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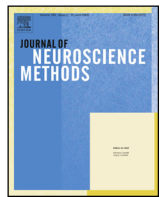

Cell culture chamber with gas supply for prolonged recording of human neuronal cells on microelectrode array

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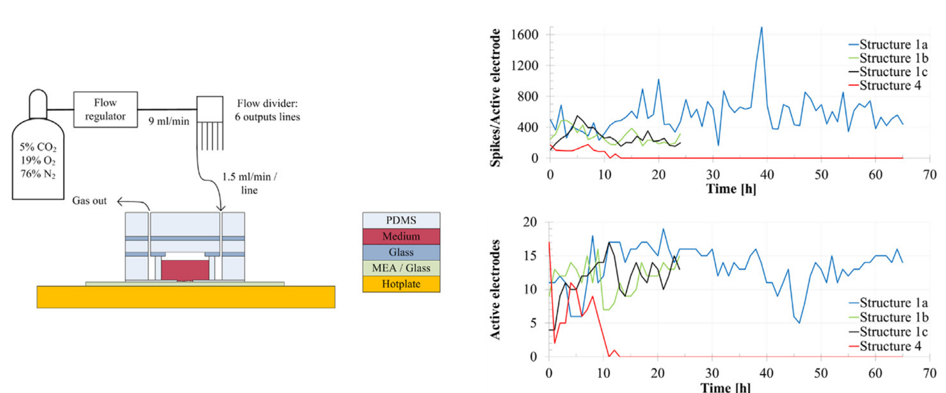
HIGHLIGHTS

- Simple culture chamber for prolonged continuous MEA recordings outside an incubator.
- Human neuronal cells express strong signaling over three days recording.
- Culture chamber maintains stable pH and osmolarity and keeps evaporation low.
- Only a low flow rate of dry gas is applied to keep the cells vital outside an incubator.
- Culture chamber suitable for use inside and outside an incubator.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Typically, live cell analyses are performed outside an incubator in an ambient air, where the lack of sufficient CO_2 supply results in a fast change of pH and the high evaporation causes concentration drifts in the culture medium. That limits the experiment time for tens of minutes. In many applications, e.g. in neurotoxicity studies, a prolonged measurement of extracellular activity is, however, essential.
New method: We demonstrate a simple cell culture chamber that enables stable culture conditions during prolonged extracellular recordings on a microelectrode array (MEA) outside an incubator. The proposed chamber consists of a gas permeable silicone structure that enables gas transfer into the chamber.
Results: We show that the culture chamber supports the growth of the human embryonic stem cell (hESC)-derived neurons both inside and outside an incubator. The structure provides very low evaporation, stable pH and osmolarity, and maintains strong signaling of hESC-derived neuronal networks over three-day MEA experiments.
Comparison with existing methods: Existing systems are typically complex including continuous perfusion of medium or relatively large amount of gas to supply. The proposed chamber requires only a supply of very low flow rate (1.5 ml/min) of non-humidified 5% CO_2 gas. Utilizing dry gas supply makes the proposed chamber simple to use.
Conclusion: Using the proposed culture structure on top of MEA, we can maintain hESC-derived

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neural networks over three days outside an incubator. Technically, the structure requires very low flow rate of dry gas supporting, however, low evaporation and maintaining the pH of the culture.

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1. Introduction

Live cell analyses are typically performed outside an incubator in an ambient air with low CO_2 concentration and low humidity. The lack of sufficient CO_2 supply results in a fast change of pH of the cell culture medium and the high evaporation causes concentration drifts. These facts limit the experiment time to tens of minutes particularly in applications which use bicarbonate buffer in culture medium. In many applications, e.g. in neurotoxicity studies, prolonged measurement of extracellular activity is, however, essential.

Extracellular activity of cells can be recorded using microelectrode arrays (MEA). MEA technology has become an important tool for electrophysiological studies of cells *in vitro* since its invention in early 1970's (Thomas et al., 1972). Since then, the technology has significantly improved and the number of application areas has increased substantially. Still, there is an unmet need to create a stable and controllable environment in MEA systems, particularly for prolonged measurements. That need is especially recognized in the fields of neuronal and cardiac drug screening, toxicity analysis, network learning and detection of chronic responses in cultures (Kehat et al., 2002; Johnstone et al., 2010; Ylä-Outinen et al., 2010). Those studies would benefit long-term (from hours to days) continuous measurement periods to gain detailed information about dynamics of the signaling (Ylä-Outinen et al., 2010; Odawara et al., 2016). Also, the fast development in the stem cell field, especially in the field of human embryonic stem cells (hESC) has broaden up new areas such as studies of human brain development and disease modelling that will surely benefit from prolonged MEA measurements (Narkilahti, 2014; Nat, 2011).

