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An aerator for brain slice experiments in individual cell culture plate wells

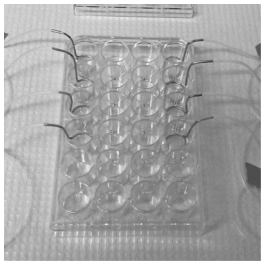
David M. Dorris[a], Caitlin A. Hauser[a], Caitlin E. Minnehan[a], John Meitzen[a,b,*]

[a] Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA
[b] W.M. Keck Center for Behavioral Biology, Center for Human Health and the Environment, and Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC, USA

HIGHLIGHTS

- We have developed a new aerator designed to fit into a single well of a standard 24-well cell culture plate.
- The aerator keeps brain slices viable and stationary, and is inexpensive to produce.
- The aerator enables individual manipulation of living acute brain slices or potentially other tissues in low solution volumes.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Ex vivo acute living brain slices are a broadly employed and powerful experimental preparation. Most new technology regarding this tissue has involved the chamber used when performing electrophysiological experiments. Alternatively we instead focus on the creation of a simple, versatile aerator designed to allow maintenance and manipulation of acute brain slices and potentially other tissue in a multi-well cell culture plate.
New method: Here we present an easily manufactured aerator designed to fit into a 24-well cell culture plate. It features a nylon mesh and a single microhole to enable gas delivery without compromising tissue stability. The aerator is designed to be individually controlled, allowing both high throughput and single well experiments.
Results: The aerator was validated by testing material leach, dissolved oxygen delivery, brain slice viability and neuronal electrophysiology. Example experiments are also presented, including a test of whether β1-adrenergic receptor activation regulates gene expression in *ex vivo* dorsal striatum using qPCR.
Comparison with existing methods: Key differences include enhanced control over gas delivery to individual wells containing brain slices, decreased necessary volume, a sample restraint to reduce movement artifacts, the potential to be sterilized, the avoidance of materials that absorb water and small biological molecules, minimal production costs, and increased experimental throughput.
Conclusion: This new aerator is of high utility and will be useful for experiments involving brain slices and other potentially tissue samples in 24-well cell culture plates.

* Corresponding author at: Dept. of Biological Sciences, NC State University, 144 David Clark Labs, Campus Box 7617, Raleigh, NC 27695-7617, USA. Tel.: +1 919 515 4496; fax: +1 919 515 5327.
E-mail addresses: jemeitze@ncsu.edu, jmeitzen@gmail.com (J. Meitzen).

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1. Introduction

Ex vivo acute living brain slices are a broadly employed and powerful experimental approach (Collingridge, 1995; Khurana and Li, 2013; Li and McIlwain, 1957; McIlwain et al., 1951; Yamamoto and McIlwain, 1966). Though most prominently associated with electrophysiological recordings, *ex vivo* brain slices have been used to test hypotheses across the entire continuum of neuroscience, using genetic, molecular, tissue culture, pharmacological, immunocytochemical, anatomical and many other approaches.

Brain slices are prepared by rapidly extracting the brain, exposing it to either warm or cold oxygenated artificial cerebrospinal fluid (ACSF), and sectioning the brain slices using a vibratome (Colbert, 2006; Huang and Uusisaari, 2013; Madison and Edson, 2001). Oxygenation is typically provided *via* carbogen gas (95% O_2/5% CO_2). After creation, brain slices are then usually maintained and then recorded from in one of two basic chamber types. The first chamber type submerges the tissue in relatively large volumes of oxygenated ACSF (Blake et al., 2007; Buskila et al., 2014; Croning and Haddad, 1998; Fujii and Toita, 1991; Hajos and Mody, 2009; Koerner and Cotman, 1983; Moyer and Brown, 1998; Nicoll and Alger, 1981; Sakmann et al., 1989; Thomas et al., 2013; Tominaga et al., 2000; White et al., 1978). The second is interface- or membrane-style chamber that places the tissue at the convergence/interface between oxygenated ACSF and humidified O_2/CO_2 (Dingledine et al., 1980; Haas et al., 1979; Hajos et al., 2009; Hill and Greenfield, 2011; Knowles, 1985; Li and McIlwain, 1957; Matthies et al., 1997; Tcheng and Gillette, 1996). The design and creation of brain slice chambers has been the subject of intense effort, especially regarding microfluidics (Ahrar et al., 2013; Huang et al., 2012; Mohammed et al., 2008; Scott et al., 2013; Thomas et al., 2013), and oxygen delivery (Blake et al., 2007; Choi et al., 2007; Hill and Greenfield, 2011; Oppegard et al., 2009; Rambani et al., 2009).

Less attention has been directed toward designing chambers for *ex vivo* manipulation of individual brain slices for the purposes other than electrophysiology, and the maintenance and storage of brain slices, especially in low volumes of ACSF. Here we aim to fill this gap by presenting a new aerator designed to allow easy manipulation and maintenance of brain slices and potentially other tissues in low volumes in multi-well cell culture plates. Multi-well cell culture plates, and in particular 24-well plates were chosen given their versatility and prevalence.

