**Affordable Diffusion Microchamber Array Designs for Isolating Microbes in Classrooms and Laboratories**

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**INTRODUCTION**

Small innovations in instrumentation often have larger-than-expected impacts on both experimental research and science education, and one example of this interesting tendency, worth communicating to students of microbiology today, is a diffusion microchamber array (DMA) device, the first of which was named the ichip (1). For over a century, the plating method using petri dishes (2) has been a workhorse in microbiology, but it has a key limitation with regard to the number of taxa it can feasibly cultivate (3). This limitation motivated the research group of Slava Epstein to design the ichip, which enables the free exchange of environmental solutions (e.g., soils, sediments, rivers, lakes, etc.) with microbial cells across a semipermeable membrane. The diffusion-based design simultaneously prevents the contamination of the cultures in the membrane-bound microchambers while trapping the often-motile cells inside. In other words, a DMA device enables investigators to “contact-culture” isolated cells on or between membranes by creating contained microhabitats while controlling the conditions and inoculum of those microhabitats, whereby the environmental solution flows freely to nourish target cells. As such, classrooms and laboratories offering activities using DMA devices more closely align their learning outcomes with items 13, 20, 21, 22, 27, and 33 of the American Society for Microbiology (ASM) 2012 Recommended Curriculum Guidelines for Undergraduate Microbiology Education (4).

The history and current development of DMA devices to improve the functionality of such devices are additional recommended learning outcomes of the following activity (see Appendix 1 in the supplemental material). Although highly innovative, the design of the original ichip restricts its implementation in a classroom context. These restrictions include the following.

1. The high density of plugs as they are arrayed on the ichip and the small size of the microchambers on or in which microbes grow make the design generally unwieldy.
2. The size and shape are poorly interoperable with common labware and tools, such as multichannel pipettes and well plates.
3. During the assembly and disassembly of these small, dense DMA devices, there is a high risk of cross-contamination.
4. The ichip’s complex and non-open-source manufacture specifications naturally prevent both their affordability and customization.

We propose here key changes to the design of the ichip DMA device, and we call the resulting suite of devices “iplates,” a portmanteau of “isolation” and “plate,” because iplates mimic the size and shape of standard 96-well plates (Fig. 1A) and have the same capabilities for isolation as ichips (Fig. 1B). Iplates have key improvements to the original ichip for use in the classroom and the microbiology laboratory. Iplates have fewer microchambers than the ichip but are interoperable with common microbiological labware because they align flush with 96-well plates, enabling more rapid transfers when situated directly over multiwell plates while using 96-pin replicators. The wider spacing of microchambers lowers the risks of cross-contamination between microchambers during assembly and disassembly, and the larger microchambers are more easily viewable under a microscope and provide sufficient surface area and volume into and across which isolates may freely grow. Additionally, the open-source design and inexpensive materials allow affordable manufacture and customization, which will reveal the fullest capabilities of DMA devices by way of investigators’ imaginative combinations of media, microbes,
and protocols. The suite of iplates does not reinvent the technology in use (5) but expands upon it, and Appendix 2 offers a recommended notation for documenting, communicating, and subsequently replicating novel experimental designs therein.

**PROCEDURE**

**Manufacture**

The primary materials needed for iplate manufacture are Delrin (1/8-in. acetal homopolymer; Ensinger-Hyde, Pennsylvania), firm silicone (1/8-in. red/orange commercial-grade 60A), a membrane (dialysis tubing, ≤14-kDa cutoff, such as product no. D9402 from Sigma-Aldrich), and stainless steel bolts and screws. Delrin has been used in biological laboratories by us, Robin Sen (personal communication), and others because of the material’s resilience to autoclaving or chemical sterilization, low moisture absorption, and very low toxicity to cells. Hard silicone is similarly appropriate for use in a DMA device, and the bolts and screws create a strong contact with Delrin or steel that is virtually air-tight. The Delrin sheets are cut with a laser cutter using a single template file for all plates (Fig. S4). Silicone sheets will ignite under laser cutting and therefore require cutting and punching by hand. A complete description of the specifications of manufacture and requisite components can be found in Appendix 3.

**Incubation**

The Delrin and silicone plates are interleaved with membranes to form a single membrane-bound microchamber or, alternatively, a “layer cake” of several plates that creates an array of stacks whose microchambers are connected by the vertically permeable membranes. The microchambers are composed of 5% agar and minimal medium, pipetted when molten into the through-holes of Delrin or silicone plates during assembly. Amending the gel with C sources and N sources is optional, because such nutrients are ostensibly provided by the soil solution or other environmental media in which the iplate is incubated. It is recommended, however, to saturate membranes in a liquid medium such as yeast extract or sterilized extracts from the target environment and allowed to dry, because pure cellulose unsaturated with such nutrients will tend to “mop up” growth factors intended to nourish target cells. A complete description of the protocols for inoculation and incubation of iplates can be found in Appendix 4.
SAFETY ISSUES

An instructor or technician should supervise all activities of students from manufacture to incubation using DMA devices. The operation of a laser cutter requires personal protective equipment and ventilation and the heating of plastics and metals, with minor risks of burns and scrapes when finished parts are handled. However, owing to their commonplace usage, these machines are operated in designated workspaces with appropriate safety procedures. Additionally, the sterilization of device parts may expose students and investigators to caustic chemicals and environmental samples (freshwater, soils, etc.), but these chemicals and samples pose no elevated risk when classes or research is conducted at properly equipped and certified laboratories. Finally, as in any laboratory setting where students are working with isolates of unknown identity, standard safety protocols for working with unknown environmental microbes should be followed.

CONCLUSION

Researchers in the field of microbiology today have more tools than ever before to create realistic in situ microhabitats to grow, isolate, and analyze microorganisms of interest, but DMA devices are not completely available to students and instructors. The manufacture and design of iplates are activities proposed to expand the use of DMA devices and to further improve education and discovery in microbiology, such as Jo Handelsman’s Small World Initiative (6). The resulting suite of iplates will enable students and instructors to integrate DMA devices into the undergraduate and graduate microbiology curriculum.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.9 MB.

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