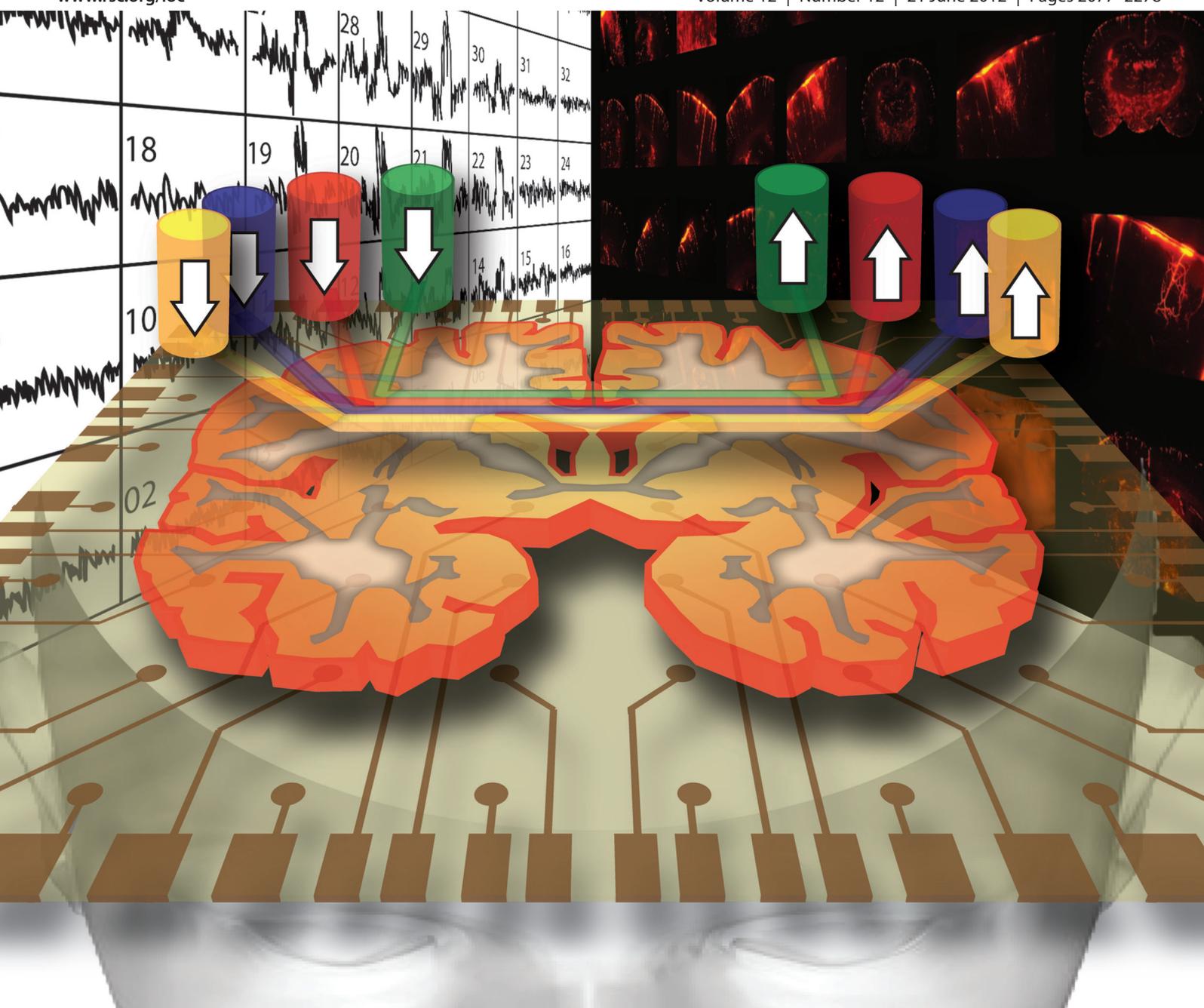


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Brain slice on a chip: opportunities and challenges of applying microfluidic technology to intact tissues†

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Isolated brain tissue, especially brain slices, are valuable experimental tools for studying neuronal function at the network, cellular, synaptic, and single channel levels. Neuroscientists have refined the methods for preserving brain slice viability and function and converged on principles that strongly resemble the approach taken by engineers in developing microfluidic devices. With respect to brain slices, microfluidic technology may 1) overcome the traditional limitations of conventional interface and submerged slice chambers and improve oxygen/nutrient penetration into slices, 2) provide better spatiotemporal control over solution flow/drug delivery to specific slice regions, and 3) permit successful integration with modern optical and electrophysiological techniques. In this review, we highlight the unique advantages of microfluidic devices for *in vitro* brain slice research, describe recent advances in the integration of microfluidic devices with optical and electrophysiological instrumentation, and discuss clinical applications of microfluidic technology as applied to brain slices and other non-neuronal tissues. We hope that this review will serve as an interdisciplinary guide for both neuroscientists studying brain tissue *in vitro* and engineers as they further develop microfluidic chamber technology for neuroscience research.

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Introduction

Isolating brain tissue specimens under *in vitro* conditions is a powerful experimental approach that allows neuroscientists to have exquisite control over experimental conditions and access to neural networks, individual cells, processes, and synapses. However, this reductionist approach isolates the neural tissue from its normal delivery of nutrients, endocrine and trophic



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factors, synaptic inputs, regulatory molecules, and dissolved gasses, and establishes unwanted gradients in the tissues due to diffusion limitations.

Since the development of the first brain tissue preparation (thin slice or large block), neuroscientists have refined their *in vitro* methods to improve tissue viability, better mimic *in vivo* conditions, and develop novel experimental approaches. Microfluidics and brain slice preparations have always been a good experimental match. Although neuroscientists haven't always used microfluidics jargon, the tools they have developed for keeping brain slices viable have (perhaps unknowingly) utilized microfluidic principles. The use of laminar flow has been particularly exploited in historical brain slice chambers as it allows access to the top of the slice with electrophysiology instruments while maintaining a constant perfusion over the top and sides of the slice. This is achieved by carefully balancing the input and output flow rates across a miniature open-top chamber, thereby producing a thin fluid stream that results in laminar flow due to its small cross section and high velocity.

The time is ripe for a dialogue between neuroscientists and biomedical engineers because both groups are working towards the same goal from different perspectives. The application of microfluidic techniques and principles to isolated brain tissue is an important area of common ground where opportunities exist to address scientific and experimental problems. The goal of this review is to identify the challenges and opportunities that can be addressed by microfluidic technology and increase awareness in the neuroscience community with respect to available biomedical engineering tools and techniques. The review culminates with highlights of technical approaches that have started the push towards clinically relevant applications, including the use of other non-neuronal tissue models.

Experimental challenges associated with *in vitro* brain slice experiments

For the past half century, *in vitro* brain slice studies have added great insights into the understanding of the function of the central nervous system.^{1,2} In the 1960's, resting membrane potentials were first recorded from cortical slices *in vitro* by McIlwain and colleagues,^{3–5} and synaptic long-term potentiation was observed in hippocampal slices *in vitro*.⁶ Unlike *in vivo* brain studies that require live animals to be anesthetized and

maintained, thin brain slice preparations are advantageous because they preserve local network and anatomical features, allow access for microscopic and electrophysiological approaches, allow for alterations in solutions bathing the slices, and are free from non-specific influences due to anesthetics. Over the years, the evolution of the brain slice technique has been noted and debated with respect to recording chamber size, buffer flow rate and composition, slicing methods, temperature, animal pretreatment, and other features.^{2,7,8} More recently, there is a strong motivation to adapt the basic slice chamber technique to accommodate new technologies and experimental approaches (e.g., microfluidics, imaging, multichannel recording). Although these chambers are derived from different technical bases and theoretical concepts, their motivation to improve brain slice viability and function centers around several basic principles. There are three major problems that conventional brain slice chambers attempt to address, with varying degrees of success: (1) efficient delivery of oxygen and nutrients, and waste removal; (2) fine control over local neurochemical environments; (3) access to modern microscopic and electrophysiological techniques (Fig. 1). Each of these problems will be outlined in the following sections:

Efficient oxygen and nutrient delivery, and waste product removal

The *in vivo* brain is one of the most highly vascularized structures in the body. Each neuron in the neurovascular unit is no more than one cell away from the nearest blood vessel⁹ and blood flow is highly regulated to match oxygen supply to the high metabolic demand of active neuronal networks.¹⁰ For example, highly active brain areas such as the visual cortex are 'on' continuously throughout the waking hours, and overall the brain uses a disproportionate amount of the calories that are consumed each day. The brain cannot maintain proper function without adequate oxygen for even a few minutes.¹¹ These characteristics illustrate the major obstacle in keeping *ex vivo* brain tissue viable: maintaining proper oxygen and nutrient levels without the aid of the intrinsic vascular system. Once brain tissue is removed, the endogenous delivery and regulatory mechanisms are completely disrupted, which produces unwanted consequences.