We first describe in detail the design and operation of the aerator. We then validate the device using inductively coupled plasma optical emission spectrometry (ICP-OES) to test for material leach, dissolved oxygen measurements to test for gas delivery, infrared-differential interference contrast (IR-DIC) microscopy and tetrazolium chloride (TTC) staining to test brain slice viability, and electrophysiology to test neuronal physiology. We then present example applications, including immunocytochemistry and a test of whether β1-adrenergic receptor activation in *ex vivo* dorsal striatum regulates gene expression using real-time polymerase chain reaction (qPCR). We conclude that new aerator is of high utility and will be useful for experiments involving brain slices and other potentially other tissue samples in 24-well cell culture plates.

2. Materials and methods

2.1. Aerator design

The aerator is constructed from 18 ga stainless steel tubing (Part no. HTX-18R-30, 304 SS Hypo Tube 18 ga. Regular Wall, Component Supply Company, Florida, http://www.componentsupplycompany.com/), with a nylon mesh (Cat no. 64-0198, Warner Instruments, Connecticut, https://www.warneronline.com/) used to restrain brain slices (or potentially other tissues) (Fig. 1A). At the bottom is a 15 mm inner diameter loop with a single 0.343 mm hole in the top of the loop, opposite where the end of the tube meets a 90 degree turn in the tube. The open end is crimped. The shaft runs approximately 20 mm to where it meets another 90 degree bend. After this bend, the shaft runs approximately 10 mm where it remains open for gas line attachment. While the bottom loop can be manufactured with or without a nylon mesh, we find that the mesh is useful for restraining samples at the bottom of the well. The mesh is currently constructed of nylon because of its relative inertness and long use with brain slices. However, the mesh could potential be constructed of other materials such as polyester or polypropylene.

Once constructed, the bubbler is attached to a gas line controlled by a manifold and placed in a culture well (Fig. 1B and C). 2 mL of solution in the well was found to be optimal and was used in all the experiments reported here, however, the well could also hold 1 or 3 mL of solution. 2 mL was chosen for the working volume as increased cavitation was observed with ≤1 mL volume, and 3 mL increased the risk of spillover and aerosol contamination of other wells. Once in solution the gas (in our case, 95% O_2/5% CO_2) was turned on using the manifold. We found that the microhole in the bottom loop of the aerator provided adequate oxygenation with minimal sample disruption, and thus we did not place a tubing crimp to regulate gas flow. Additionally, when multiple aerators are in use (Fig. 1D), gas exposure was more rigorously controlled by using a common manifold than individual crimps.

We find that this basic design met our requirements for the aerator. First, the aerator needed to fit snugly into the well of a 24 well cell culture plate. This was achieved *via* the 15 mm diameter rounded bottom. Second, the brain slice must be kept stationary. This was achieved *via* the nylon mesh affixed to the top of the rounded bottom, and the single microhole used for gas delivery. Third, the aerator needed to be individually controlled in exclusion to all other wells. This criterion was met by creating an aerator with individually-controlled operation. Fourth, we wanted the aerator to be made out of a material that can potential be sterilized, that does not absorb water or small molecules, and with minimal release of endocrine disruptors. We thus considered three broad materials: plastic, glass, and finally stainless steel. Plastic was our initial choice, due to the relative ease of manufacture and the potential possibility of construction using 3-D printers. However, many of the plastics employed for 3-D printer construction are toxic to neurons (Hyde et al., 2014). Our primary consideration was polydimethlysiloxane (PDMS), which has been employed in other multi-well culture plate aerators and brain slice chambers (Blake et al., 2007; Oppegard et al., 2009, 2010). PDMS has many advantages for aeration, however, PDMS is known to absorb water and small biological molecules (Heo et al., 2007; Mukhopadhyay, 2007; Randall and Doyle, 2005; Roman et al., 2005). This makes it unsuitable for many experiments involving living tissue, and especially brain slices. These considerations, along with the potential release of additional endocrine disruptors (Gore and Patisaul, 2010) led us to consider other materials, although we note that the presented schematic could still potentially be used with 3-D printers. The second material we evaluated was glass. However, we quickly ran into severe challenges with manufacturing. That, along with the possibility of leached materials (Doremus, 1994; Kay, 2004; Nunamaker et al., 2013), led us to our current material, stainless steel. We found stainless steel to be advantageous in that it was relatively easy to manipulate, was already commonly used in many biomedical applications, was relatively inexpensive and was able to be sterilized. One potential drawback to stainless steel is like glass and some plastics is the potential for the leaching of heavy metals in solution. We directly tested this using ICP-OES, and aerators actively employed in the laboratory between 6 and 12 months.