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Published in final edited form as:
Stem Cell Rev. 2017 June ; 13(3): 381–406. doi:10.1007/s12015-017-9738-0.

Microphysiological Human Brain and Neural Systems on a Chip: Potential Alternatives to Small Animal Models and Emerging Platforms for Drug Discovery and Personalized Medicine

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Abstract

Translational challenges associated with reductionist modeling approaches, as well as ethical concerns and economic implications associated with small animal testing, drive the need for developing microphysiological neural systems for modeling human neurological diseases, disorders and injuries. Here, we provide a comprehensive review of microphysiological neural systems on a chip (NSCs) for modeling higher order trajectories in the human nervous system. Societal, economic, and national security impacts of neurological diseases, disorders and injuries are highlighted to identify critical NSC application spaces. Hierarchical design and manufacturing of NSCs are discussed with distinction of surface- and bulk-based systems. Three broad NSC classes are identified and reviewed: microfluidic NSCs, compartmentalized NSCs, and hydrogel NSCs. Emerging areas and future directions are highlighted, including the application of 3D printing to design and manufacturing of next-generation NSCs, the use of stem cells for constructing patient-specific NSCs, and the application of human NSCs to 'personalized neurology'. Technical hurdles and remaining challenges are discussed. This review identifies the state-of-the-art design methodologies, manufacturing approaches, and performance capabilities of NSCs. This work suggests NSCs appear poised to revolutionize the modeling of human neurological diseases, disorders and injuries.

Keywords

organ on a chip; brain on a chip; neural system on a chip; blood-brain barrier; 3D printing; 3D hydrogel

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Conflict of interest statement: No competing financial interests exist.

Author Disclosure Statement: No competing financial interests exist.

1. Introduction

1.1 Societal Impacts of Neurological Diseases and Disorders

Neurological diseases, disorders and injuries (NDDIs) cause significant mortality rates and quality of life losses worldwide.[1] For example, the World Health Organization has recently determined that 8 of 10 disorders in the highest disability class are neurological disorders.[2] According to the American Academy of Neurology, genetic and infectious diseases and disorders of the nervous system currently affect over 6.4 million people in the United States (US) alone. In fact, stroke and Alzheimer's disease are currently the third and sixth leading cause of death in the US, respectively.[3,4] Furthermore, the number of Americans diagnosed with Alzheimer's disease is rising and expected to reach 16 million by 2050.[5] Neurological disorders resulting from injury also pose critical problems. For example, according to the Centers for Disease Control and Prevention each year over 1.7 million people experience a traumatic brain injury (TBI) in the US. As a result, 3.3 – 5.3 million people are currently living with a TBI.[6] Several recent studies have also estimated that *ca.* 20,000 new cases of spinal cord injury occur annually in the US with *ca.* 270,000 currently living survivors.[6] In addition to TBIs and spinal cord injuries, it has been estimated that over 200,000 peripheral nerve repair procedures are performed annually in the US to treat NDDIs that affect the peripheral nervous system.[7]

Importantly, NDDIs also have significant impact on the economy and national security. For example, the direct healthcare costs associated with stroke and TBI alone in the US have been estimated to be greater than $46 billion US dollars (USD) per year with indirect costs associated with work and productivity loss estimated near $91 billion USD per year.[6] Additionally, the high cost and time associated with drug development for NDDIs also contributes to both direct and indirect healthcare costs.[8] High incidence of neurological disorders and injuries among the warfighter and veterans is also of great concern and has far reaching implications toward national security. For example, TBI and posttraumatic stress disorder (PTSD) have been recognized as the most common problem requiring medical intervention.[9] Ultimately, considering the likelihood of continued civil conflict worldwide[10] and trend toward an aging population,[11] the prevalence of NDDIs is projected to further increase. Thus, there is a critical need for novel cost-effective molecular, cellular, and device-based medicines and therapies for the prevention and treatment of NDDIs.[12]

1.2 Limitations of State of the Art Models for Neurological Diseases, Disorders and Injuries

Historically, medicines and therapies for NDDIs have been developed by extending discoveries based on tissue culture and small animal models to clinical application. For example, two-dimensional (2D) monolayer cultures remain a standard model for many applications, such as high throughput drug screening. However, such 'reductionist approaches' often fail to replicate higher order features and trajectories of the *in vivo* nervous system, and although incorporating more cell types or use of human cells can somewhat improve the realism of such models, this approach sacrifices robustness.[13,14] This tradeoff between robustness and realism as well as overall limitations in achievable complexity currently impede the use of reductionist approaches for modeling higher order trajectories of the nervous system.

Presently, three-dimensional (3D) histotypic and organotypic slice cultures, such as brain slice cultures, and small animal models are gold standards for modeling higher order features and trajectories of the *in vivo* nervous system.[13,14] However, such models are typically not compatible with human cells, which can challenge the translational impact of associated results due to cross-species differences. Furthermore, ethical concerns and high cost associated with animal testing also drive the need for alternative approaches. Driven by these limitations, 'microphysiological neural systems' (MPNS) appear poised to advance our fundamental understanding of higher order structure, function, and disease of the human nervous system, therein providing novel platforms for developing next-generation medicines and therapies for NDDIs.

1.3 Microphysiological Neural Systems on a Chip

MPNS are defined as 3D biological constructs that reproduce higher order features, parameters and trajectories of the *in vivo* nervous system. Importantly, MPNS design and manufacturing processes are compatible with human cells endowing them with unparalleled translational value. In general, microphysiological systems (MPS) can be broadly categorized as: 1) scaffold-free or 2) scaffold-based.[15] A discussion of scaffold-free MPS is beyond the scope of this review; various excellent reviews can be found elsewhere.[16,17] Alternatively, scaffold-based MPS are versatile platforms based on a tissue chip concept. Tissue chips are micro-fabricated devices that mimic human physiological responses. Tissue chips typically integrate scaffolding, mechanical cues, biochemical cues, and topographical cues to recreate physiological conditions. Recently, chip-based MPS have been applied to a number of tissue and organ systems and now show significant promise in modeling higher order trajectories of the brain and nervous system.

Chip-based MPNS, hereinafter referred to as neural systems on a chip (NSCs), are scaffold-based 2D or 3D culture systems that possess higher order structure or functionality of the nervous system. In addition to compatibility with human cells, the concept of constructing biological architecture on a versatile functional substrate (*i.e.* a chip) provides flexibility, robustness, and efficiency in controlling system parameters, monitoring and stimulation.[18–20] Furthermore, NSCs are also highly attractive from a design and manufacturing perspective. For example, NSCs can be constructed using state of the art computer-aided design and robotic-assisted biofabrication approaches. Researchers have recently demonstrated that novel NSCs can be created using 3D printing techniques.[21] Given such desirable characteristics, highly biomimetic NSCs are now emerging to model higher order features and trajectories of the human nervous system. Here, we provide a critical review of the state of the art of NSCs. NSC designs, manufacturing approaches, and applications to pathophysiological modeling of NDDIs are comprehensively reviewed. We also highlight emerging trends and techniques, technical challenges, and future directions.