For example, one of the fundamental limitations of *in vitro* brain tissue experiments is tissue or slice thickness. Since oxygen delivery is exclusively due to diffusion, there will fundamentally always be very large oxygen concentration gradients throughout the slice^{12–14} or more intact isolated tissue.¹⁵ Early studies showed that solution flow above and below the slice is optimal for delivery of oxygen, nutrients, and drugs throughout the slice, and that there was a 300–400 μm limit on slice thickness.¹⁶ Oxygen gradients are considered "acceptable", as long as the oxygen partial pressure (PO_2) at the center of the tissue is sufficient to meet metabolic demand. This is a serious flaw because neuronal function is highly dependent on tissue PO_2 .^{17–19}

To increase oxygen penetration and increase tissue PO_2 , methods are used to increase PO_2 at the brain tissue surface. For example, hyperoxygenated solutions are routinely used despite the fact that hyperoxygenation alters neuronal physiology and induces acute cell death.^{14,20} Alternatively, brain slices can be maintained in a suffused chamber with solution flowing



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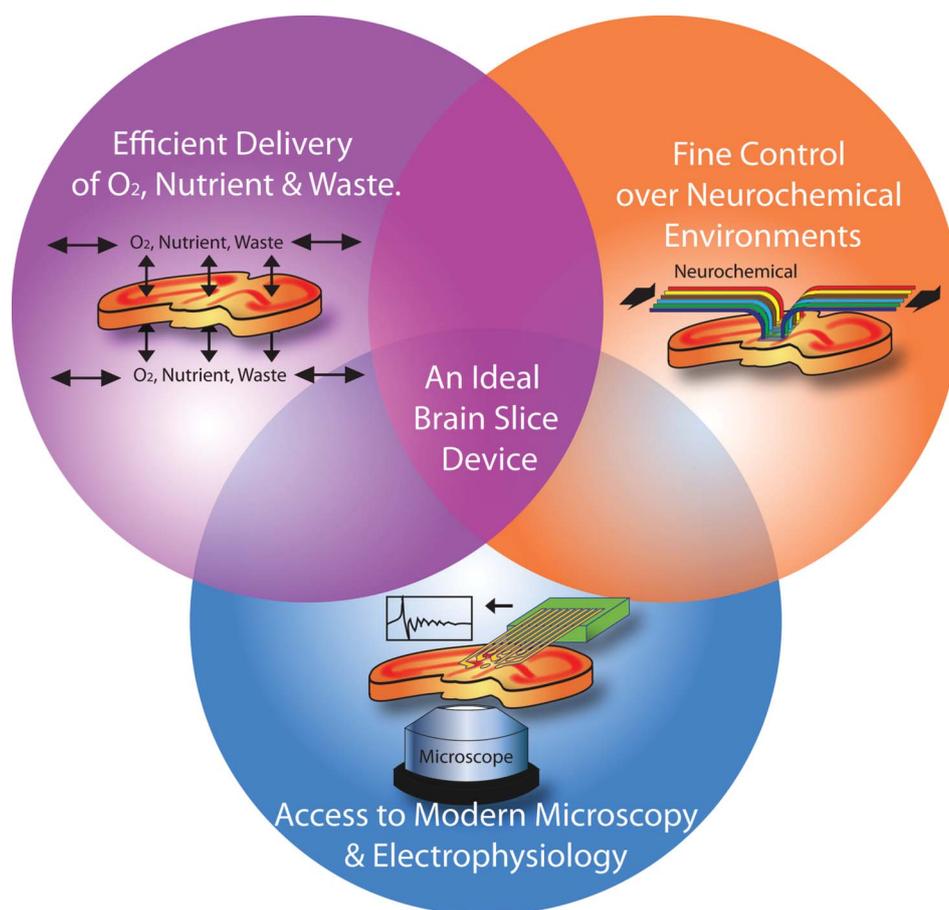


Fig. 1 Venn diagram showing three major goals of *in vitro* brain slice chambers. The goals are to provide efficient nutrient/oxygen delivery and metabolic waste removal (purple circle, upper left), permit high resolution spatiotemporal control over local neurochemical environment (tan circle, upper right), and *in situ* access to high-resolution microscopes and microelectrodes (blue circle, lower middle). Individual and combined goals have motivated the creation of novel microfluidic devices. An ideal device for brain slice studies will accomplish all three goals (center overlap area).

underneath the slice and the top of the slice exposed to vapor-saturated oxygen-rich atmosphere (referred to as “Haas” or “interface” chambers). This configuration allows one slice surface to be exposed directly to highly oxygenated air while still remaining hydrated. Under these conditions, brain slices retain physiological and morphological characteristics better than submerged brain slices.^{21,22} Emerging progress in conventional and microfluidic brain slice chambers will be extensively reviewed in later sections.

Another strategy for maintaining sufficient tissue oxygenation is to decrease metabolic demand by simply lowering the temperature of the preparation. While viability is improved, the cost is that overall neural function is likely altered substantially since channel kinetics is highly temperature-dependent. Alternatively, differences in tissue metabolic rate and susceptibility to low oxygen levels can also be exploited. In particular, fetal or postnatal brain slices *in vitro* have a lower metabolic rate, higher tissue PO_2 levels, and higher resistance to hypoxic conditions compared to adult brain slices.¹³

Fine control over neurochemical environment

Neural networks in the brain are highly organized into different modules that are domain specific in function, such as speech and

vision.²³ To understand the corresponding neuronal activity, it is of great interest to apply localized delivery to a specific region on the slice. This approach is especially valuable for assessing neural phenomena or diseases that vary spatiotemporally. For instance, seizures and cortical spreading depression (CSD) originate and spread from a specific focus. Spatiotemporal information of such phenomena can be better interpreted, when chemical stimuli are focally perfused and the information passed to surrounding neurons only *via* network signaling.²⁴ However, such a feature is lacking in conventional slice chambers, where a large portion or even the entire slice is bathed in a uniform environment. In many *in vitro* brain slice experiments, investigators wish to apply drugs to slices or change solutions within slices with high spatiotemporal resolution. Therefore, it would be of great experimental significance to achieve a fine control over the neurochemical environment of brain slices.

Conventional bath application methods involve changing the solution bathing the entire brain slice. This experimental approach is easy and the entire slice can be equilibrated to the same concentration of the chemical. However, bath application is relatively slow and doesn't allow for precise application to specific regions of the slice. On the other hand, several methods are available to apply chemicals or solutions to more limited regions of the slice: pressure or iontophoretic ejection from a

single- or multibarrel glass micropipettes, parallel-flow pipe systems and U-tube applicators, photolysis of neurotransmitter precursors or chemically caged neurotransmitters, *etc.*²⁵ Limitations of chemical ejection methods include loss of control once the chemical is ejected, irregular or unknown diffusion/metabolism within the interstitial space, poor temporal resolution, leakage of chemical when no pressure or current is applied, tip clogging, and mechanical damage when devices are inserted into slices.²⁶ Methods for photolysis of caged compounds require the use of relatively expensive equipment and materials, and the activation field is usually limited to being a single uniform circular spot within the tissue.²⁷ Microfluidic devices can potentially address many of these limitations, as we extensively review in the next sections. For instance, laminar solution flow around slices can be carefully controlled with fluid stream widths less than 100 μm ,²⁸ different solutions can be rapidly changed within a fluid stream or several streams emitted in a pulsatile manner.²⁹ In addition, microfluidic chambers are relatively inexpensive to produce and can be modified to accommodate large regions of brain slices (*e.g.*, slice hemisections³⁰).

Access to modern microscopy and electrophysiology techniques

For many *in vivo* studies, the neuronal structures of interest are located deep within the brain with limited access for visualization, stimulation, and recording techniques. In contrast, *in vitro* brain slices lie flat in a chamber with neural circuits and neuroanatomical features exposed on the slice surface. By allowing high magnification objectives to be placed close to the slice surface, this configuration enables the application of high resolution light, fluorescent, confocal, multiphoton, and infrared imaging techniques. Many brain slice chambers are modified to allow a shallow approach angle for placement of intra- and extracellular recording electrodes. Finally, the flat cut surface of brain slices allows slices to be placed on two-dimensional multielectrode arrays (MEA).³¹ The development of interface amenable MEAs for brain slices is accelerating their use in various applications,³² as will be discussed later in this review.

As can be seen from these three fundamental challenges of studying brain slices *in vitro*, there are a number of advantages that microfluidics and other microtechnologies could offer. Before we go into the application of microfluidics towards solving these problems, we will review the traditional approaches that neuroscientists have developed, to help gain a better historical perspective on this field. It should be quickly appreciated that neuroscientists have frequently been drawn to microfluidic principles throughout the development of brain slice culture methods.

