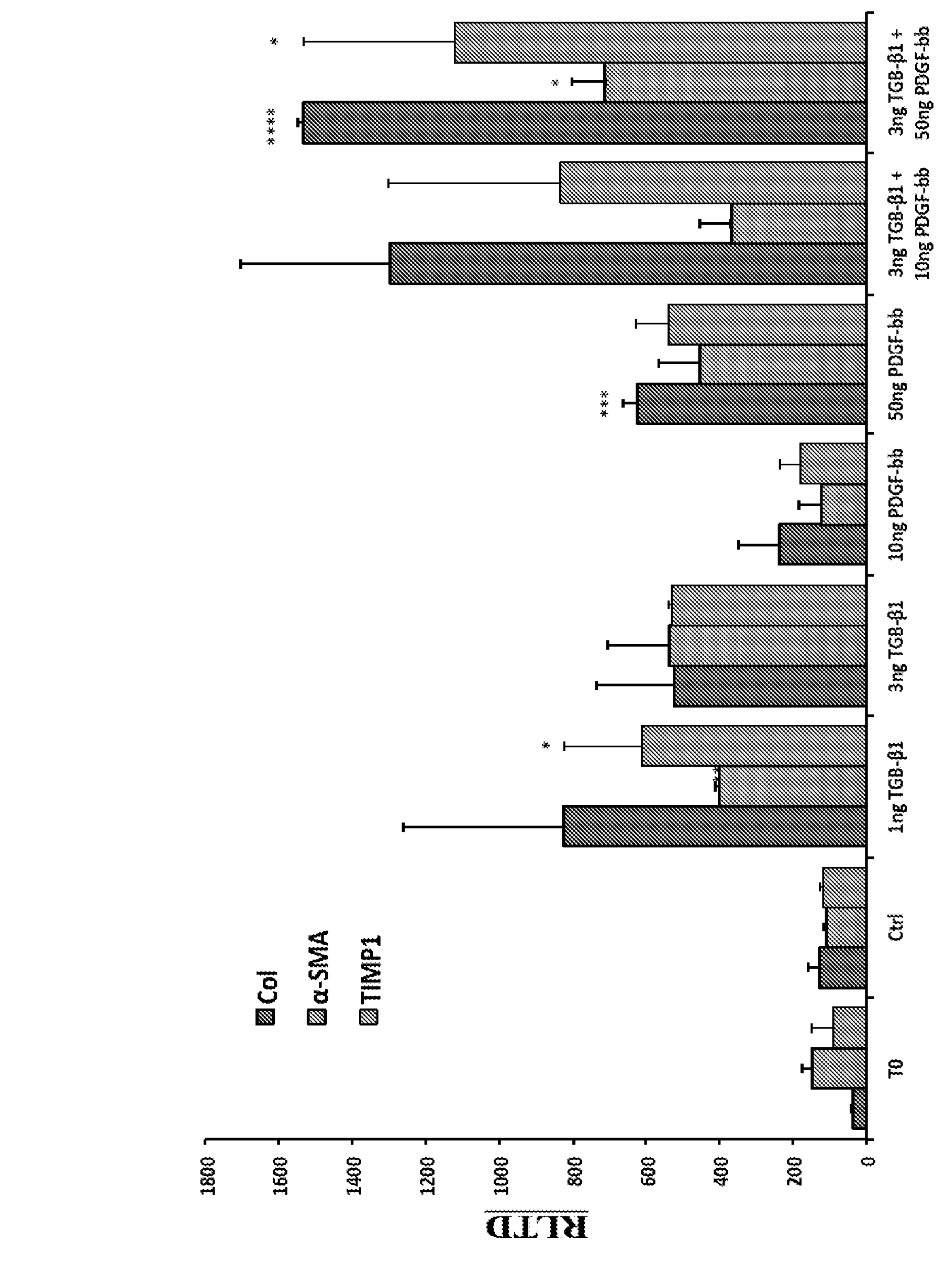


Figure 30


Col
α-SMA
TIMP1
RLTD
0
200
400
600
800
1000
1200
1400
1600
1800
T0
Ctrl
1ng TGB-β1
3ng TGB-β1
10ng PDGF-bb
50ng PDGF-bb
3ng TGB-β1 + 10ng PDGF-bb
3ng TGB-β1 + 50ng PDGF-bb