2. Bio-inspired Design of Neural Systems on a Chip for Structure, Function and Disease

Design and engineering of NSCs is non-trivial as reproducing higher order neurophysiology or -pathophysiology requires realistic modeling of human neural anatomy, circuitry, and microenvironmental parameters. Furthermore, the hierarchal structure and function of the nervous system imposes additional design challenges. As a result, NSCs are designed using a 'structure-function-disease' heuristic. The typical bio-inspired design approach is a multi-step process consisting of identifying the required anatomical and functional features needed for controlling system parameters toward a desired higher order trajectory.

2.1 Hierarchical Design toward Higher Order Neural Trajectories

NSCs typically possess one or more higher order *anatomical features*. As shown in Figure 1.A, such features include: 1) cellular heterogeneity; 2) clustering of multiple cell types; 3) spatial alignment of cell bodies; 4) spatial alignment of neurites; 5) controlled distribution of extracellular matrix (ECM); and 6) three-dimensionality. These features form the basis of complex *anatomical systems* found in NSCs. As shown in Figure 1.B, such systems include: 1) circuits of neuronal cells (*e.g.* used in Parkinson's or Alzheimer's disease modeling); 2) ensembles of neuronal and glial cells (*e.g.* used in brain tumor modeling); 3) ensembles of neuronal and other non-neuronal cells (*e.g.* used in neuromuscular junction modeling); and 4) ensembles of glial and other non-neuronal cells (*e.g.* used in models of the blood-brain barrier (BBB)).

In addition to higher order anatomical features and systems, NSCs typically possess one or more higher order *functional or augmented features.* As shown in Figure 1.C, such features include: 1) fluidic channels, 2) controlled drug release systems, and 3) electroactive components. These features serve to program and control higher order *microenvironmental parameters* including: 1) mass transport of solutes; 2) static and dynamic mechanical stresses; and 3) spatiotemporal distributions of biochemical cues. Such features also enable the stimulation and monitoring of biology. Ultimately, as shown in Figure 2, the *microenvironmental parameters* established by *functional or augmented features* govern the higher order *trajectories* of realistic *anatomical systems.* Typical trajectories include: 1) cell and neurite outgrowth; 2) cell migration; 3) cell signaling; 4) circuit mapping; 5) phenotypic outcomes; and 6) gene expression. Thus, NSCs are a disruptive cell culture platform for the study of human NDDIs. However, the extent and flexibility by which the structure-function-disease design heuristic can be implemented depends on both the scaffold design and the manufacturing approach.

3. Manufacturing Approaches for Neural Systems on a Chip

Although design widely varies, NSCs are derived from the following components: 1) microchannels, 2) microchambers, 3) functionalized microdomains, 4) ECM, and 5) cells (see Figure 3). Microchannels have diverse application-dependent function. For example, microchannels are most commonly used to guide neurite outgrowth.[22,23] In some cases, they also serve as scaffolds for assembling non-neuronal cells.[24] In addition, microchannels are commonly used for fluid handling to provide perfusion[25], gradients of biochemical cues[26], and mechanical actuation[27]. Microchambers are commonly used to spatially-isolate different cell types. Thus, they are useful components for guiding the formation of heterogeneous tissues.[28] Functionalized microdomains are commonly used to spatially-control biochemical cue distribution, such as the patterning of ECM or growth factors. As a result, they are effective for controlling cell seeding locations and neurite outgrowth directions.[29]

Importantly, the ability to both make and assemble these building blocks is highly dependent on the manufacturing approach used. Thus, since these building blocks serve as the basis for constructing higher order anatomical and functional features, the manufacturing approach has a direct impact on the type of microenvironmental parameters that can be controlled and the resultant trajectories that can be modeled. Overall, NSC manufacturing consists of three steps: design, fabrication, and integration with biology (*i.e.* surface functionalization and cell seeding). However, these steps widely differ depending on the manufacturing process used and may include: 1) manual techniques, 2) computer-aided design (CAD), 3) medical imaging, and 4) computer-aided manufacturing (CAM). As a result, the manufacturing process influences the robustness of the resultant NSC platform as discussed in greater detail in the following sections.

3.1 Manufacturing Processes

As shown in both Figure 4 and Table 1, five primary manufacturing processes are used to construct NSCs: 1) photolithography, 2) soft lithography, 3) contact printing, 4) laser patterning, and 5) 3D printing. The following techniques are briefly reviewed below.

3.1.1 Photolithography—Photolithography is a core process of microfabrication.[30] The base material for photolithography is typically silicon. As shown in Figure 4.A, the first step involves oxidizing the wafer's top surface followed by coating with a thin photoresist layer. A laser or photomask is subsequently used to selectively expose specific locations of the wafer to UV light, which initiates a photochemical reaction in the photoresist. The photoresist can then be selectively removed to expose specific locations of the wafer to subsequent chemical etchants. For example, hydrofluoric acid is often used to remove the exposed silicon oxide. The final step involves removing, also referred to as 'stripping', the remainder of photoresist from the wafer by exposure to chemical solutions, such as sulfuric acid.[30] We note that many variations to the process have been developed and reviewed elsewhere.[30,31] *Advantages:* High precision lasers and photomasks designed with CAD software allow highly precise designs. Subsequent etching or material deposition steps yield complex electronic systems. *Disadvantages:* Photoresist curing and material removal steps typically require high temperature, extreme pH, and exposure to radiation. As a result, the manufacturing process does not support *simultaneous* integration with biology during platform fabrication.

3.1.2 Soft Lithography—Soft lithography is a type of polymer casting process and has been extensively used for the fabrication of microfluidic devices.[32] The process begins with creation of a rigid mold, commonly referred to as a master, using photolithography. This process transfers the geometric pattern of the photomask to the 'master'. As shown in Figure 4.B, an elastomeric polymer, commonly polydimethylsiloxane (PDMS), is then cast onto the master and solidified via crosslinking. The solidified elastomeric material, which contains an imprint of the master, is then released from the mold. Typical features include open microchannels, chambers, pads, and pillars. To form microfluidic devices, the solid elastomeric molds are bonded to rigid substrates, such as glass or plastic, thereby forming sealed channels. *Advantages:* Soft lithography is a high precision manufacturing process due to the use of photolithography for creating the master. Thus, it enables precision manufacturing of NSC building blocks, such as microchannels. The photomask creation step is also compatible with CAD resources. *Disadvantages:* Given soft lithography utilizes photolithography for master creation, the disadvantages of photolithography apply to soft lithography. Elastomer crosslinking also typically requires elevated temperatures and is impeded by moisture. As a result, similar to photolithography, the manufacturing process does not support *simultaneous* integration with biology during platform fabrication.