Conventional brain slice chambers

By the late 1980s, relatively standardized brain slice chambers had been developed to house or culture brain slices. These chambers are typically bulky in size and cause hyperoxygenated artificial cerebrospinal fluid (aCSF) or nutrient-containing culture solution to flow over or around brain slices. Some chambers are intended for short-term (6–8 h) experiments while chambers for organotypic slice cultures allow experiments that last for days.

(1) Interface slice chambers. As illustrated in Fig. 2(A1), the interface chamber (referred to as ‘Haas’ or ‘Oslo’ type chamber^{1,33}) anchors the brain slice at the interface between air (usually humidified 95% O_2 /5% CO_2) and hyperoxygenated aCSF. Brain slices rest on a nylon mesh or filter paper that allows for nutrient/waste exchange with the aCSF from underneath. The top surface of the slice is directly exposed to a gas at a high PO_2 , which provides sufficient oxygen penetration. However, aCSF exchange through the supporting mesh or filter is not sufficient enough to maintain effective nutrient/waste turnover³⁴; also, gravity compression and surface dehumidification can alter slice physiological and optical properties over time.³⁴

(2) Submerged slice chambers. The submerged slice chamber (also referred to as superfusion chamber²²) has recently regained popularity for brain slice studies because it allows for better control of aCSF flow over the brain slice.³⁴ As illustrated in Fig. 2(A2), the brain slice is anchored on a supporting surface and entirely submerged in hyperoxygenated aCSF, allowing for effective diffusion through both its surfaces (some chambers also place a supporting mesh on top of the slice). Submerged slice chambers permit faster exchange of drugs or toxins compared to interface chambers.¹⁹ Also, submerged slices are advantageous for optical and electrophysiological recordings because morphology is better preserved.²² It is also possible to adapt high magnification water immersion microscope lenses for working with submerged slices.³⁴ As a major drawback, however, oxygen exchange with brain slices in submerged chambers is less efficient.^{22,34}

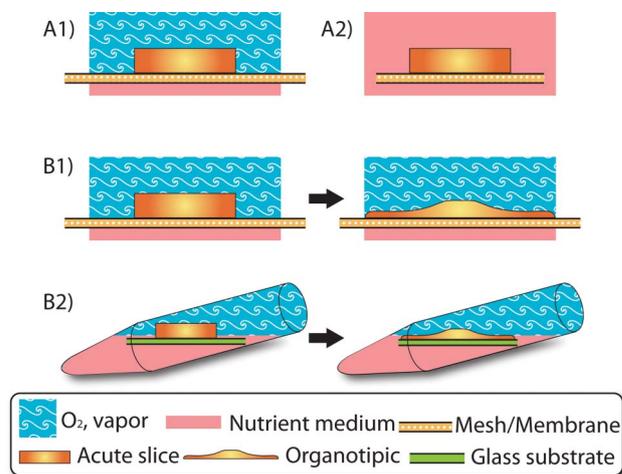


Fig. 2 Examples of conventional brain slice chambers. (A1) Interface chamber - the brain slice exchanges oxygen by directly exposure to oxygen-rich humidified air. The exchange of nutrients and metabolic waste products occur through a porous support interface, such as a mesh. (A2) Submerged slice chamber - the brain slice exchanges oxygen, nutrients, and waste products through fluid superfusing the slice. (B) Organotypic slice chambers - brain slices are allowed to settle into a thin layer of cells over a period ranging from days to months. This can be accomplished by incubating slices in an interface chamber (B1) or rotary tube (B2).

(3) Organotypic slice cultures. Acute brain slices are often used for recording immediately after slicing. They have been quite popular to neuroscientists, because the preparation is straightforward and the cytoarchitecture is mostly retained as in intact brain.³⁵ However, current techniques can only sustain brain slice viability for a few hours, which precludes the ability to study neurological processes that take place over days, such as neurogenesis³⁶ and synaptogenesis.³⁷ To overcome these limitations, organotypic slice cultures were developed to maintain slice viability for weeks and months.³⁸ During the culturing process, slice morphology and cytoarchitecture are altered as the tissue spreads out and settles into a thin layer of cells, resulting in the development of new neuronal structures, and degeneration of certain neuronal fibers. Fortunately, very few changes in the original network connections are observed.³⁸ Moreover, new neuronal structures and functions that would develop in organotypic cultures are similar to those that develop in the intact brain.^{35,39,40} Besides the unique compatibility of organotypic slice to long-term study, thinning down to 1–4 cell layers makes it amendable to high magnification imaging objectives that require extremely small working distance.

Despite the significant attraction of using organotypic slice culture, its preparation methods can be very demanding and time intensive. One preparation method is simply to culture the freshly cut slice in an interface chamber for a few weeks⁴¹ (Fig. 2(B1)). Another preparation method is to attach the fresh slice onto a glass coverslip and culture it in a dedicated roller-tube system for two weeks.⁴² The roller-tube, half-filled with media, is inclined so that the slice is partially immersed in aCSF and partially exposed to oxygen (Fig. 2(B2)). As the system that holds the tubes rotates like a Ferris wheel, the slice makes alternative contacts with oxygen and media periodically to ensure thorough exchange with both.

A number of design elements and principles learned from these conventional brain slice chambers have carried over into the design of microfluidic chambers. The remainder of this review will highlight relatively recent efforts that have been made by the microfluidics community to address the fundamental challenges of brain slice viability, fine fluidic control, and the integration of physiologic instrumentation.

Microfluidic devices enhance brain slice viability

As described earlier, effective oxygen penetration is vital for maintaining viable brain slices and supporting metabolic demand.^{19,43} By improving oxygen penetration efficiency, microfluidic devices have shown the capacity to enhance brain slice viability and function compared to conventional brain slice chambers.

Microfluidic interface and submerged slice chambers

Unlike conventional bulky interface or submerged slice chambers, microfluidic devices are typically much smaller (*e.g.*, 1–2 cm) and can be readily fabricated and plugged into a customized holder, such as the linear configuration shown in Fig. 3A. The versatility and flexibility of microfluidic device allows them to be integrated into a wide range of experimental configurations.

On a much smaller scale, microfluidic devices can incorporate several principles from conventional brain slice chambers, such

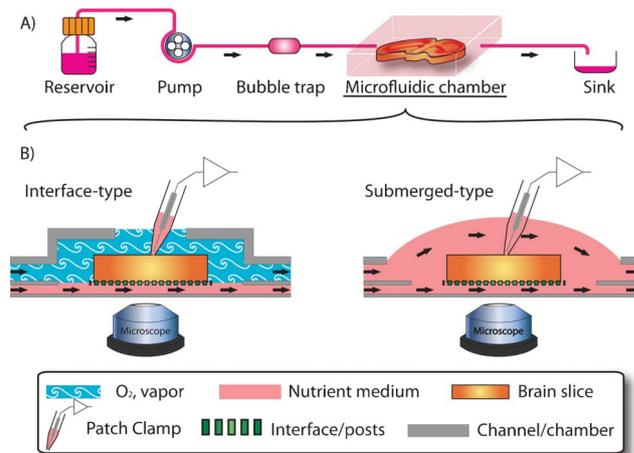


Fig. 3 Microfluidic brain slice devices. (A) Schematic diagram of microfluidic device integrated into a typical linear experimental set-up. (B) Microfluidic devices can integrate principles of conventional brain slice chambers and resemble interface and submerged slice chambers on a much smaller scale. Miniaturization allows for more effective gas/nutrient exchange, economical solution use, compatibility with small working distances, and highly versatile designs.

as the basic interface⁴⁴ and submerged slice chamber designs (Fig. 3B). Microfluidic devices have several additional advantages, such as: (1) the smaller chamber size. All of the spaces around the slice, especially the underlying space, are miniaturized to a sub-millimeter size range so that laminar flow is established and the device remains amenable to microscopes with reasonable working distance optics; (2) the chamber is mostly closed and its shape is tailored so the laminar flow can be better confined; (3) the supporting interface can also be made with micro-fabricated structures, such as microchannels⁴⁵ and microposts.^{30,44,46,47} With these modifications, a high speed laminar flow can be maintained to increase superfusion efficiency. Microfluidic devices also provide a robust system for rapid perfusion of drugs or chemicals across the whole slice.^{48,49} Like the conventional chambers, micro-superfusion chambers confirm that increasing solution flow rate increases oxygen penetration to a certain extent.¹⁹ However, larger flow rates produce increased solution velocity, which can cause correspondingly large shear forces that have the potential to damage tissues.¹⁹ Therefore, it is important to continue to develop microfluidic devices that can improve the gas/nutrient exchange efficiency without relying solely on solution flow rate, especially for improving the viability of thicker brain slices.