n=2

Figure 31

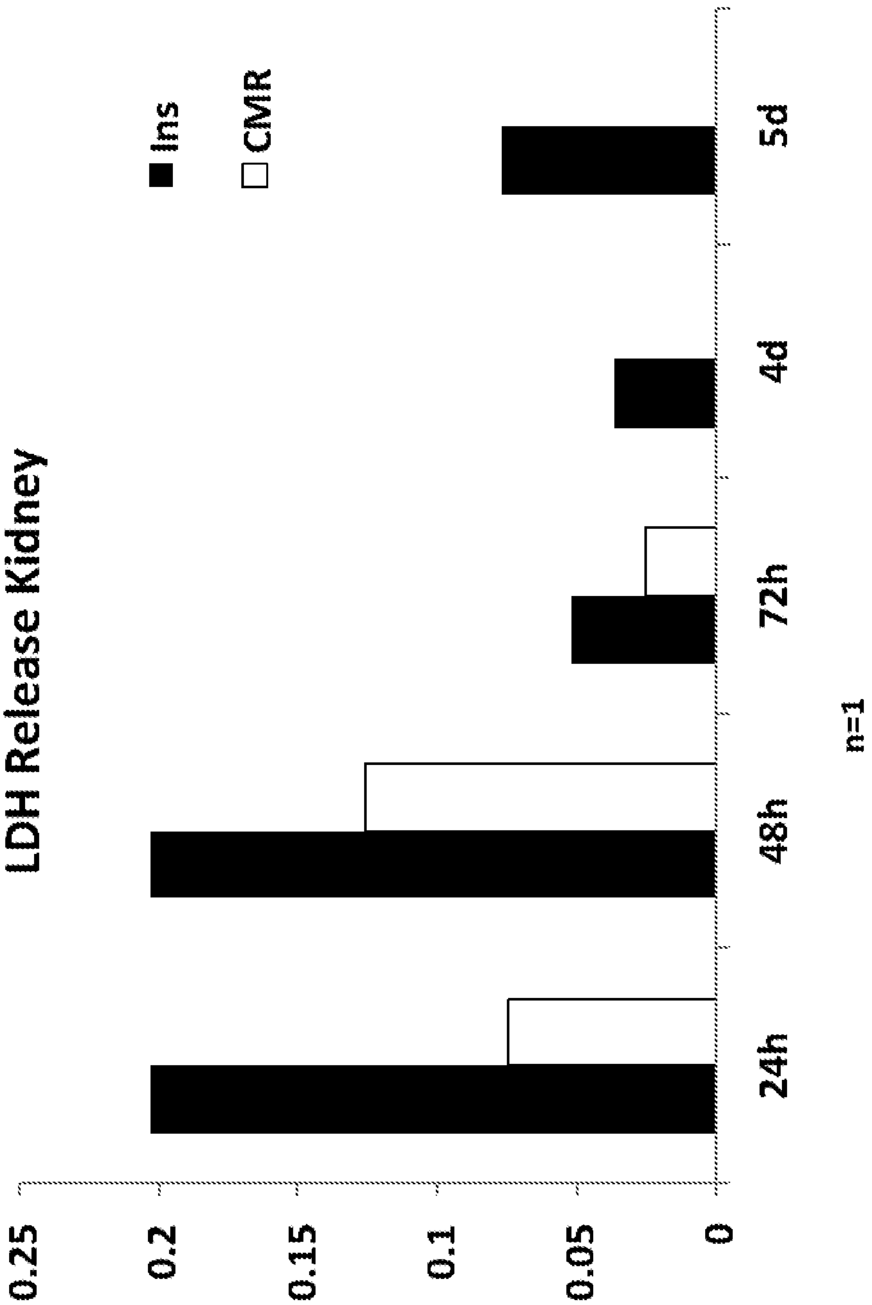

LDH Release Kidney
Ins
CMR
0.25
0.2
0.15
0.1
0.05
0
24h
48h
72h
4d
5d
n=1

Figure 32

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/053310

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00 C12M1/32 C12M1/00 C12M3/06 C12M1/34
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01L C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 422 270 A (CASPI RACHEL R [US]) 6 June 1995 (1995-06-06) column 6 sentence 10 - sentence 40 figures 1,2 -----	1-62
Y	US 2003/017582 A1 (KIM ENOCH [US] ET AL) 23 January 2003 (2003-01-23) paragraph [0015] - paragraph [0022] figures 1-3,6 -----	1-62
Y	WO 03/078565 A1 (SURFACE LOGIX INC [US]) 25 September 2003 (2003-09-25) page 6, line 6 - line 29 figures 1-2 -----	1-62
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 2017	01/02/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bayer, Martin

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/161480 A1 (IMP INNOVATIONS LTD; KIRSTEN MARIE LIS [GB]; GOEHRING NATALIA [GB]) 29 December 2011 (2011-12-29) par. 7-10, 21-26, 28-36 figures 1,2 -----	1-62
Y	WO 2006/097749 A1 (ROBIO SYSTEMS LTD [GB]; DODGSON JOHN ROBERT [GB]; AUSTEN MALCOLM [GB]) 21 September 2006 (2006-09-21) paragraph [0016] - paragraph [0044] paragraph [0067] figures 1, 2 -----	1-62
Y	WO 03/083044 A2 (CONDROS INC [US]; UNIV JOHNS HOPKINS [US]; FRONDOZA CARMELITA G [US];) 9 October 2003 (2003-10-09) examples 5, 6 -----	1-62
X,P	WO 2016/166315 A1 (INSPHERO AG [CH]) 20 October 2016 (2016-10-20) claims 1-20 page 35, line 17 - line 22 -----	1-62

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2016/053310

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5422270	A	06-06-1995	NONE		
US 2003017582	A1	23-01-2003	NONE		
WO 03078565	A1	25-09-2003	AU 2003213834	A1	29-09-2003
			US 2004002131	A1	01-01-2004
			WO 03078565	A1	25-09-2003
WO 2011161480	A1	29-12-2011	NONE		
WO 2006097749	A1	21-09-2006	NONE		
WO 03083044	A2	09-10-2003	AU 2003223416	A1	13-10-2003
			US 2005147959	A1	07-07-2005
			WO 03083044	A2	09-10-2003
WO 2016166315	A1	20-10-2016	NONE		