3.1.3 Contact Printing—Contact printing is a material deposition process and has been used extensively for functionalizing substrates in cell culture applications. The process begins with creation of an elastomeric stamp using soft lithography. As shown in Figure 4.C, the stamp is then coated with the desired adsorbate through exposure to an analyte-containing solution. Subsequently, the stamp is brought into mechanical contact with the substrate, thereby transferring the adsorbate from the stamp to the substrate. After transfer, the stamp is removed, resulting in a substrate functionalization pattern that matches the geometric pattern of the stamp. *Advantages:* Contact printing has similar advantages to soft lithography (*e.g.* precision and compatibility with CAD). Contact printing is also compatible with a wide range of analytes as the transfer mechanism is based on adsorption.[29,33] Contact printing can also create periodic functionalized microdomains across macroscopic length scales.[33] *Disadvantages:* Repeated contact printing on the same substrate is challenging as the mechanical contact step is typically done manually. Contact printing can only be used to deposit small molecules and biomacromolecules, but faces challenges with depositing larger biologics, such as animal cells. Contact printing can only facilitate deposition of thin material layers.

3.1.4 Laser Patterning—Laser patterning in the scope of this review is a technique used to selectively bind biomolecules to a hydrogel in 3D.[34,35] Although varying mechanisms exist, all processes involve the laser-triggered reaction of photolabile groups within a hydrogel (see Figure 4.D). The most common technique is to bind mono- or di-acrylated peptides to polyethylene glycol diacrylate (PEGDA) hydrogels given PEGDA hydrogels inherently contain unreacted acrylate groups from the curing step.[34,36] Another approach involves the laser-triggered cleavage of photolabile bonds, which exposes new reactive groups for selectively bonding biomolecules to the hydrogel.[35] *Advantages:* The ability to spatially control 3D hydrogel chemistry offers unique advantages for directing cell growth in hydrogel NSCs.[35] *Disadvantages:* Only hydrogels and biomolecules functionalized with photoreactive groups can be used, which significantly limits material availability.

3.1.5 3D Printing—3D printing is a biomanufacturing process and has been used extensively in tissue engineering applications. Various types of 3D printing processes exist, including stereolithography, inkjet printing, micro-extrusion printing and laser-assisted bioprinting. A detailed description of the individual processes can be found elsewhere.[37,38] While multiple types of 3D printing processes exist, they differ in terms of material deposition mechanism, process physics, material compatibility, multi-material printing capability, manufacturing speed, and precision. As shown in Figure 4.E, the first step to 3D printing is constructing the path information that describes the motion and triggering of the printing tool (*e.g.* a laser or extruder) from a 3D digital model. The next step in 3D printing is the formulation of a printable material, commonly referred to as an "ink". Subsequently, the ink is loaded into a dispensing tool or holding reservoir and the printing process is initiated. This enables the conversion of the 3D digital model to a physical object. *Advantages:* Digital models, and thus printer path information, can be derived from medical imaging data. Unlike contact printing, 3D printing is a CAM process. This aspect affords repeatability and robustness in multi-layer and -material assembly. Certain types of 3D printing, such as micro-extrusion printing, are compatible with a diverse materials palette including thermosets, thermoplastics, composites, hydrogels, and solutions.[38] 3D printing offers a one-pot biomanufacturing approach for directly interweaving biology with scaffold and functional materials. *Disadvantages:* 3D printing is currently a serial processing technique. Thus, throughput can be limited for large parts that contain intricate path geometries. However, we note that the development of advanced dies that accommodate the simultaneous printing of multiple parts can address this limitation similar to prior advancements in injection molding processes.

4. Classes of Neural Systems on a Chip

NSCs are scaffold-based architectures. Thus, they require cell seeding either on the surface or within the bulk of an exogenous material. As a result, NSCs differ with respect to the degrees of freedom for resultant trajectories, such as growth and migration. Importantly, the desired features and the manufacturing approach influence the scaffold design. In general, NSCs fall into one of two categories: 1) surface-based designs and 2) bulk-based designs. Surface-based designs are those that seed cells *on the surface* of an exogenous material. Thus, mono- or multi-layer cell growth, cell migration, cell-cell interactions and cell-matrix interactions occurs in 2D at the solid-liquid interface between the exogenous material and growth medium. Microfluidic and compartmentalized NSCs are the most common type of surface-based designs. In contrast, bulk-based designs seed cells *within the bulk* of an exogenous material. Thus, cell growth, cell migration, cell-cell interactions and cell-matrix interactions occurs in 3D within the bulk of the growth medium-infused exogenous material. Hydrogel NSCs are the most common type of bulk-based designs.

4.1 Microfluidic Neural Systems on a Chip

Microfluidic NSCs are surface-based designs based on isolated or interacting microfluidic channels (see Figure 5). The microfluidic channels are typically rectangular and range 1 – 500 μm in width and 2 – 25 mm in length. The microfluidic channels typically have three walls that arise from the bulk polymer material (*e.g.* PDMS) and one wall (the bottom wall) that arises from the substrate (*e.g.* glass or tissue culture plastic). In some cases, the bottom wall consists of a porous membrane that enables the cells in two adjacent microchannels to chemically interact. For example, one common design involves two co-directional microchannels separated by a polycarbonate transwell membrane.[39–41] *Advantages:* The ability to use transparent materials for microfluidic construction provides ease in optical characterization and stimulation techniques. Microfluidic NSCs also support fluid handling, which has various advantages including establishing: 1) convective flow of nutrients and biochemical cues[42], 2) diffusive profiles of biochemical cues[26], and 3) mechanical cues[43], such as shear stress or dynamic scaffold deformation. Additionally, the ability to utilize a wide range of substrates, such as conductive materials[44], offers the ability to establish unique functional and augmented features for stimulation and monitoring. *Limitations:* Given microfluidic NSCs are surface-based designs, the degrees of freedom for cell growth and migration are limited relative to bulk-based designs (*i.e.* to 2D instead of 3D). High order trajectories related to or derived from 3D cell growth, cell migration, cell-cell interactions and cell-matrix interactions are difficult to model using microfluidic NSCs. Further, microfluidic NSCs typically utilize simplistic surface functionalization approaches, such as surface coating with adsorbed ECM components. Thus, it is relatively challenging to replicate native cell-matrix interactions using microfluidic NSCs.