A number of groups have developed dual-superfusion chambers, which effectively superfuse the thicker slice with solution on both the top and bottom surfaces.^{30,45,46,50} Typically, the chamber is closed and forms two laminar flows above and under the slice. Polydimethylsiloxane (PDMS) made microchannels⁴⁵ or microposts^{30,46} are used to support and mechanically stabilize brain slices, and provide more surface area for superfusion⁵¹ (Fig. 4A). Arrays of sparsely arranged microposts gently anchor the brain slice by clamping it from both sides. Unlike conventional interface chambers that partially block the bottom brain slice surface and compromise gas/nutrient exchange, microposts apply very minimal contact with the brain slice.

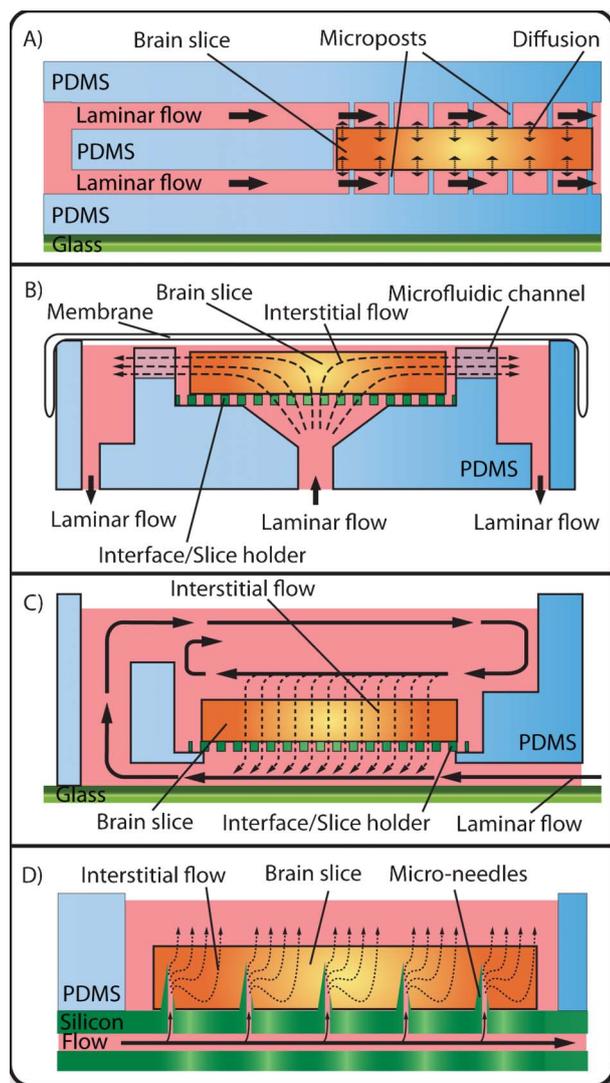


Fig. 4 Microfluidic devices using dual-side diffusion (A) and interstitial flow (B–D) to enhance oxygen/nutrient exchange. (A) A dual-side microfluidic device uses two layers of microposts to non-invasively anchor the brain slice.³⁰ The pillars are small enough to allow laminar solution flows on both sides of the brain slice. (B) An interface microfluidic device uses channels below the slice to “pull” interstitial flow of medium through the slice from the top to the bottom surface.⁵² (C) A submerged slice device that uses a flow rate difference (fast below, slow on top) to enhance solution flow through the interstitium and anchor the slice.⁵³ (D) Three-dimensional microneedles⁵⁴ or microposts penetrate the slice and deliver solution to the middle of the slice.

Thus, the majority of the brain slice is exposed to aCSF on both sides. As a result, viable thick acute slices ($> 400 \mu\text{m}$) can be physiologically recorded for hours.³⁰

Interstitial flow type device

There is a strong interest in working with relatively thicker ($> 400 \mu\text{m}$) brain slices for longer periods of time because they preserve more cytoarchitecture and network integrity.^{52,55,56} Unfortunately, diffusion-dependent conventional brain slice chambers are only able to sustain the viability of brain slices

for no more than a few hours,^{55,56} most likely due to low gas/nutrient exchange in the inner layers of the tissue.

One strategy for overcoming this limitation is to introduce solution flow through the interstitial space of the tissue.^{52,57} Three different ways of guiding oxygen/nutrient rich solution to interstitially pass throughout the slice thickness are shown in Fig. 4. Interstitial solution flow is induced by forcing laminar flow to pass through a slice as it is anchored within the flow pathway that runs from the bottom surface to the upper edge of the slice (Fig. 4B). The gas-permeable film sealing the top of the device also acts as a source of oxygen diffusion. As a result, a viable culture of $700 \mu\text{m}$ thick brain slice has been maintained for 5 days.⁵²

The Bernoulli effect can also be used to induce interstitial flow in brain slices.⁵³ Bernoulli’s principle states that for a non-viscous solution flow, increasing solution velocity decreases the solution’s pressure energy. In microfluidic chambers where solution is flowing laminarily within a constricted space, the area with higher speed creates a lower pressure as compared to the area with lower speed. Due to the reduced pressure, solution surrounding the slice is sucked through the slice towards the opposite side of the slice where there is faster flow. For example, a microfluidic device can be designed so that solution flow is fast underneath the slice, but slow in the space above the slice (Fig. 4C). Consequently, due to the Bernoulli effect, solution is pulled downwards through interstitial space within the slice and the permeable supporting interface.⁵³ The net downward pressure on the slice also acts to mechanically stabilize the slice on the membrane.

Three-dimensional penetrating devices can also be used to increase interstitial solution flows (Fig. 4D). Such techniques employ an array of hollow microneedles⁵⁴ or microposts⁵⁸ made of silicon by micro-fabrication. While also anchoring and penetrating into a thick brain slices, these microneedles or microposts create direct tunnels for solution to inject into the inner layers of the brain slice.

Microfluidic devices enable fine control of neurochemical environment

As we mentioned in the previous section, localized stimulation within brain slices is a common goal for neuroscientists. Having the perfusion localized controlled within a size scale of 100s of microns is a capability that microfluidics is particularly well suited for. For a long time, however, neuroscientists have been applying microfluidic principles in their traditional chamber setups in attempt to solve such problems. As a classic example, a micro-syringe needle was used to perfuse drugs into a standard brain slice bath (Fig. 5A).⁵⁹ With the proper gauge size and flow rate, the perfusion stream remains localized through laminar flow effects (Fig. 5A). As a result, the perfusion staining is well confined in the area that contacts the perfusion stream, inducing focal ischemia. As convenient as it is, this strategy lacks the capacity for designable control, such as adjusting the stream size or position.

On the contrary, microfluidics is well-known for its fine controllability. For example, a hydrodynamic focusing device was developed and tested for inducing focal stimulation of sodium-free solution to regulate network activity in one portion

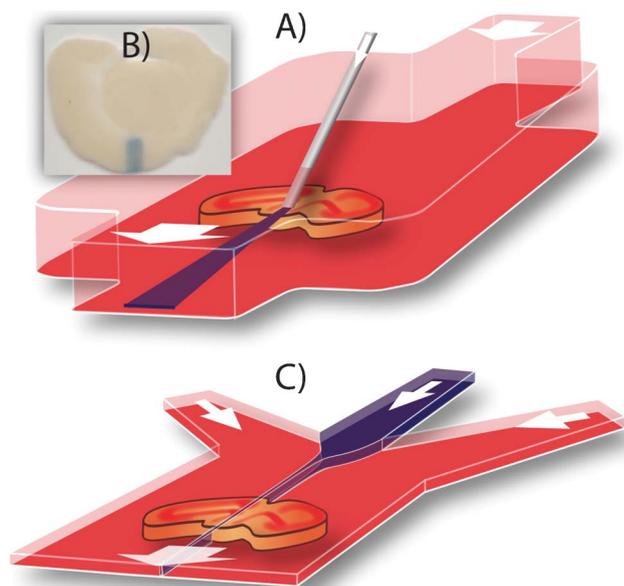


Fig. 5 Focal perfusion within a conventional slice chamber and a microfluidic device. (A) Focal ischemia in a conventional slice chamber was implemented with a syringe needle to focally apply a laminar flow (in blue) of oxygen-glucose deprived solution to a small portion of a brain slice.⁵⁹ (B) Color stain on the brain slice represents the region of focal ischemic damage.⁵⁹ (C) In a microfluidic device, focal superfusion of a sodium-free solution (in blue) was applied by directing a thin stream of laminar flow along the midline of a brainstem slice.³⁰

of a slice.³⁰ The perfusion solution from the middle inlet is hydrodynamically focused by aCSF from the other two inlets, a well characterized feature of laminar flows (Fig. 5B). The width of the perfusion stream, and also the perfusion area, can be widely adjusted by controlling the flow rates of the input fluids.

