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The use of three-dimensional printing to produce *in vitro* slice chambers

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HIGHLIGHTS

- We tested a variety of 3D printed plastics for chambers for patch clamp and cell culture.
- Nylon 618 and Shapeways detail plastics were toxic to cells in culture.
- ABS, PLA, T-Glase and Nylon 680 can be used depending on the length of exposure.

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ABSTRACT

Background: In recent years, 3D printing technology has become inexpensive and simple enough that any lab can own and use one of these printers.
New method: We explored the potential use of 3D printers for quickly and easily producing *in vitro* slice chambers for patch clamp electrophysiology. Slice chambers were produced using five available plastics: ABS, PLA, Nylon 618, Nylon 680, and T-glase. These "lab-made" chambers were also made using stereolithography through a professional printing service (Shapeways). This study measured intrinsic membrane properties of neurons in the brain stem pedunculopontine nucleus (PPN) and layer V pyramidal neurons in retrosplenial cortex.
Results: Nylon 680 and T-glase significantly hyperpolarized PPN neurons. ABS increased input resistance, decreased action potential amplitude, and increased firing frequency in pyramidal cortical neurons. To test long term exposure to each plastic, human neuroblastoma SHSY5Y cell cultures were exposed to each plastic for 1 week. ABS decreased cell counts while Nylon 618 and Shapeways plastics eliminated cells. Primary mouse pituitary cultures were also tested for 24-h exposure. ABS decreased cell counts while Nylon 618 and Shapeways plastics dramatically decreased cell counts.
Comparison to existing methods: Chambers can be quickly and inexpensively printed in the lab. ABS, PLA, Nylon 680, and T-glase plastics would suffice for many experiments instead of commercially produced slice chambers.
Conclusions: While these technologies are still in their infancy, they represent a powerful addition to the lab environment. With careful selection of print material, slice chambers can be quickly and inexpensively manufactured in the lab.

1. Introduction

3D printing as a technology has been around for nearly 30 years, beginning with some of the first attempts by Charles Hull in 1984 (Hull, 1986). In the intervening years, the technology has advanced with many different approaches to producing a physical object from a digital model. Most of these technologies have been controlled by patent restriction. However, these patents are beginning to expire, leading to a new wave of open source 3D printing technologies available to the casual user. This allows basic plastic parts to be quickly produced in the lab for a fraction of the cost normally associated with laboratory supplies. The purpose of this study was to provide a basic introduction to the rapidly advancing field of 3D printing and its potential application to slice electrophysiology.

3D printing is a form of additive manufacture. The physical object is built up from successive layers of material (plastic, metal,

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etc.). This is opposed to more traditional subtractive manufacturing techniques where material is removed from a block of starting material through cutting, shaping, and grinding. A number of technologies exist for 3D manufacture. Some examples include fused deposition modeling (FDM), selective laser sintering (SLS), and stereolithography. Each method has its own advantages and disadvantages. Currently, only FDM and stereolithography are readily available to the average consumer.

Fused deposition modeling (FDM) is the most basic additive technique. Molten material is extruded through a nozzle head onto a printing bed where it immediately hardens, essentially acting as a hot glue gun. The object is then built up layer by layer to its final form. This is the most common deposition method seen in consumer and do-it-yourself 3D printers. Typically, a thermoplastic such as polylactic acid (PLA) or acrylonitrile butadiene styrene (ABS) is used as the extrusion material. It is fed into the extrusion head as either 1.75 or 3 mm diameter plastic filament from a spool. The extrusion material is forced through a nozzle orifice between 200 and 500 μm in diameter and deposited onto the printing platform. One of the major shortcomings of this process is that the printer cannot print extreme overhangs without some variety of support material. Various methods have been devised to solve this problem, but they are beyond the scope of this study. This study will mainly focus on FDM printing.

Other methods of 3D printing include SLS and stereolithography. SLS involves depositing thin layers of plastic, metal, ceramic, or glass powder on the printing surface. A high power laser then selectively fuses the powder to draw a layer of the printed object. A new layer of powder is deposited followed by laser sintering. These steps alternate until the completed object can be removed from the surrounding unsintered powder. Because the surrounding powder provides a support structure, highly complex objects can be printed using SLS, although, this technology is still mainly confined to the realm of industrial manufacturing. Stereolithography uses selective exposure of a light curable photopolymer resin. Each layer of the printed object is produced by selectively exposing areas of the resin to a scanning laser or a standard computer projector. Consumer versions of this type of printer have only become available in the last two years. One example is the Form 1 printer (Form Labs, Somerville, MA). The advantage of that printing method is that it is one of the most precise printing methods with layers as thin as 25 μm and features as small as 300 μm. The main disadvantage is that the photopolymers are generally toxic and the printed object size is limited due to small build volumes.