4.1.1 Manufacturing of Microfluidic Neural Systems on a Chip—Microfluidic NSCs consist of a microchannel-containing elastomeric cast bonded to a rigid substrate. Microfluidic NSCs are typically manufactured using a combination of photolithography and soft lithography (see Sections 3.1.1 and 3.1.2, respectively).[45,46] However, we also note that 3D printing has recently emerged as a technique for manufacturing microfluidic devices and NSCs (see Section 3.1.5).[21,47–50] In cases of microfluidic NSCs that contain convective fluid flow, microneedles are inserted to the inlet and outlet ports to interface with fluid handling components and instrumentation (*e.g.* tubing and pumps).

Microfluidic NSCs are typically functionalized by first exposing the fluidic channels to ECM-containing solutions in a static or flow mode. Subsequently, the coated fluidic channels are exposed to cell suspensions in a static or flow mode to seed cells on the coated surfaces, thereby resulting in a cell mono-layer. Cellular growth strongly follows topographical cues. If required, the process may be repeated to form multi-layers in the same microchannel (*e.g.* to establish a feeder layer).

4.1.2 Hierarchical Design of Microfluidic Neural Systems on a Chip—Given the ease of designing and fabricating microchannels via soft lithography, active fluid handling capability, and flexibility with using alternative substrate materials, various higher order *anatomical features* of native neural systems can be reproduced using microfluidic NSCs (see Table 2). For example, microfluidic NSCs have been used to affect the: 1) clustering of different cell types; 2) spatial alignment of cell bodies; and 3) spatial alignment of neurites. Microfluidic NSCs have also been designed to reproduce higher order *functional and augmented features* given the ability to integrate: 1) fluidic channels, and 2) electroactive components.[25,44,51–54] For example, Bianco *et al.* developed a microfluidic system in which neuroinflammation could be simultaneously monitored by microscopy and electrophysiological recordings.[55] As a result, microfluidic NSCs offer programing and control of various higher order *microenvironmental parameters* such as: 1) convective mass transport of solutes; 2) actuation of static and dynamic mechanical stresses; 3) spatiotemporal distributions of biochemical cues; and 4) stimulation and monitoring of cells. For example, Griep *et al.* have demonstrated that shear stress (5.8×10^{-1} Pa) is an important parameter for improving endothelial cell function through tight junction formation in BBB models.[40] As shown in Figure 5.A, Park *et al.* used a microfluidic device to control spatiotemporal distributions of three different cytokines (Shh, FGF8, and BMP4) ranging from 0 – 500 ng/ml to affect the differentiation of neural progenitor cells.[56] Various studies have also used microfluidics to support the perfusion of tissues for long term studies.[42,57,58] Thus, microfluidic NSCs are useful platforms for modeling higher order *trajectories* of native neural systems such as: 1) neurite outgrowth; 2) cell migration; 3) cell signaling; and 4) gene expression. Chung *et al.* have used a microfluidic device to direct the proliferation and differentiation of human neural stem cells via gradients of epidermal growth factor, fibroblast growth factor 2, and platelet-derived growth factor established by laminar flow and diffusive mixing.[26] As shown in Figure 5.B, Booth *et al.* developed a BBB model that showed endothelial cells began to express tight junction in flowing media at 2.6 μL/min after three days via zonula occludens-1 (ZO-1) imaging.[39] Deosarkar *et al.* also showed that endothelial cells exhibited tight junction formation, as measured by the expression of ZO-1 in microfluidic BBB models, and allowed endfeet-like neonatal astrocyte-endothelial cell interactions through a porous interface.[59] Although beyond the scope of this review, we note that microfluidic tissue chips have been applied toward a number of organ systems.[18,19,60] As a result, the microfluidic-based tissue chip design is commonly referred to as an organ-on-a-chip; however, we caution the reader that the 'organ-on-a-chip' concept does not strictly apply to microfluidic designs as alternative design and manufacturing approaches now exist for constructing tissue chips (*e.g.* 3D printing[24] and molding[61]).

4.2 Compartmentalized Neural Systems on a Chip

Compartmentalized NSCs are surface-based designs based on interconnected compartmentalized culture chambers (see Figure 6). The design supports the manual addition of media to individual chambers for studies in static fluid or under gravity-perfusion. Compartmentalized NSCs typically have channels ranging from 1 – 5 mm in width and 5 – 20 mm in length for compartmentalizing cell bodies, and microchannels ranging from 1 – 5 μm in width and 100 – 1,000 μm in length for directing axonal growth or creating diffusive gradients.[22,23] For example, the most commonly used design consists of two compartmentalization channels separated by microchannels, as shown in Figure 6.A.[22,62–66] In contrast to microchannels found in microfluidic NSCs, microchannels in compartmentalized NSCs may not contain a top wall, such as in Campenot chamber designs. Similar to microfluidic NSCs, the bottom wall is formed by the underlying substrate (*e.g.* a 35 mm dish). Importantly, the primary distinction between microfluidic and compartmentalized NSCs is the absence of active fluid handling in the latter design. Compartmentalized NSCs also offer the ability to incorporate controlled release systems within the cell compartments, such as loaded hydrogels or microparticles, as the millimeter-sized chambers accommodate the manual incorporation of controlled release systems and passive release mechanisms.[62,67,68] Compartmentalized NSCs are maintained using conventional manual cell culture techniques. *Advantages:* The separation of cell chambers via microchannels enables the co-culture of multiple cell types as well as the use of multiple media types and biochemical cues in the same platform. This affords the ability to study the interaction between cells that require significantly different biochemical cues as well as to restrict cellular interaction to neurite-based signaling and transport.[29,69,70] Further, this feature enables the selective stimulation and inoculation of cells in a single chamber, which has importance for fundamental circuit mapping studies and modeling a wide range of NDDIs. *Limitations:* The type of cell-cell interactions are relatively restricted to those that occur through neurites. Similar to microfluidic NSCs, the fact that compartmentalized NSCs are surface-based designs makes high order trajectories related to or derived from 3D cell growth, cell migration, cell-cell interactions and cell-matrix interactions difficult to model.

4.2.1 Manufacturing of Compartmentalized Neural Systems on a Chip— Compartmentalized NSCs consist of a multi-chamber bonded to a rigid substrate. Depending on the design, the multi-chamber is typically composed of either PDMS or Teflon (*e.g.* Campenot chamber designs), but can also be made of soft materials, such as hydrogels. Thus, the microchannels and microchambers are typically fabricated via molding processes, as PDMS and Teflon can be molded at relatively low temperatures using soft lithography (see Section 3.1.2), extrusion, pressing or injection molding. It was also recently demonstrated that compartmentalized NSCs can be fabricated using 3D printing, which enables the rapid prototyping of microchannel and microchamber design and geometry.[24] Although the chamber-substrate bonding process is typically done manually, 3D printing approaches have recently emerged as a one-pot bottom-up biomanufacturing process for compartmentalized NSCs.[24]

Compartmentalized NSCs are typically functionalized prior to chamber-substrate bonding using standard manual surface coating approaches (*i.e.* exposure to solutions containing soluble ECM components). Thus, contact printing can also be used to create patterned functionalized microdomains prior to chamber-substrate bonding (see Section 3.1.3). Cell seeding is accomplished by conventional manual plating techniques (*i.e.* pipetting of cell suspensions into the cell compartments). Similar to microfluidic NSCs, cellular growth strongly follows topographical cues.