Upright micro-perfusion probes

'Micro-perfusion probes' are devices designed to deliver soluble chemicals in a microfluidic manner to the extracellular space of brain tissues from a location slightly above the surface of the slice. The classic format, glass micropipettes, which inject laminar flow of soluble chemicals through a micro-scale aperture, have been widely used for over six decades.⁶⁰ The solution is delivered by positioning the micropipette tip, which is located in an upright configuration immediately above the slice. A modified version of the micropipette, the theta pipette⁶¹ (Fig. 6A), can inject two different solutions side-by-side (other pipettes can have 3–5 injection ports), or can be used to confine solution delivery just to the tip by injecting fluid from one port and immediately removing it with the other, in a 'push-pull' configuration. Glass micropipettes are extremely popular tools for neuroscientists because they share the same fabrication and operation equipment with conventional patch clamp instruments, which are readily available in many neuroscience labs. However, control of flow dynamics is quite limited with glass micropipettes because of their poor designability and fabrication consistency.

More complex microfluidic micro-perfusion probes can be designed to apply solution through microscale apertures, which are microfabricated and located with great precision. These

devices use controllable hydrodynamic envelopes,⁶² whose basic principle is illustrated in Fig. 6B. Typically, two apertures are micro-fabricated on a planar tip, which is precisely positioned to form a small gap between the probe and the slice surface. The solution is injected through one aperture into this gap, and immediately aspirated through the other aperture, forming a hydrodynamically-shaped envelope that perfuses the slice locally.⁶³ The envelope shape is determined by the gap dimension and the flow rates through the two apertures^{62,63} (Fig. 6B). In this way, the micro-perfusion is confined to desired regions without diffusion of injected chemicals into surrounding media. Increasing aperture quantity can be used to introduce more parameters to control the hydrodynamics, creating a diverse set of spatial profiles of micro-perfusion. A quadrupolar and more advanced profiles have been explored with four-aperture-containing⁶⁴ (Fig. 6C) and six-aperture-containing probes.⁶⁵

Like the glass micropipette, micro-perfusion probes can be positioned relative to any spot above the slice for localized chemical delivery. It is also possible to move the probe precisely to dynamically perfuse at a series of locations. However, the operation machineries are large and they block the optical path and impede the placement of other instruments and probes.

Underlying micro-perfusion device

To circumvent the problem of increasingly limited space above the slice using micro-perfusion probes, the perfusion element can alternatively be arranged beneath the brain slice in a planar layer. For example, a standard perfusion chamber is attached onto a planar microfluidic layer, which consists of microchannels and microscale *via* holes (Fig. 7A). This device was developed to deliver neurotransmitters that are pumped through the microchannels and then perfused into brain slice through *vias* (Fig. 7A).⁶⁷ An iteration on this design was developed with a micro-dispenser that attaches as an additional layer containing pneumatic micro-valves that can precisely control timing, duration and intensity of the perfusion event (Fig. 7B).²⁹ Similar micro-perfusion devices were designed with microscale *vias* or hollow microposts⁴⁷ that inject ultrafine fluid volume to a focal point of stimulation. However, the released fluid is not hydrodynamically confined and usually diffuses in a gradient profile that expands in size.^{47,68}

In a brain slice study of CSD, active suction was added to the perfusion ports to introduce a confined flow envelope for focal stimulation²⁴ (Fig. 7C). This device contains several suction ports surrounding each injection port. Similar to those in perfusion probes, laminar flows are confined to an envelope (as small as a 100 μm spot), running from the injection to the suction ports. Under this setup, multiple CSD events can be induced and observed with controlled timing and location. A variety of spatial profiles can be achieved by manipulating the port geometries and the dynamics of the underlying flow (Fig. 7C).

The underlying layers of these micro-perfusion devices are usually made of transparent PDMS, which has good optical properties. Its thickness is only in a range of a couple hundred microns; therefore, it is compatible with visualization from underneath. On the other hand, the geometrical design of the perfusion ports and delivery channels can be quite flexible, and

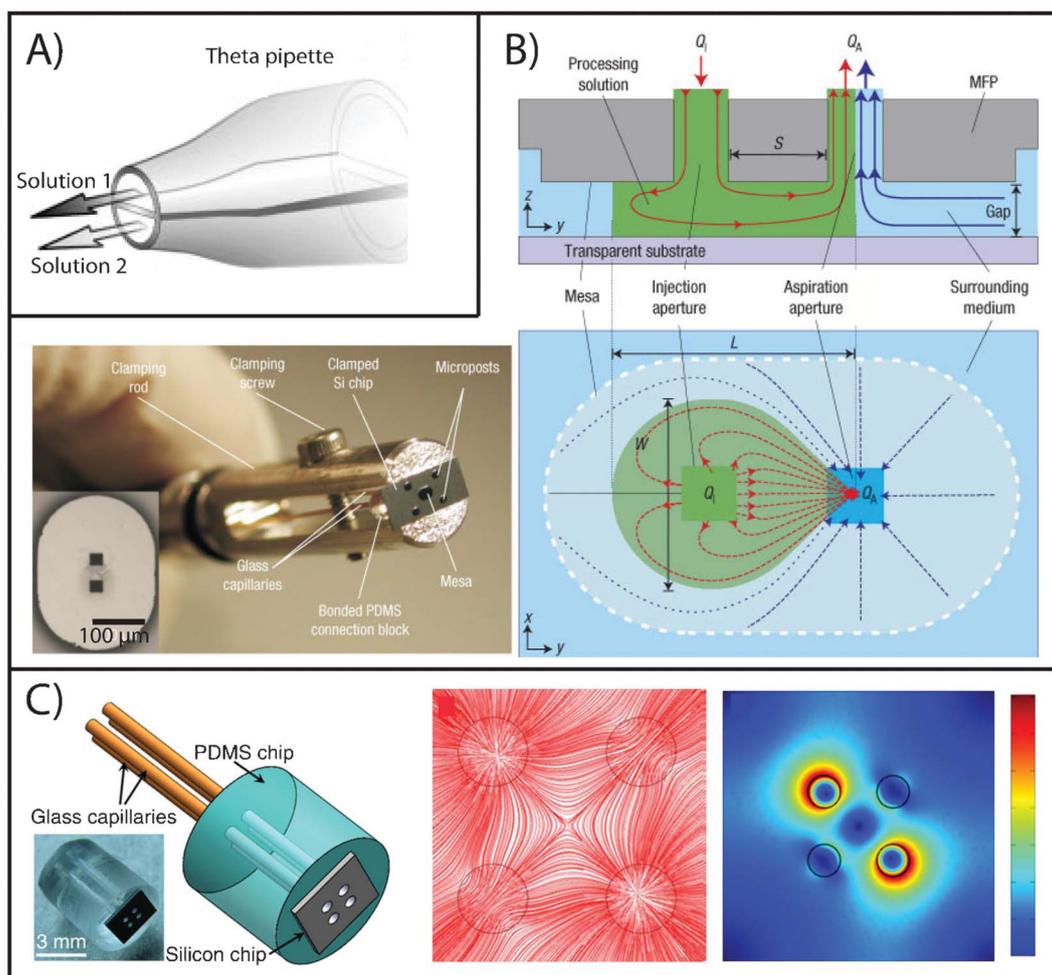


Fig. 6 Upright micro-perfusion probes. (A) Schematic diagram of a double-barreled glass theta pipette that can apply two different solutions to a specific slice region. Theta pipettes can be modified into a “push-pull” configuration to further localize solution application to a small slice region. Reproduced and adapted from ref. 61 with permission from the American Physiological Society. (B) Lower left: a photo of a microfluidic probe shows two micro-scale apertures connected to separate glass capillaries. Right two panels: by applying injection flow to one aperture and suction to the other one, a hydrodynamic envelope is formed to confine laminar flows. Reproduced and adapted from ref. 66 with permission from the Nature Publishing Group. (C) Left: schematic diagram of a quadropole probe. Right: by manipulating flow dynamics in four apertures, complex concentration and velocity dynamics are formed. Reproduced and adapted from ref. 64 with permission from the Nature Publishing Group.

controlled multiple stimulations can be easily realized. Unlike the perfusion probes that can be re-positioned during the experiment, the underlying devices are limited in positioning and have to rely on a prearranged alignment of the perfusion ports.

Microfluidic devices with integrated electrophysiology

Many microfluidic devices, despite their intricate engineering capabilities and elegant design, are developed without an appreciation of the ultimate end user, in our case, the neuroscientist. The typical neuroscience laboratory still has at its heart, the light (or fluorescent) microscope, and those that do brain slice physiology usually add electrophysiology tools to their microscopy setups. The majority of microfluidic approaches are at least amenable to microscopy tools, due to the optically clear materials they are made of. It remains a challenge though to allow both microscopy and electrophysiological access to the slice within a microfluidic platform. A number of groups have addressed this challenge by making

devices that allow the use of traditional electrophysiology tools, or by integrating microfabricated sensors into the device itself.

