

"Incubator-Free" Vessels For Cell Culture Which Do Not Use An Air Intermediate For Gas Regulation

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OTHER INFORMATION

KEYWORDS

cell culture, hands free cell culture,

incubator free cell culture, gas

permeable, gas impermeable, 3-D

cell culture, organoid, pH regulation,

oxygen regulation, reduced

contamination, temperature

regulation, gasket

CATEGORIZED AS

- ▶ **Biotechnology**
- ▶ Other
- ▶ **Engineering**
- ▶ Engineering

RELATED CASES

2023-919-0, 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 as well as in glove boxes. 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 unnatural growth of cells and well-to-well variability 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, and 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. 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.

Another 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

A group within the Braingeneers Group at UC Santa Cruz has designed cell culture vessels that are configured to regulate 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 (including 3-D cell cultures,) bacteria, organoids, etc.

The design includes an air-free cell culture compartment that could be part of a microfluidic chip, a fluidic circuit that perfuses the cells with media - this could be a one-way, single pass flow or a recirculating perfusion of media.

At least one wall of the compartment are made from a nonporous polymer film that is permable to oxygen and carbon dioxide, potentially other gases that is nontoxic to cells. The film is in contact with the culture media such that gas diffusion across the membrane may occur. The other side of the membrane is open to the outside - whether the outside is the room or a mixed gas other than regular air. This film should be relatively impermeable to water vapor. This side of the film might also be in contact with a fluid in which gases are soluble and this fluid is in contact with the outside.

Aside from the one wall, all other walls should be gas-impermeable.

Tech ID 2023-918 describes a dual gasket that seals the vessel. It must be biocompatible and impermeable to carbon dioxide, oxygen, and water vapor in both directions. It can also be used elsewhere in the system. One design is an inner gasket made of a cell compatible

elastomeric material such as fluorsilicone or silicone and an outer gasket that is impermeable to oxygen, carbon dioxide and water, such as butyl rubber.

APPLICATIONS

- ▶ Long term cell culture (weeks/months)
- ▶ Culture of electrically active cells (cardio, neuronal, muscular cells)
- ▶ Culture of cells under high pressure (effective with 3-D cultures)
- ▶ Organoid culture

ADVANTAGES

- ▶ Reduces evaporation relative to current solutions
- ▶ Better regulates dissolved gas concentrations
- ▶ Eliminates the need for an incubator - freeing up overall lab space, allowing use of equipment outside of harsh incubator conditions, and reducing the need for frequent access to the incubator
- ▶ Reduces the chance of microbial contamination - leads to experiments with longer timecourses and reduces cross-contamination

INTELLECTUAL PROPERTY INFORMATION

Country	Type	Number	Dated	Case
Patent Cooperation Treaty	Published Application	WO/2025/024652	01/30/2025	2023-919

Additional Patent Pending

RELATED MATERIALS

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