4.2.2 Hierarchical Design of Compartmentalized Neural Systems on a Chip— Given the ability to co-culture multiple cell types and program cell-cell interactions via guided neurite outgrowth, various higher order *anatomical features* of native neural systems can be reproduced using compartmentalized NSCs (see Table 3). For example, compartmentalized NSCs have been used to affect the: 1) formation of heterogeneous tissues; 2) clustering of multiple cell types; 3) spatial alignment of cell bodies; and 4) spatial alignment of neurites. For example, as shown in Figure 6.B, Ionescu *et al.* developed a compartmentalized NSC for study of the neuromuscular junction, which enabled the monitoring of muscle-neuron communication.[71] Berdichevsky *et al.* used a compartmentalized NSC to co-culture cortical and hippocampal neurons separated by microgrooves to monitor the development of neurite pathways.[72] A compartmentalized NSC containing a cell-laden hydrogel component was used by Shin *et al.* to co-culture endothelial and neural progenitor cells in a 3D environment allowing them to study the effect of vasculature on neural progenitor cell differentiation.[73] Compartmentalized NSCs have also been designed to reproduce higher order *functional and augmented features* given the ability to integrate: 1) controlled drug release systems, and 2) electroactive components. For example, although not a controlled drug release system, Millet *et al.* demonstrated a microfluidic-based surface functionalization approach for compartmentalized NSCs to enable the study of laminin and poly-L-lysine gradients on neurite outgrowth.[29] Johnson *et* *al.* demonstrated that 3D printing could be used to guide neurite outgrowth in compartmentalized NSCs onto conductive grids to support transmission electron microscopy measurements of single axons.[21] As a result, compartmentalized NSCs offer programing and control of various higher order *microenvironmental parameters* such as: 1) spatiotemporal distributions of biochemical cues; and 2) stimulation and monitoring of cells. For example, Johnson *et al.* developed a 3D printed compartmentalized NSC with spatially segregated distributions of nerve growth factor and Schwann cell media to study Schwann cell-axon self-assembly and axon-to-cell viral spread.[24] Ch'ng *et al.* used a compartmentalized NSC to study the spread of viral infection between neuronal and epithelial cells via axonal transport.[74] Thus, compartmentalized NSCs are useful platforms for modeling higher order *trajectories* of native neural systems such as: 1) neurite outgrowth and tissue self-assembly; 2) cell migration; 3) cell signaling; 4) circuit mapping, and 5) gene expression profile. For example, one of the most common uses of compartmentalized NSCs is to establish aligned neurite outgrowth. This is a useful technique for both studying neurite physiology and pathophysiology, such as structure, transport, degradation, regeneration, depolarization, as well as establishing neural circuits.[75] Southam *et al.* directed neurite outgrowth from a neuronal and glial compartment into a chamber containing skeletal muscle cells to form neuromuscular junctions.[28] Liu *et al.* used a compartmentalized NSC to track pseudorabies virus transport between cell bodies and axons.[69] A similar compartmentalized NSC was used by Taylor *et al.* to track viral spread from infected neurons along neurites through size exclusive barriers into a separate epithelial cell-containing compartment using fluorescence microscopy.[76] Bérangère *et al.* have shown that compartmentalized NSCs can also be used to study the effect of biochemical cues on neurite pathophysiology, such as the dying back of axons exposed to β-amyloid (Aβ).[77] Compartmentalized NSCs have also provided useful platforms for investigating the effect of mechanical injury on neural system components. Siddique *et al.* developed a compartmentalized NSC that supports co-culture of spinal cord and peripheral nerves for studying the effect of growth factors on axonal regeneration following mechanical injury.[78] Koyuncu *et al.* showed although pseudorabies virus induces protein synthesis to enable retrograde virus transport, damaging axons prior to infection decreases virion transport, suggesting that virus particles and damage signals compete for retrograde transport.[79] Shin *et al.* used gene expression analysis to study the effect of neural progenitor-endothelial cell interaction on neural progenitor cell morphology and differentiation.[73]

4.3 Hydrogel Neural Systems on a Chip

Hydrogel NSCs are bulk-based designs involving the growth of suspended cells within a 3D hydrogel scaffold (see Figure 7). Hydrogel NSCs are typically a few hundred micrometers in thickness and may extend millimeters to centimeters in width. As shown in Table 1, hydrogel based NSCs currently make up less than 10% of research efforts, however, this is likely to increase with the emergence of biomanufacturing approaches, such as 3D printing. Hydrogels are formed from either synthetic or natural polymers. This feature enables the selection and design of hydrogels to mimic the native human ECM of neural tissue. For example, many naturally derived hydrogels used for NSCs are based on collagen, hyaluronic acid, or matrigel.[50,80,81] Primary cells and cell lines are used to construct hydrogel NSCs. Hydrogel NSCs are also compatible with spheroids, which enables further design of biomimetic cell-cell interactions. *Advantages:* Hydrogel NSCs are 3D architectures. Thus, they enable the study of high order trajectories related to or derived from 3D cell growth, cell migration, cell-cell interactions and cell-matrix interactions.[35] An important distinguishing feature of hydrogel NSCs is the flexibility to design heterogeneous tissues through: 1) the suspension of multiple cell types within a single hydrogel phase, 2) the adjacent crosslinking of cell-laden hydrogels that contain different cell types, or 3) the adjacent crosslinking of hydrogels that contain different ECM compositions. Such features enable hydrogel NSCs to examine the effect of multiple higher order parameters, such as ECM composition and biochemical cue distributions, on the growth of heterogeneous neural tissues. Given hydrogel NSCs consist of 3D architectures and are derived from extrudable cell- and biochemical cue-laden hydrogels, 3D printing approaches can be leveraged to model novel higher order trajectories of the human nervous system. *Limitations:* The physical dimensions of hydrogel NSCs are constrained by the ratio of the bioconversion rate to the diffusion rate, which can be described by the Damköhler number. For hydrogel NSCs, the effective diffusivity of the rate-limiting substrate in the cell-laden hydrogel limits the maximal thickness to a couple hundred micrometers. Thus, hydrogel NSCs that exceed this value will develop an internal necrotic zone extending from the center of the hydrogel to a certain critical distance over which the concentration of the limiting substrate is below the threshold to sustain cell viability. To overcome this barrier, efforts are now ongoing to vascularize cell-laden hydrogel matrices.[82]