Open access microfluidic devices

Most tissue slice laboratory environments are already well equipped with a wide variety of electrophysiology probes. These include patch clamp pipettes, sharp extracellular recording electrodes, whole nerve suction electrodes, stimulating and electrochemistry probes. While most general-purpose microfluidic devices rely on closed channels to constrain fluid flow, many of the microfluidic devices dedicated to brain tissue slices take special considerations to provide ‘open access’ to the slice so that it can be integrated with the neuroscientists’ multitude of probe instruments. There are a number of devices that have been described in the previous sections that fit this approach. These devices have microfluidic spatial delivery integrated into the bottom of the chip, allowing the top of the slice to be open to the external environment for insertion of electrodes. In Fig. 7a,

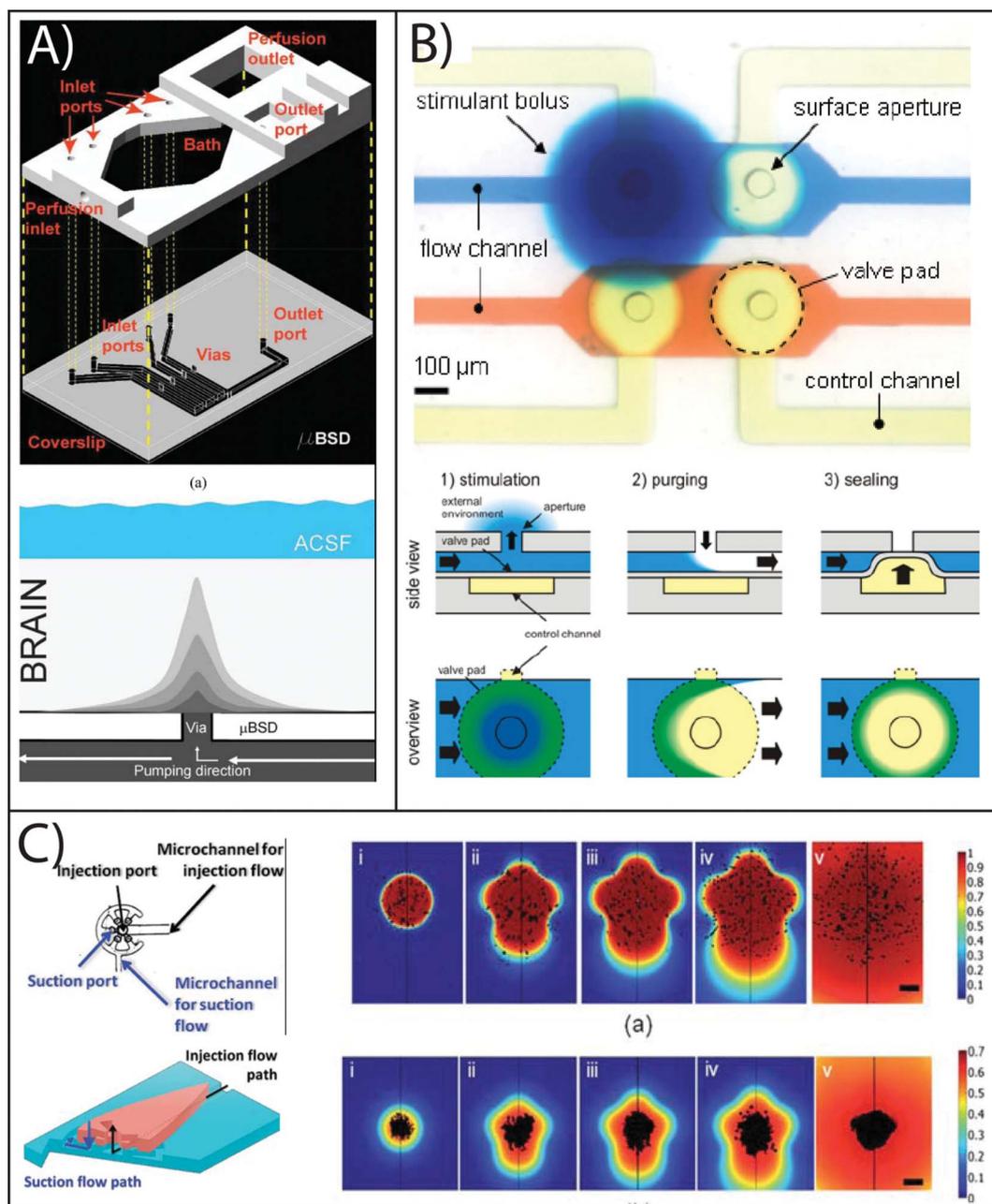


Fig. 7 Underlying microfluidic perfusion chambers. (A) Upper panel: a PDMS layer with an array of microfluidic channels docked to a standard perfusion chamber. Lower panel, side view: in each microfluidic channel, perfusion solution was passively pumped into brain slice through a *via* from underneath. Reproduced and adapted from ref. 67 with permission from the Royal Society of Chemistry. (B) Upper panel, top view: a multilayered micro-dispenser device applies a stimulant bolus from underneath. Lower panel: control of pneumatic valves and flow dynamics maneuvers the stimulation. Reproduced and adapted from ref. 29 with permission from the Chemical and Biological Microsystems Society. (C) Left panel: a micro-dispenser combines a center injection port and surrounding suction ports. Right panel: manipulating the flow dynamics of ports results in a variety of concentration and velocity profiles. Reproduced and adapted from ref. 24 with permission from the Royal Society of Chemistry.

the microfluidic device⁶⁷ was designed as an ‘add on’ for the base of a traditional diamond shaped laminar flow brain slice chamber. This approach leaves the original chamber virtually untouched, maintaining its accessibility to physiology instruments. Fig. 7C illustrates a similar approach, but in this case the instrumentation used is primarily optical, as CSD is induced locally in the slice and can be monitored through phase contrast microscopy.²⁴ This type of approach is particularly powerful in

translational research, as CSD is a clinically relevant problem that exhibits itself in a number of traumatic brain injury (TBI) scenarios. An *in vitro* model that combines both focal CSD induction with microfluidic drug delivery and high power imaging has potential as a screening tool for pharmacological treatments to limit the impact of adverse TBI events.

Fig. 8A shows an alternative approach, where a thin layer of media is maintained over an organotypic slice through the use of a

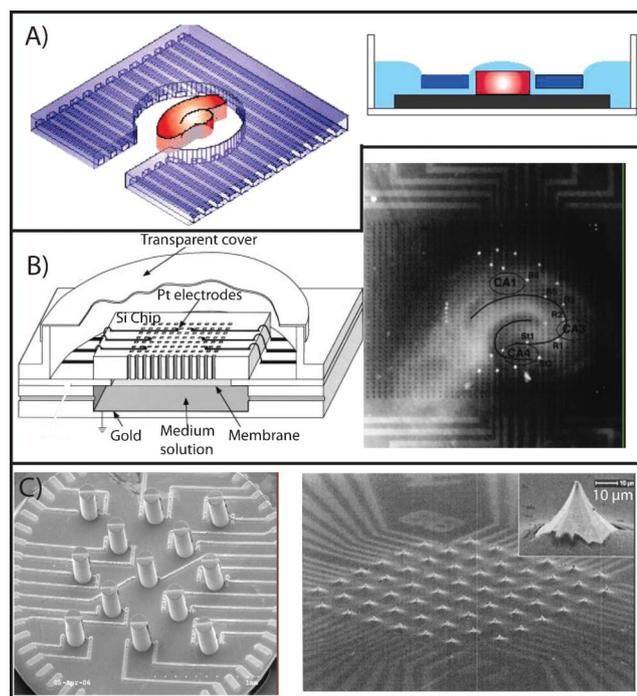


Fig. 8 Progress in microelectrode research for brain slices. (A) Left: a schematic diagram of a PDMS microfluidic device with an array of fluidic channels. Right: the PDMS-made device naturally sealed to MEA surface. The composed channels facilitated the medium exchange. Reproduced and adapted from ref. 69 with permission from Elsevier. (B) Left: a schematic diagram of a microelectrode array with an array of superfusion ports on the silicon base, which were connected with the medium. Right: a microscopic image of the device with a brain slice on the electrodes. Reproduced and adapted from ref. 70 with permission from IEEE. (C) Electronic microscopic images of 3D MEAs. Conductive electrodes were represented in phase bright. Reproduced and adapted from ref. 58 and 71 with permission from IOP Publishing and Elsevier respectively.