We explored 3D printing due to the costs associated with maintaining electrophysiology components such as slice chambers. Some reagents contaminate chambers and can be particularly difficult to remove, e.g. leptin. Thus, we searched for ways to easily and inexpensively make disposable chambers. Components could be made in house using available machining methods, but due to lead time and cost issues we decided to explore consumer 3D printing methods. In order to test the compatibility of 3D printed objects with live cell experiments, we conducted a number of patch clamp and cell culture experiments in the presence of 3D printed thermopolymer plastics and professionally printed photopolymers.

We examined intrinsic membrane and firing properties in neurons of the mesopontine pedunculopontine nucleus (PPN) and pyramidal neurons in retrosplenial cortical layer V. The PPN is mainly involved in waking and paradoxical sleep (Steriade and McCarley, 1990). As part of the reticular activating system (RAS), the PPN modulates ascending projections to the thalamus as well as descending projections through the pons and medulla. The nucleus is composed of populations of glutamatergic, cholinergic, and GABAergic neurons (Wang and Morales, 2009). Retrosplenial cortex is generally associated with memory and projects to the hippocampal formation (Wyss and Van Groen, 1992).

2. Methods

2.1. 3D printing

All parts were printed on a Revolution XL printer (QU-BD, Little Rock, AR) equipped with a heated printing bed at 90 °C. The extruder was equipped with a 400 μm nozzle using 1.75 mm plastic filament. Exact printing temperatures can be found in the supplementary materials. The printing bed is made of 3/8 in. thick basalt (a volcanic glass) to prevent sagging or warping when heated. Natural color free ABS (acrylonitrile butadiene styrene) and PLA (polylactic acid) plastic filament was from ProtoParadigm (Hermiston, OR). Nylon 618, 680, and T-glase (polyethylene terephthalate polymer) were provided by Taulman3D (St. Louis, MO). Chambers were also printed using the online Shapeways (New York, NY) printing service using the transparent detail plastic, which is an acrylic photopolymer. This plastic was selected due to its being watertight. The control chamber was a RC-26 polycarbonate chamber from Warner Instruments (Hamden, CT).

All parts were designed using Inventor 2014 software (Autodesk, San Rafael, CA). Slice chambers for patch clamp experiments were designed to fit the P-1 Series 20 chamber platform from Warner Instruments. The chamber uses a coverslip bottom to allow for transmitted IR illumination while recording from cells (Fig. 1A). Sample disks were printed from each available media. Each disk was 10 mm wide by 2 mm tall. Each printed object had an individual layer height of 0.1 mm. Files were exported from inventor in the STL format and were imported into the open source G-code generator, Slic3r (Ranellucci, 2014). G-code from Slic3r was then interpreted by the printer control software, Repetier-Host (Hot-World GmbH & Co. KG, Willich, Germany). The control software interfaced with the Azteeg X3 controller on the printer using repetier firmware. A thin coat of ABS 'glue' was applied to the printing bed to ABS, PLA, Nylon 618, and Nylon 680 for better filament adhesion. The glue was later removed with acetone. The glue was made by dissolving ABS filament in acetone until it reached a thick consistency. T-glase adhered to the printing bed without assistance. Small objects (less than a 1 cm × 1 cm footprint) printed in Nylon tended to detach from the platform even with ABS glue, so that the G-code settings in Slic3r were altered to include a 5 mm skirt on the first printing layer to increase the object's surface area. The skirt was later removed with a scalpel prior to use. We should note that 3D printing community has devised other bed options for working with difficult plastics such as Nylon. These include using wood printing beds or covering a glass/metal bed with standard blue painters tape. However, we found the addition of a skirt in conjunction with ABS glue was sufficient for working with nylon on our printer.

2.2. Electrophysiology

2.2.1. Slice preparation

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences and were in agreement with the National Institutes of Health guidelines for the care and use of laboratory animals. Rat pups aged 8–16 days were taken from adult timed-pregnant Sprague-Dawley dams. Pups were anesthetized with ketamine (70 mg/kg, i.m.) until the tail pinch reflex was absent. Pups were decapitated and the brain was rapidly removed and cooled in oxygenated sucrose-artificial cerebrospinal fluid (sucrose-aCSF). The sucrose-aCSF consisted of (in mM): sucrose, 233.7; $NaHCO_3$, 26; KCl, 3; $MgCl_2$, 8; $CaCl_2$, 0.5; glucose, 20; ascorbic acid, 0.4; and sodium pyruvate, 2. Sagittal sections (400 μm) containing the PPN nucleus were cut under cooled oxygenated sucrose-aCSF with a Leica VT1200 vibratome (Leica Biosystems, Buffalo Grove, IL) with a Huber mini-chiller (Huber, Offenburg, Germany), and allowed to