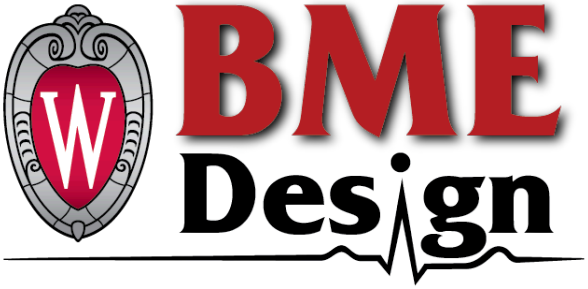

Multi-chamber culture insert for connected neuronal aggregates

Product Design Specification

Client: Dr. Jason Brant

Advisor: Dr. Mehmet Orman

Section: 305

Team:

Greta Mahlke (Co-Team Leader)
Sam Brandt (Co-Team Leader)
Eli Von Holtum (Communicator)
Safia Syed (BSAC)
Sri Krishnan (BPAG)
Sophia Mazer (BWIG)

Date: 09/17/2024

Function

Common devices used in biological research, including well plates and petri dishes, are readily accessible, provide easy platforms for addition of cells, media, and reagents, and allow for observation using standard laboratory microscopes. However, they fail in their ability to model the complex physiological environments of the human body. In recent decades, there has been a large push toward the development of microphysiological systems used for research, for example, the Organ-on-a-Chip platforms [1]. The ability to model complex tissue environments is incredibly important for the study of the auditory pathway because of its dynamic environment. 2D systems completely isolate cells from the complex physical forces, specialized microenvironments, and 3D neural networks that are vital to the transduction of sound to the brain [2]. The multi-chamber culture insert device provides a more dynamic platform to study the growth and interactions of neuronal cell bodies on a 3D platform. The device allows for growth and interaction of neuronal cell aggregates in a structured manner. The device separates a well plate well into distinct chambers each holding a neuronal aggregate. The device allows for growth of neurons between chambers, but separates the media in each chamber, allowing for independent treatments of aggregates.

Client requirements

- The insert must create three to four distinct chambers.
- The insert must keep the media separate between chambers.
- The insert must accommodate extracellular matrix (ECM) filled channels connecting the neuronal aggregates in each chamber.

Design requirements

1. **Physical and Operational Characteristics**
    a. **Performance requirements**
        i. The insert must fit into a standard 12-well plate.
        ii. The insert must create three to four distinct chambers.
        iii. The insert must keep the media separate between chambers with no measurable transfer, while maintaining ample media exposure to individual aggregates.
        iv. The insert must accommodate ECM filled channels connecting the neuronal aggregates in each chamber.
        v. The chambers must be accessible by pipette.
        vi. The insert must allow for post-treatment imaging of cells; the insert must either be transparent or easily disassembled to allow for imaging. Disassembling of insert cannot damage culture structures.

b. **Safety**
    i. The insert itself does not pose any inherent danger to others; however, the most caution should be taken whilst fabricating and loading the insert.
    ii. Proper PPE must be worn while working with cells, reagents, and media.
    iii. ECM must avoid extreme temperatures, direct sunlight, and strong oxidizing agents, as they are incompatible [3].
    iv. Cell culture and ECM must be handled in a biosafety cabinet, as proper ventilation is required [4].
    v. Agarose, a polymer that forms into a gel used for neuronal aggregate channels, is generally non-toxic. Regardless, proper PPE is needed, and caution should be taken when using high heat with it [5].
c. **Accuracy and Reliability**
    i. Neuronal aggregates must be linked together through channels, such as ECM filled agarose columns, but the medium within each chamber must stay separate.
    ii. Neuronal aggregates must not float. They must sit at their column, or no axons enter the lumen.
        1. Out of 8 axons, at least 6 must enter the lumen, growing in both directions from the internal aggregates.
    iii. Aggregates must be within 200 μm of the target through each feed in 90% of chambers.
    iv. At least 90% of constructs must be recovered, have their tract intact, and the chamber from which they originated must be known.
    v. The insert must have evaporation control so as to not lose medium over time, and for successful cell cultures.
    vi. Viability of aggregates must be equal or better than a free-floating control.
d. **Life in Service**
    i. The insert and neuronal aggregates must be able to survive weeks in a sterile environment at 37°C, containing 5% carbon dioxide.
    ii. ECM remains stable for a minimum of three months from its date of shipment, when stored at -70°C [3].
    iii. Agarose gel columns must remain moist, and are stable at room temp.
e. **Shelf Life**
    i. The inserts will be replicated from the master mold, which must remain in a dark and dry environment to slow down degradation of the insert mold [6].
    ii. The insert must last for up to 3-4 weeks in order to fully carry out scientific experiments and maintain continuous cell culture of neuronal aggregates [7].