series of microfluidic channels connecting the internal ‘mini-well’ with the surrounding bulk media reservoir.⁶⁹ Interestingly, the microchannels served as conduits for axons to extend out of the slice into the adjacent compartment. This is similar to the approach that Taylor *et al.*⁷² used to guide and isolate developing axons from their cell bodies in dissociated culture. This idea has been extended further to include microchannels that connect two microwells, each containing an organotypic slice.⁷³ In this study, the slices were able to send developing axons towards one another and establish newly formed functional connections. This provides an interesting approach to studying interactions between brain networks, as individual brain areas can be isolated and then induced to form new connections. More provocative is the idea that this approach could be potentially used to connect a brain tissue slice to another organ’s cultured tissue slice, or even one of the emerging ‘organ on a chip’ devices that are being developed to form new models of a number of different tissue types. For example, microfluidic devices have been developed to maintain medullary slices that contain the breathing regulatory centers of the brain.³⁰ These slices maintain a rhythmic respiratory output that could theoretically be used to trigger the ‘breathing’ activity of a lung on a chip device.⁷⁴

Yet another approach involves opening the sides of a microfluidic channel, such that the slice can be accessed along its entire length by inserting a probe parallel to its cut surfaces.⁴⁶ In this study, a microfluidic chamber was produced that had small opening in the sides of its main channel, adjacent to the slice perfusion region. By using hydrophobic materials and balancing the input and output flows, a virtual wall of fluid was produced where the sides of the slice were exposed. This allowed the insertion of a microfabricated multi-electrode array (MEA) into the slice so that multiple parts of the slice could be monitored simultaneously. This approach allows for monitoring of communication between multiple areas in the slice and the calculation of electrical sources and sinks *via* current source density analysis. This approach also mimics the insertion of MEAs *in vivo*,⁷⁵ and could be used as a model to investigate the design of MEAs for potential clinical uses.

Microperfusion with integrated MEAs

Traditional electrophysiological techniques primarily utilize single channel electrodes for recording or stimulation. Although insertable MEAs, as described above, can be used in place of single channel probes, an attractive alternative is to use micro-fabrication techniques to integrate multiple sensors into the microfluidic chamber. This is attractive for a number of reasons, primarily in that it allows the chamber to be completely enclosed, making fluid flows more easily controlled. Secondly though, the integration of microelectrode sensor fabrication techniques lend themselves to the inclusion of sensor arrays with high channel counts that can be accessed simultaneously. This confers the ability to dramatically increase the throughput of experiments, since multiple neural circuits can be accessed in parallel, rather than sequentially as is normally done with traditional single electrode approaches. This parallel approach reduces the variability in the measured response between sequential recordings and makes it possible to interpret the precise timing relationships between neural ensembles.⁴⁶ Additionally, it makes it possible to measure network activity in response to experimental conditions that may irreversibly alter the slice physiology, which would make sequential measurements difficult to interpret. Lastly, it would greatly enhance the utility of even short term viability in different slice models, as information could be gained that would be equivalent to performing sequential measurements on a slice that would have to remain viable for a much longer length of time. This would make it possible to perform meaningful experiments on brain slices taken from aged animals or perform experiments at elevated physiological temperatures.

MEA technology has been developed for numerous *in vitro* cell culture assays, and has been covered extensively in a number of reviews.⁷⁶ Due to the wide availability of cell culture MEAs, there have been a number of attempts to develop microfluidic devices for culturing brain slices that are merely built upon an existing MEA structure. The device in Fig. 8A, discussed in the last section, was further modified to be able to be attached directly to the surface of a planar MEA, allowing the organotypic slice to be recorded from at dozens of locations simultaneously. The process of attachment is fairly straightforward in this approach, as the microfluidic device is made from PDMS, which spontaneously adheres to the insulating top surface of the MEA. Fig. 8B shows a

slight variant on this approach, in which an MEA is built on top of an interstitial perfusion device, similar to those described in Fig. 4. A number of groups have taken this concept even further, by making 3D MEAs that penetrate into the bottom surface of the slice, as shown in Fig. 8C. These devices were developed in an attempt to solve one of the limitations of planar MEAs; the decreased viability of the bottom layers of the slice. In planar MEAs, the bottom of the slice lies directly on the MEA surface. It is commonly thought that the slicing process kills a layer of cells approximately 50 μm deep on the slice surface. Compounding this is that the presence of the MEA further inhibits nutrient diffusion into the bottom layer of the slice. A number of studies have developed raised MEAs⁷¹ that penetrate into the slice, presumably into more viable tissue towards the center of the slice. Rajaraman and colleagues took this concept a step further by making hollow microperfusion electrodes that could simultaneously deliver fluid to the middle of the slice and record neural activity.⁵⁸

Translational studies in non-brain tissue

The rationale for using microfluidic devices for maintenance of brain slices is well motivated by the desire to maintain intact networks in thick samples and the need to integrate electrophysiology instrumentation. The work described in this review on microfluidic brain slice techniques has also created opportunities for leveraging this technology for creating devices for studying other types of tissue slices. While the specific needs of different types of tissues will vary from application to application, the most basic of features are largely the same; to maintain adequate perfusion of thick slices and to deliver chemical stimuli. This section outlines recent work in the emerging area of microfluidics for non-brain tissue slices.

Microperfusion chip for biopsy samples

One of the areas where tissue slice devices have the most immediate need is for the maintenance of human tissues, typically taken at biopsy. The motivation to develop methods for maintaining viable, intact human tissue samples stems from our current inability to produce 3D tissue cultures of most primary human cell types and the relative scarcity of certain tissue types from both normal and human disease states. There have been several studies that have recently looked at microfluidic devices employing simple laminar flow for maintaining tissue biopsy slices. Fig. 9A illustrates a basic laminar flow device that was originally developed for use in an animal model of liver tissue biopsies. The device was shown to maintain viable tissue slices from biopsies for over 70 h. The design was also capable of performing tissue manipulations within the device, including cellular disaggregation. This device was further developed to be amenable to actual human biopsies, focusing on both normal and neoplastic colorectal tissue biopsies.⁷⁷ This study showed that the normal and tumor biopsies could be maintained for greater than 70 h and produced expression of proteins in response to hypoxic changes in their local environment that are typical of their tissue phenotype. This type of approach has particular relevance to cancer tissue biopsies, as it could provide a human model that replicates the tumor microenvironment and is amenable to increased throughput methods for screening potential chemotherapeutic agents.

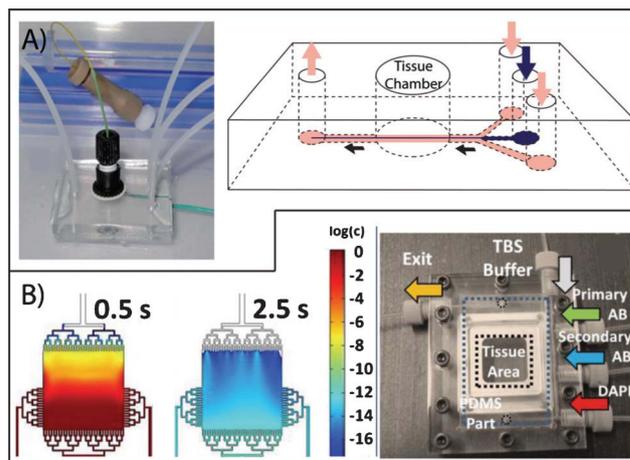


Fig. 9 Microfluidic perfusion chamber used for non-brain tissue samples. (A) A setup of micro-perfusion system for tissue biopsy culture and interrogation is shown (left panel) with a schematic diagram of the device (right panel). Flow direction is indicated with arrows. Reproduced and adapted from ref. 77 with permission from the Royal Society of Chemistry. (B) Simulation of a microfluidic gradient generator shows that a complex concentration gradient can be adjusted in a couple of seconds (left panel). A photo of a microfluidic device that incorporated the gradient generator for rapid perfusion of cancer tissue slices (right panel). Reproduced and adapted from ref. 78 with permission from the Chemical and Biological Microsystems Society.

Cancer detection

Another application of microfluidics for use with tissue biopsies doesn't deal with the maintenance of the slice, but rather the analysis of the biopsy characteristics. Fig. 9B shows a device for producing gradients of immunohistochemical reagents for rapid histopathology analysis of tumor biopsies.⁷⁸ This study outlines the potential for tissue slice microfluidic devices to have utility as a clinical diagnostic technology. In a related article from the same group, it was shown that antibody based tumor diagnostic assays could be performed in 20 min compared to 2 h needed for classical immunohistochemical protocols.⁷⁹ The results of their microfluidic assay were in agreement with the clinical laboratory results from the same samples. They further postulated that by using a microfluidic device that incubation times could be substantially reduced due to short diffusion distances. This would have a cumulative effect in any process that involved multiple staining/incubation/wash steps, and has the potential to dramatically speed up clinical analysis of tumor biopsies. This would open up the possibility of producing disposable, fast assays that could be used at the surgical intervention site, providing much needed information to the clinical team.