4.3.1 Manufacturing of Hydrogel Neural Systems on a Chip—Hydrogel NSCs are composed one or more compositionally-unique 3D hydrogel domains. Cells are first propagated to reach high cell density and subsequently suspended in a nutrient-rich uncrosslinked hydrogel. Given uncrosslinked hydrogels are typically incapable of maintaining free-standing 3D structures, molding approaches are commonly used. For example, an uncrosslinked cell-laden hydrogel is first added to the mold cavity, subsequently crosslinked, and then removed. In addition to molding, 3D printing can also be used to additively assemble 3D hydrogel structures (see Section 3.1.5). 3D printed hydrogel NSCs are first constructed by developing printable hydrogel bio-inks that contain the desired cell type and biochemical cues. Subsequently, the 'bio-inks' are loaded into dispensing tools, such as cartridges syringes, or cast on energy absorbing plates for 3D printing. 3D printing provides a one-pot manufacturing process for hydrogel NSCs as it is possible to print both support materials and bio-inks using the same printing system.[38]

In contrast to microfluidic and compartmentalized NSCs, which require post-processing steps to integrate biology through functionalization and cell seeding steps, hydrogel NSCs do not require such post-processing. This unique aspect of hydrogel NSCs arises due to bio-compatible manufacturing (*i.e.* biomanufacturing) processes, such as 3D printing.

4.3.2 Hierarchical Design of Hydrogel Neural Systems on a Chip—Given the ability to additively assemble hydrogels via 3D printing, use hydrogels as carriers for multiple components (*e.g.* cells and biochemical cues), and assemble adjacent hydrogel systems of unique composition, various higher order *anatomical features* of native neural systems can be reproduced using hydrogel NSCs (see Table 4). For example, hydrogel NSCs have been used to affect the: 1) formation of heterogeneous tissues; 2) clustering of multiple cell types; 3) controlled distribution of extracellular matrix; and 4) construction of three-dimensional systems. Hydrogel NSCs also contain higher order *functional and augmented features* given the ability to integrate: 1) fluidic channels, and 2) controlled drug release systems. For example, Lee *et al.* combined a microfluidic flow chamber with a hydrogel NSC to model gliomas.[25] These functional features enable the programing and control of various higher order *microenvironmental parameters* such as: 1) mass transport of solutes (*e.g.* gases and biomolecules) and 2) spatiotemporal distributions of biochemical cues (*e.g.* rate and profile). For example, in that same study, Lee *et al.* leveraged fluidic flow through hydrogels to control the mass transport of fresh media and growth factors to glioma cells.[25] As shown in Figure 7.A, Luo *et al.* have shown that hydrogel NSCs can be created with controlled distributions of biochemical cues for directing cell growth.[35] Thus, hydrogel NSCs are useful platforms for modeling higher order *trajectories* of native neural systems, such as: 1) tissue self-assembly; 2) neurite outgrowth, 3) cell migration; 4) phenotypic outcomes, and 5) gene expression. For example, as shown in Figure 7.B, Lozano *et al.* demonstrated hydrogel NSCs composed of biomimetic layered brain-like structures could be used to examine the neurite outgrowth from cortical neurons between adjacent hydrogels.[50] Gu *et al.* demonstrated 3D printed hydrogel NSCs could be used to examine the differentiation of neural progenitor cells into neurons and glia using gene expression analysis as shown in Figure 7.C.[81]

5. Applications of Neural Systems on a Chip

NSCs have various applications given their ability to model a variety of higher order physiological and pathophysiological phenotypes and trajectories of the human nervous system. The applications of NSCs can be broadly classified as: fundamental research (*e.g.* modeling of complex neural systems or disease phenotypes), drug discovery (*e.g.* creating biomimetic models of NDDIs derived from human cells as high throughput drug screening platforms), or personalized medicine (*e.g.* creating patient-specific disease models as personalized drug screening platforms). As a result, NSCs have a significant impact on basic, translational (*i.e.* applied), and clinical research in neuroscience, neurology, and neural engineering. Below, we highlight the application of NSCs to various disease, disorder, and injury models.

5.1 Alzheimer's Disease

Alzheimer's disease (AD) is a prevalent and serious neurological disorder and is currently the sixth leading cause of death in the US.[5] Our current understanding of AD suggests it is driven by extracellular deposition of Aβ and intracellular accumulation of tau proteins.[83] As a result, the presence of Aβ plaques, tau tangles, oxidative stress, and brain inflammation are hallmark characteristics of AD pathology.[83] Researchers are now using NSCs to create platforms to study higher order pathophysiological trajectories associated with AD. As shown in Figure 8.A for example, Song *et al.* used a compartmentalized NSC to show that Aβ is transmitted through neural connections toward an attempt to improve our understanding of the mechanism by which Aβ plaques lead to loss of synapsis.[65] Stoothoff *et al.* used a compartmentalized NSC to show that differences in tau levels change the mitochondrial distribution within a cell and affect axon transport dynamics.[68] Kunze *et al.* developed a microfluidic NSC that allowed them to spatially control the concentration of hyperphosphorylated tau proteins throughout a cell population, thereby creating an AD model with co-cultured "healthy" and "diseased" tissues.[84] Choi *et al.* used a microfluidic NSC to determine that Aβ fibrils had little neurotoxic effect, but oligomeric Aβ assemblies resulted in atrophy.[85] A microfluidic NSC designed by Park *et al.* based on applying interstitial flow to 3D neurospheroids showed that Aβ was significantly more destructive under flowing conditions than static conditions.[43]

5.2 Parkinson's Disease

Parkinson's disease (PD) affects over 10 million people worldwide.[86] Our current understanding of PD suggests it is driven by the progressive impairment and deterioration of dopaminergic neurons in the substantia nigra.[87] As a result, the presence abnormal protein aggregates, known as Lewy bodies, are hallmark characteristics of PD pathology.[87] Although still emerging, researchers are now using NSCs to create platforms to study higher order pathophysiological trajectories associated with PD. For example, Lu *et al.* fabricated a compartmentalized NSC to study 6-hydroxydopamine-mediated axonal degradation, which proceeds the degeneration of dopaminergic neurons in PD.[88] This NSC also allowed researchers to study mitochondrial transport dynamics in conditions replicating PD.[88]