Usually, cells on a MEA-plate are cultured in a humidified incubator with 37 °C and 5% CO_2. However, to record cellular activities, the MEA-plate is placed on a signal amplifier, which is not typically compatible with humid environments. Thus, MEA recordings are performed outside the incubator in ambient air in lower temperature, humidity, and CO_2 concentration, which drastically change the culture conditions. This limits the experiment time typically to tens of minutes.

Changes in the culture conditions affect the cell behavior. For example, evaporation of the culture medium is often significant due to the low ambient humidity. Evaporation increases the osmolarity of the culture medium, and particularly in small volume systems (tens of μl), osmolarity shifts can significantly influence the cell growth as reported by Heo et al. (2007). The evaporation can be reduced by sealing the culture chamber, for example, using a fluorinated ethylene-propylene (FEP) (Potter and Demarse, 2001) or a polydimethylsiloxane (PDMS) (Blau et al., 2009) membrane. These materials have low water vapor permeability but are gas permeable, which is an advantage when supplying CO_2 and other gases through the material. Without additional CO_2 supply, pH of the medium start to increase immediately and pH cannot be maintained in physiological level more than few tens of minutes (Potter and Demarse, 2001; Blau et al., 2009). Therefore, sufficient supply of CO_2 is the most critical parameter for maintaining pH in medium supplemented with sodium bicarbonate, such as DMEM/F12/Neurobasal, a usual medium base for neuronal cultures (Lappalainen et al., 2010; Salimi et al., 2014; Ylä-Outinen et al., 2010). Typically, 5% concentration of CO_2 maintains the pH of the culture medium in at a physiologically relevant level. Sufficient CO_2 supply maintains the pH of a healthy culture between 7.2–7.4, while insufficient CO_2 supply increases the pH of the culture medium to a level harmful for the cells cells (Potter and Demarse, 2001). On the other hand, other media compounds, such as HEPES buffered medium solutions may not require CO_2 to maintain the pH but are reported to be slightly phototoxic (Lepe-Zuniga et al., 1987). Furthermore, HEPES buffered medium may not support the culture of some type of cells.

To overcome challenges to maintain the cultures during long-term MEA recordings, few research groups have developed several types of bioreactors and environment chambers (Mukai et al., 2003; Blau and Ziegler, 2001; Li et al., 2011; Saalfrank et al., 2015; Biffi et al., 2012). In the study of Mukai et al. (2003) a MEA system was implemented inside a humidified incubator with continuous perfusion of medium. However, MEA systems are typically not recommended to be placed inside the humid environment because of a high risk of corrosion. In other studies (Blau and Ziegler, 2001; Li et al., 2011), continuous or periodic perfusion of medium was required to maintain the small volume of culture medium in a closed system. Periodic and/or continuous perfusion was also required in the study of Saalfrank et al. (2015) in which they were able to perform the continuous MEA recordings up to 70 days. However, they maintained the pH of the culture medium in ambient CO_2 using HEPES buffered medium. An environment chamber introduced by Biffi et al. (2012) required the use of a high flow (70 ml/min) rate of humidified gas with high concentration (10%) of CO_2 to maintain the culture inside a large environment chamber. Overall, settings described above include several limitations, such as a requirement for continuous medium perfusion during the long pre-cultivation period that typically takes 3–8 weeks for hESC-derived neuronal cell cultures before actual electrophysiological measurements. Also, increased complexity and incompatibility with electronics because of the humidified gas, and a substantial supply or lack of CO_2 limit the use of these systems.

In this paper, we introduce a compact cell culture chamber for prolonged MEA measurements outside an incubator. The compact structure is placed reversibly on a MEA-plate and it includes a large medium reservoir. Therefore, cells can be cultured in the structure in an incubator prior to actual MEA experiments and normal medium exchange periods can be maintained. During the MEA recordings, the proposed structure requires only a supply of very low flow rate (1.5 ml/min) of non-humidified (dry) 5% CO_2 gas. CO_2 is transferred through a gas permeable but water vapor-tight silicone structure to the culture medium.

The proposed gas supply structure is compared with three other implementations designed for similar use. Computational simulations are utilized to analyze the distribution of CO_2 concentrations. Evaporation, pH, and osmolarity of the culture medium are experimentally evaluated. Finally, the functionality of the proposed structure is demonstrated in a continuous three-day MEA measurement. We show that the proposed structure with a continuous low flow rate of non-humidified CO_2 supply provides a stable cell culture environment for continuous and successful MEA recordings of hESC-derived neuronal networks while retaining physiologically relevant pH and osmolarity levels.

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