Micro-tissues

Tissue-slice-derived microfluidic techniques have begun to extend their application to *in vitro* micro-tissues, such as that of the pancreatic islet, vascular system, capillary, and bone systems. On one hand, these individual microscale tissues often consists of no more than a few thousand cells. Having many similarities to *in vitro* cell cultures, they are easier to acquire, maintain, manipulate, and scale up than primary slice tissues. On the other hand, their cytoarchitecture and functions are complete

enough for many pharmacological studies, which provides motivation for creating microfluidic perfusion assays with high throughput and spatiotemporal resolution. As a classic example, hundreds of individual islets were isolated from pancreatic tissue and separately immobilized in microfluidic chambers employing micro-wells,^{80,81} micro-channels,⁸² or micro-plugs.⁸³ Highly-dynamic metabolic responses, such as that of insulin secretion in response to glucose oscillations, can be well resolved using microfluidics-based perfusion, providing detailed insights into diabetic pathophysiology.⁸⁴ Microfluidic devices have also been employed to grow and support micro-tissues from dissociated cells culture and co-cultures.⁸⁵ Such approaches are predominantly used in vascular research,⁸⁶ in that endothelial cells form vessel-like tubes compartmentalized by microfluidic architectures. Endothelial cell migration⁸⁷ and cancer metastasis⁸⁸ have been assayed by perfusing growth factors and chemokines. Microfluidic-based superfusion chambers that re-construct three-dimensional bone⁸⁹ and capillary⁹⁰ micro-tissues from cell co-culture have recently been explored to mimic ischemic environments for tissue engineering assays.

Screening and toxicology

One of the most sought after applications for tissue microfluidic devices has been the possibility of using them as screening tools for drug development and toxicology. The marriage of the high throughput capabilities of microfluidic technology with the *in vivo*-like attributes of tissue slices would make an ideal platform for investigating the kinetic, metabolic and toxicity profiles for large libraries of drug candidates. Add to this platform the ability to integrate human tissues from biopsies and the possibilities for potential screening applications only increases. Towards this lofty goal, a number of recent studies have looked at microfluidic systems for use in pharmacological and toxicological studies in excised tissue slices. The majority of these are directed at either the liver or the heart, both common candidates for drug screening. Similar to the interstitial devices described in Fig. 4D, Khong and colleagues developed a chamber that incorporated an array of microneedles that helped to deliver nutrients to the interior of thick slices of rat liver.⁹¹ Van Midwoud and colleagues followed this with the development of a microfluidic ‘perfusion’ system (*i.e.*, the fluid flow goes around the sides of the slice) to culture 100 μm thick liver slices.⁹² Their design also incorporated thin membranes that take advantage of the gas permeability of PDMS to oxygenate the solution flowing around the slice. Cheah *et al.*, took a microfluidic perfusion approach to maintaining heart tissue samples, and also added real-time electrochemical monitoring by using an open-access approach, similar to those described previously in this review.⁹³ In this study they were able to maintain the tissue for up to 5 h while assessing reactive oxygen species release from the tissue, which plays a major role in ischemia-reperfusion injury.

Limitations/Future Directions

The future looks bright as neuroscientists and biomedical engineers collaborate to use microfluidic devices for addressing scientific questions using *in vitro* brain tissue preparations. Several compelling questions regarding microfluidic chambers

are currently being investigated, such as testing whether: (1) microfluidic devices improve oxygen/nutrient penetration and viability of slices from different parts of the brain; (2) microfluidic chambers allow thick slices to retain their structure and remain viable for days and weeks without settling into a thin organotypic slice; (3) different solutions can be focused onto small regions of brain slices and control the composition of interstitial fluid with high spatial resolution; (4) microfluidic devices permit co-culture of neurons with other tissues, both neuronal and non-neuronal in origin; (5) microfluidic devices can be developed for high throughput drug/toxin screening applications in neural and non-neural tissues.

However, significant scientific and technical challenges inherent with microfluidics still need to be overcome. The microfluidic approach has several inherent limitations. For example, solution flow in very small channels can introduce significant shear stress,⁹⁴ resulting in potential damage to the surface of the tissue sample. Small channels in microfluidic chambers are also highly susceptible to trapping air bubbles⁹⁵ that disrupt flow around slices and possibly damage the tissue they contact. Likewise, microfluidic devices are frequently fabricated with PDMS elastomer, which has many excellent properties (*e.g.*, biocompatible, flexible, inexpensive, clear, *etc.*) but also some potential drawbacks. PDMS absorbs proteins and hydrophobic small molecules (*e.g.*, estrogen),⁹⁵ and potentially many other substances commonly used in brain slice experiments. For example, if tetrodotoxin (fast sodium channel blocker) was used to suppress action potentials in a brain slice within a microfluidic chamber made of PDMS, the tetrodotoxin could diffuse into the PDMS and leach out in an uncontrolled manner in subsequent experiments using the same microfluidic chamber. Thus, there is a need to design brain slice microfluidic chambers that are either disposable, capable of being completely cleaned, or sealed with a coating to prevent diffusion. Standard inexpensive methods for sealing PDMS (and other materials) need to be developed because the high gas-permeability of PDMS limits its application in aerobic-sensitive assays in which PO_2 needs to be precisely controlled.⁹⁶

A second challenge is the nature of the brain slice itself. Brain slices of similar size and thickness can be cut from any part of the central nervous system (see table 1 in ref. 2), but there may be critical differences between slices from different regions. For example, cortical, hippocampal, and brainstem slices from adult rodents are acutely viable for several hours *in vitro* and can be transformed into organotypic cultured slices that last for several days. In contrast, it is not as easy to maintain motoneuron function in similar transverse slices cut from the spinal cord of adult rodents, due to the relatively high metabolic demand and low calcium buffering within motoneurons, and the severing of large dendrites that are oriented rostrocaudally (discussed in ref. 97). To obtain viable transverse spinal cord slices from adult mammals, unique methods have been developed that involve animal/tissue pretreatment protocols and different solutions for tissue isolation and cutting.^{97–100}

It's possible that principles associated with microfluidic technology discussed in this review may be applied to brain slices that are notoriously difficult to isolate and maintain under *in vitro* conditions. For example, enhanced penetration of oxygen and nutrients may compensate for higher metabolic rates due to

species differences or the presence of more gray matter vs. white matter. Also, microfluidic technology may allow large portions of tissue to survive *in vitro* and thereby better preserve dendrite structure and integrity.

A third major challenge is the critical issue of optical access for microfluidic chambers. Almost all brain slice physiology experimental setups require the integration of imaging, the very least of which involves the use of stereoscopic microscopes for identifying morphological landmarks within the slices. While the majority of the reviewed devices allow for optical access for rudimentary monitoring, none of them were designed specifically with optics in mind. This is going to be a crucial area for future development, as the slice community will undoubtedly continue to explore the myriad of revolutionary optical techniques that are emerging in the neurosciences.¹⁰¹ While many of the emerging optical techniques, such as TIRF (total internal reflectance fluorescence) do not lend themselves well to slice applications, as they only image at the surface, techniques such as multi-photon imaging are becoming crucial for looking deep into tissues. Since microfluidic devices often involve components both below and above the slice, optical methods will likely require long working distance lenses and will have to account for the numerous changes in index of refraction caused by the presence of microfluidic layers. Furthermore, the new field of optogenetics has created the ability to also use light for optical stimulation.¹⁰² Optogenetics involves the use of light activated channels in neural tissue to provide spatiotemporal activation through optical modulation. Thus, the materials used to make microfluidic chambers and the size/thickness of various layers of microfluidic chambers will need to be optimized to take full advantage of the experimental power that imaging and optogenetics have to offer.

Scientists are generally eager to embrace new technologies that help them address important and novel scientific questions. However, new technologies come at a cost, and there can be a reluctance to move away from tried-and-true methodologies (e.g., subfused Haas slice chamber). It often takes considerable effort and money to develop or learn new techniques, scientific momentum within a lab can be disrupted for long periods of time, results that differ from those obtained from well-established techniques may be suspect or artifactual, and skepticism by colleagues hampers publication of results and access to funding. This seemingly paradoxical tradeoff creates a need to balance any new technology with the demands of the scientist that is going to use it. Microfluidic technology may have great potential for scientific advances, but neuroscientists need to be informed and convinced that these new methods will solve real problems in the lab. One notable step in the right direction is the development of the 'microfluidic add-on'⁶⁷ that simply adds a planar microfluidic network to a standard diamond shaped slice perfusion chamber to improve chemical localization capabilities. This type of approach may be crucial in the advancement of the field as it lessens the barrier for utilizing microfluidic technology and leverages existing approaches that are familiar to many neuroscientists.

We hope this review can be a guide for both engineers and neuroscientists alike, providing a much needed perspective into the challenges that have faced the brain slice field, and the opportunities that exist for the microfluidics field. As evidenced

by the traditional tools that neuroscientists have developed to work with brain slices, including micropipettes and laminar flow chambers, the field of microfluidics might not be so foreign after all.

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