5.3 Traumatic Brain or Nerve Injury

TBI, spinal cord injuries, and peripheral nerve injuries affect millions of people annually in the US.[1] Further, nerve injuries are especially challenging to model and treat given the wide variations in anatomy, injury mechanism, and regenerative pathways among the brain, spinal cord, and peripheral nerves. Importantly, NSCs are useful platforms for studying nerve injuries because they allow researchers to induce injuries to higher order neural anatomies and directly monitor trajectories associated with both injury and regeneration. For example, Hosie *et al.* used a compartmentalized NSC to isolate soma and axons toward the study of site-directed glutamate excitotoxicity in TBIs.[63] As shown in Figure 8.B, Siddique *et al.* have developed a compartmentalized NSC that allows researchers to manually induce nerve injuries and administer isolated treatments to axonal components in a biomimetic 3D environment.[78] Ghannad-Rezaie *et al.* created a microfluidic NSC that enabled live imaging of the neural response to peripheral axonal injury in *Drosophila* larvae.[89] Yin *et al.* developed a microfluidic NSC to study and optimize drug candidate dosages for nerve regeneration toward the goal of minimizing harmful side effects, such as tumors.[90]

6. Emerging Areas and Future Directions

6.1 3D Printing

3D printing has enabled developments across a wide range of disciplines, including electronics, materials science, and tissue engineering, and is now poised to reconceptualize the design and engineering of NSCs. For example, although soft lithography is the gold standard for creating microfluidics and microchannels, 3D printing now offers the ability to construct microfluidic networks of complex 3D geometry within a wide range of materials.[47,48] As shown in Figure 7.C, Gu *et al.* demonstrated that 3D printed neural progenitor cells in hydrogel bio-inks can be differentiated *in situ* to synapse-forming predominantly gamma-aminobutyric acid (GABA)-expressing neurons.[81] Johnson *et al.* used micro-extrusion 3D printing to construct multi-material compartmentalized NSCs containing microfluidic channels of complex geometry and embedded electroactive components, see Figure 9.A.[21] In that study, they also demonstrated viable printing of a wide range of cell types including primary embryonic neurons.[21] Another advantage of 3D printing is the ability to use medical imaging techniques, such as computed tomography (CT),[91] magnetic resonance imaging (MRI),[91] or structured-light scanning (SLS),[92] to reverse engineer anatomical geometry that would be otherwise be difficult to design and manufacture using traditional approaches. Of all 3D printing techniques, micro-extrusion 3D printing in particular lends itself to developing NSCs, due to its compatibility with processing the most expansive materials set, including solutions, cell suspensions, cell-laden hydrogels, thermoplastics, thermosets, elastomers, and composites.[38] 3D printing is also able to create 3D heterogeneous biomimetic neural tissues containing distributed ECM proteins and growth factors through the ability to control the composition of individually printed bio-inks.[38] For example, the ability to spatially distribute bio-inks containing different formulations of biochemical cues in 3D scaffolds via 3D printing was recently shown to selectively direct the growth of sensory and motor nerves.[92]

6.2 Electronic Augmentation

Creating next-generation NSCs will require the seamless integration of electroactive components for enhanced stimulation and monitoring functionality with neural anatomy. To date, efforts toward this goal have been achieved by integrating neural tissue with microelectrode arrays (MEAs). For example, Kanagasabapathi *et al.* have created MEA-coupled compartmentalized NSCs for monitoring cortical and thalamic cell connectivity.[53,54] Various studies have also used MEA-coupled microfluidic NSCs to study the effect of biochemical cues on neural networks.[44,51,52] Smith *et al.* have created a cantilever-based NSC that measures muscle contraction following the stimulation of a motoneuron.[93]

6.3 Human Cells

A major advantage of NSCs is their compatibility with human cells. Although the majority of NSCs to date have been constructed using non-human cells, human NSCs (*i.e.* NSCs constructed with human cells) are beginning to be explored. For example, Griep *et al.* used a human brain endothelial cell line (hCMEC/D3) for BBB applilcations.[40] Lee *et al.* implemented a human glioma cell line (A-172) into their microfluidic/hydrogel NSC to study the migration of glioma cells in 3D.[25] Stoothoff *et al.* used H4 human neuroglioma cells to study mitochondrial axonal transport for AD applications.[68] Yeon *et al.* cultured primary human umbilical vein endothelial cells (HUVEC) and human astrocytes for BBB drug permeability studies.[94] The ability to use human cells provides unique opportunities for preclinical drug testing, such as target identification, target validation, target-based screening, phenotypic screening, pharmacodynamics, pharmacokinetics, absorption-distribution-metabolism-excretion (ADME) studies, and toxicology testing. Thus, human NSCs provide novel platforms that could reduce the cost and time associated with drug discovery for NDDIs. For a detailed discussion, we refer the reader elsewhere to comprehensive reviews on the application of tissue chips to drug discovery.[19] The ability to utilize human stem cells is also an emerging area, which we discuss in greater detail in the following sections.

6.4 Personalized Medicine

Tissue chips have been suggested to enable future paradigms of personalized medicine and pharmacology.[19] Likewise, the ability to construct NSCs from patient-derived cells now potentially enables the 'personalized' treatment of NDDIs via target-based or phenotypic screening conducted using patient-specific NSC disease models. For example, the ability to model higher order pathophysiological phenotypes and trajectories of the human nervous system could establish highly effective treatments.[95] The continued evolution of computer-aided biomanufacturing processes, such as 3D printing, also provides novel opportunities for customization and prototyping of patient-specific NSC disease models. Ultimately, given the sustained demand for personalized medicine,[95,96] NSCs are expected to play an integral role in the future personalized treatment of NDDIs. The ability to utilize human stem cells is also critical for developing patient-specific NSCs, which we discuss in greater detail in the following sections.

6.5 Biomimicry

In vivo human neural systems consist of cells growing in soft 3D ECM in the presence of both immobilized and diffusive spatiotemporal distributions of biochemical cues. Cells also interact with 3D multi-scale topographical cues. Native neural systems are also influenced by mechanical factors, such as pulsatile fluid flow as well as vasodilation and vasoconstriction effects. Further, native neural tissue has highly controlled mechanical property matching. For example, with the exception of the meninges, neural tissue does not grow in direct contact with highly rigid materials *in vivo*. Lee *et al.* have approached this by coupling a glioma laden hydrogel with a microfluidic device, as shown in Figure 9.B.[25] Biomimetic NSCs should strive to possess each of the above features. Unfortunately, the vast majority of NSCs developed to date contain only one, or at most a few, of the aforementioned features. Thus, achieving realistic and balanced biomimicry of the *in vivo* nervous system in NSCs must be improved to achieve the most successful translational and clinical outcomes.