f. **Operating Environment**
    i. The device must maintain functionality at the standard cell culture conditions, which is approximately 37°C in a humidified 5% CO_2 incubator [7].
    ii. No cytotoxic material must make contact with the neuronal cells being cultured.
g. **Ergonomics**
    i. The insert must fit within a standard stock 12-well plate, allowing pipette access into every chamber of the insert.
    ii. The insert must be accessible in order to allow for cell feeding which can occur daily to every other day.
    iii. The insert should be manually insertable and removable from the fluidic base with standard laboratory tools as required without damaging the device itself and the neuronal aggregates [8].
h. **Size**
    i. The insert must fit inside a well of diameter 22.09 mm [9].
    ii. The insert must have a chamber wall height such that each chamber can contain 80 to 250 μL of media.
    iii. The ECM filled channels must have a lumen diameter between 100 and 300 μm.
    iv. The ECM filled channels must have an outer diameter of 400 μm.
i. **Weight**
    i. The insert must have a weight less than 15 g to minimize compressive loading on neuronal aggregates and channels.
j. **Materials**
    i. The material must not be cytotoxic, with cell viability greater than 70% relative to the control in accordance with ISO 10993-5 compatible in vitro cytotoxicity assay [10].
    ii. The material must withstand steam autoclave sterilization.
    iii. Material within the optical path must be optically transparent at wavelengths required for imaging, or the device must allow for disassembling so that the cells can be imaged.
        1. Optical transmittance should be evaluated in accordance with ASTM E1348-22 [11].
    iv. The material must maintain dimensional and chemical integrity for the entire culture period at 37℃ and 5% CO_2.
k. **Aesthetics, Appearance, and Finish**
    i. The cell culture surface of the insert must be smooth and hydrophilic.

2. **Production Characteristics**
    a. **Quantity**

i. Only one functional master mold will need to be fabricated so that an insert can be cast.
ii. Multiple inserts can be cast to accommodate multiple well-plates during concurrent studies.

b. **Target Product Cost**
i. No specific budget was provided by the client.
ii. Related designs cite polydimethylsiloxane (PDMS), a common material used in cell culture related devices, costs to be under 2-3 US dollars per cast [12] [13]. Costs of the master mold can range from 5-20 USD, depending on material [14].

3. **Miscellaneous**
a. **Standards and Specifications**
i. The insert must adhere to the dimensions listed in Standards ANSI/SLAS 1-2004 through ANSI/SLAS 4-2004 for 12-well microplates, specifically focusing on well depth and area [15].
ii. The device must withstand the mammalian tissue culturing conditions listed above as well as be compatible with device materials. Assay guidelines can be found in ASTM E3231-19 [16].
iii. If PDMS is used, contact-related cytotoxicity must be evaluated using cell viability tests outlined in ISO 10993-5 and ASTM F813-20, specifically sections 12 and 13 [10] [17].
iv. Cell culture will be conducted in a Biosafety Level II laboratory in accordance with the rules and regulations outlined by the Centers for Disease Control and Prevention and the National Institute of Health [18].

b. **Customer**
i. The customer intends for this device to aid in studying different areas of the auditory nerve system and the interactions between auditory nerves.
ii. The fabrication of this product should be easily scaled to accommodate the need for experimental replications.

c. **Patient-related concerns**
i. The device will be a research tool so there are no patient-related concerns.

d. Competition
i. There are multiple microfluidic culture platforms in use in other labs studying neuronal interactions [19].
ii. eNUVIO's OMEGA96-ACE Triple-Chamber Neuronal Co-Culture Microplate allows for the study of *in vitro* co-cultures and neuronal propagation [20].
iii. Patent US11939560B2 describes a microfluidic culture platform that separates fluids between interconnected departments allowing researchers to selectively treat specific sections of the neural network for study [21].

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