Dual Gasket System For Gas-Tight But Biocompatible Cell Culture Vessel Sealing

Tech ID: 34880 / UC Case 2023-918-0

BACKGROUND

Cell culture plates are an essential tool for cell biology research. They are used to grow cells in a controlled environment, which allows for study of the effects of different conditions on cell growth and development. The plates are typically made of plastic or glass and may have one or more wells, each of which can hold a small amount of cell culture media. The media provides the cells with the nutrients the cells need to grow and divide. Cell culture plates may be used in incubators to grow cells in a controlled environment. The incubator provides the cells with the necessary conditions for growth, including a constant temperature, humidity, and atmosphere.

Conventional cell culture plates are susceptible to evaporation, which causes increased osmolarity of cell culture media. This in turn causes unrealistic growth of cells as well as potential differences between wells due to uneven evaporation. In addition, evaporation causes increased concentration of the salts involved in electrical signaling of electrically active cell types, changing the ionic gradients across the cell membrane, thereby affecting all characteristics of the initiation, transmission (and computation) in electrically active cells such as cardiac or neuronal cells.

It is also difficult to maintain desired dissolved gas concentration with standard cell culture plates. This generally requires use of a compressed gas system, which uses gas regulators, sensors that are expensive and have limited lifetimes, and feedback control as well as a glove box for culture and/or handling.

An incubator is used to maintain the desired temperature of the cell culture plates, but the incubator impedes access to the cultures for feeding, for microscopy, etc. Furthermore, observation equipment for use inside an incubator needs to be designed to resist incubator conditions (e.g., body temperature heat and humidity). Incubators also take up significant space and packing of incubators in a laboratory is space-inefficient relative to the form factor of the cell culture plates. As the number of cell culture plates in a single incubator increases, the ability of the incubator to perform its function decreases, since there is a minimum number of times an incubator may be accessed per week per cell culture vessel. However, every time the incubator is accessed, it is unable to perform its functions for a prolonged period of time, e.g., over 30 minutes.

A further technical problem is that cell culture devices that use an air gap for gas exchange have an increased risk of microbial contamination via that air gap. This makes it difficult to perform manual cell culture experiments over the course of months without contamination. In addition, cross-contamination is more likely if multiple different experiments are being performed in the same laboratory. Thus, there is a need for cell culture vessels and systems that overcome these problems.

TECHNOLOGY DESCRIPTION

This is a mutli-material gasket construction which contains an inner cytocompatible gasket and outer gas impermeable gasket. It is to be used in cell culture vessels which require a wetted closure in which the closure must also be impermeable to carbon dioxide, oxygen and water vapor.

Sealing cell culture vessels using a closure where all of the wetted materials are compatible with cell culture, the closure is resistant to elevated pressures inside of the vessel and which does not allow carbon dioxide, oxygen or water vapor to leak in either direction across

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OTHER INFORMATION

KEYWORDS

Cell Culture, Hands free cell culture, Sealed Cell Culture, Incubator Free Cell Culture

CATEGORIZED AS

- **Biotechnology**
  - Other
- **Engineering**
  - Engineering
  - Robotics and Automation

RELATED CASES

2023-918-0, 2023-919-0

the closure.

This allows the interior of the cell culture vessel to have different oxygen and carbon dioxide concentrations from the outside. As well as being used for a closure in a cell culture vessel, this system may also be used elsewhere in a fluidic device for cell culture in order to hold a membrane in place in the case where this cannot be achieved using an adhesive or welding method (such as if the membrane needs to be replaceable.)

This device is used to create a gasketed seal between two rigid surfaces compressed together by some mechanism. The invention itself describes the design of the gasketing system

1) The inner gasket, in direct contact with the cell culture media, is made of fluorosilicone or silicone or another cell compatible elastomeric material.

2) The outer gasket, which surrounds the inner gasket and isolates it from the outside environment, is made of butyl rubber or a similar material which is relatively impermeable to oxygen, carbon dioxide and water vapor. This allows the use of an inner cell compatible gasket while also giving the closure the air impermeable properties of the outer gasket.

The cell culture vessel also includes a gas permeable film over the cell culture compartment. One side of the gas permeable film contacts cell culture media inside the cell culture compartment without an air gap in between.

APPLICATIONS

Regulating gases (e.g., oxygen) and pH (via carbon dioxide) without any direct air contact with the cell culture media or air exchange with the outside environment.

The vessels use nonporous, gas permeable membranes to exchange gas with a regulating environment without water evaporation. These vessels are also totally sealed and do not require standard incubator equipment, making them compact and versatile devices for culturing cells, bacteria, etc.

Easier measurement and visualization of cells because analysis and imaging apparatus need not be placed inside the incubator.

ADVANTAGES

1) Closures sealed with gaskets made from cell culture compatible elastomeric materials such as silicone, (plasticized) fluorocarbon polymers such as FEP or plasticized PVDF or PVC are biocompatible but also extremely permeable to oxygen and carbon dioxide, making it difficult to maintain a different gas concentration inside and outside the vessel

2) Closures sealed with gaskets made from gas impermeable materials such as butyl rubber are not permeable to oxygen and carbon dioxide

3) Closures sealed with gaskets or mating surfaces made from slightly soft, biocompatible and gas impermeable materials such as PVDF or polypropylene require high closure forces and very uniform closure forces to make a seal which remains liquid-tight under pressure.

INTELLECTUAL PROPERTY INFORMATION

Country	Type	Number	Dated	Case
Canada	Published Application	WO/2025/024650	01/30/2025	2023-918
European Patent Office	Published Application	WO/2025/024650	01/30/2025	2023-918

Additional Patent Pending

RELATED MATERIALS

- Incubator-Free Organoid Culture in a Sealed Recirculatory System - 09/05/2025

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- Perfusion Of A Semi-Permeable Tissue-Containing Block By Means Of A Pressure Gradient In A Circulatory Bioreactor
- "Incubator-Free" Vessels For Cell Culture Which Do Not Use An Air Intermediate For Gas Regulation
- Fiber Optic Light Source Scheme For Modular Remote Illumination Of Incubator-Based Live-Cell Microscopy

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