6.6 Stem Cells

The ability to construct NSCs from stem cells offers unique opportunities for studying the development and regeneration of neural systems, developing biomimetic models of human NDDIs, and creating personalized NSCs. Specifically, induced pluripotent stem cells (iPSCs)[97] provide useful tools for such applications. For example, the 'holy grail' in biomedical research is to generate a transgenic mouse model of the human illness. The MECP2 mutation in Rett syndrome or the mutant gene causing Huntington's disease are excellent examples where animal models reproduce salient features of the disease. Unfortunately, the majority of human neurological and neuropsychiatric illnesses are believed to be poly-genetic, consisting of multiple, and often unknown, gene alterations, making the generation of transgenic animals highly challenging. However, iPSCs are beginning to come to the rescue. For example, fibroblasts can be readily harvested from patients and induced to form neurons or glial cells in the dish to study their molecular changes. This approach has recently been used to reveal unexpected changes in neuron complexity in Costello syndrome,[98] a rare developmental disorder with autism traits, suggesting that NSCs derived from human iPSCs provide novel opportunities for modeling rare and complex human NDDIs. Given iPSCs are also patient-derived, NSCs constructed using iPSCs may also serve as personalized drug screening platforms where libraries of drugs can be examined regarding their ability to correct a protein or signaling pathway deranged by disease. Finally, NSCs constructed from iPSCs may eventually serve as platforms for differentiating and programming cells for neural regeneration and other cell-based therapies. For example, such cells could be differentiated into the cell of interest and corrected to express the right complement of genes in NSCs, and subsequently be collected for implantation. Since the iPSCs were initially harvested from the patient, the resultant autologous graft or cell-therapy will not elicit an immune response (*e.g.* immune rejection). In addition, the ability to construct NSCs from stem cells offers unique opportunities for understanding the development and regeneration of the nervous system. For example, NSCs constructed using neural progenitor cells derived from human embryonic stem cells have been used to study the effect of biochemical cues on the differentiation and formation of complex neurite networks.[56]

7. Technical Hurdles, Remaining Challenges and Opportunities

Although NSCs have progressed significantly over the past decade, there are still major technical hurdles and remaining challenges to overcome. It is established that cells exhibit different trajectories in 2D vs. 3D environments.[99–101] However, we still face significant manufacturing challenges associated with embedding functional and augmented features, such as fluidic channels and electroactive components, in 3D. Another challenging technical hurdle is to simultaneously program and control multiple microenvironmental parameters toward mimicking or reproducing signaling cascades. This is a critical requirement for modeling higher order trajectories associated with developing nervous systems and NDDIs. The use of NSCs for drug discovery and personalized medicine applications also involves regulatory considerations. For example, the future use of NSCs as alternatives to small animal models for preclinical drug testing may require steps equivalent to Animal Model Qualification, which is required by the FDA to rely on the evidence from animal studies regarding drug effectiveness. Alternatively, use of NSCs as patient-specific disease models intended for the diagnosis or treatment of NDDIs would subject NSCs to the regulatory requirements of biomedical devices.[102] Ultimately, both the translational impact and the regulatory barriers of NSCs are tied to the challenge of creating highly robust and reproducible NSCs. However, as noted in Sections 3 and 4, the vast majority of NSCs, with the exception of 3D printed NSCs, involve manual assembly, functionalization, or seeding steps. Thus, the creation of robust NSCs hinges on eliminating manual processing steps toward fully automated biomanufacturing processes. As discussed in Section 6, realistic biomimicry is a major driving force for NSC design. Specifically, mimicry of vascularized brain tissue remains a critical challenge. Additionally, the coupling of NSCs to hemodynamic processes, such as hemoglobin-based oxygen transport or lipoprotein-based lipid uptake, is required to advance NSCs.

8. Conclusions

NSCs appear poised to shift the paradigm for modeling human NDDIs. The ability to model higher order anatomical features, functional and augmented features, microenvironmental parameters, and ultimately, trajectories of the human nervous system is highly dependent on the NSC design (*e.g.* microfluidic, compartmentalized, or hydrogel NSCs). Emerging biomanufacturing processes, such as 3D printing, are now enabling the design and manufacturing of robust novel NSCs. The field of NSCs is currently in a developmental stage heading toward increased biomimicry, functional-augmentation, and personalized medicine and pharmacology. Opportunities exist in terms of addressing various technical and regulatory hurdles that remain toward NSC application to drug discovery and personalized medicine, including achieving more realistic biomimicry of the human nervous system and robustness in NSC manufacturing approaches.

Acknowledgments

The presented work was supported by the National Science Foundation (NSF) grant number NSF CBET-1650601.

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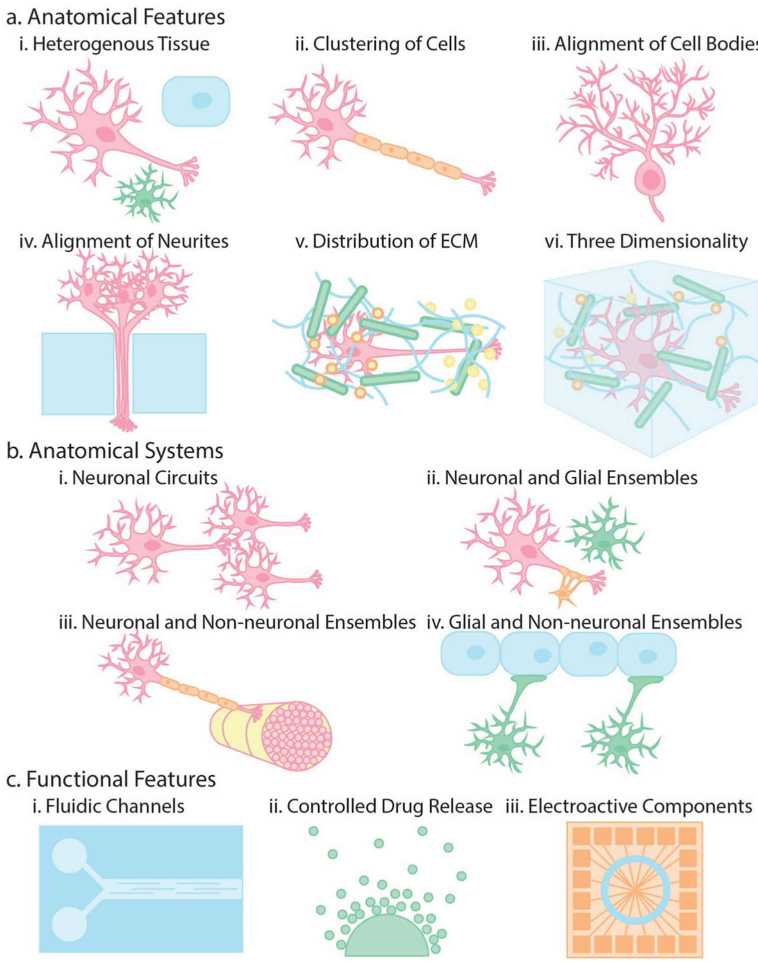

Fig. 1.

Hierarchical design of neural systems on a chip toward assembly of higher order functional neural anatomical systems. Anatomical features (**a**) are assembled into anatomical systems (**b**) which ultimately contain functional features (**c**) that control microenvironmental parameters.

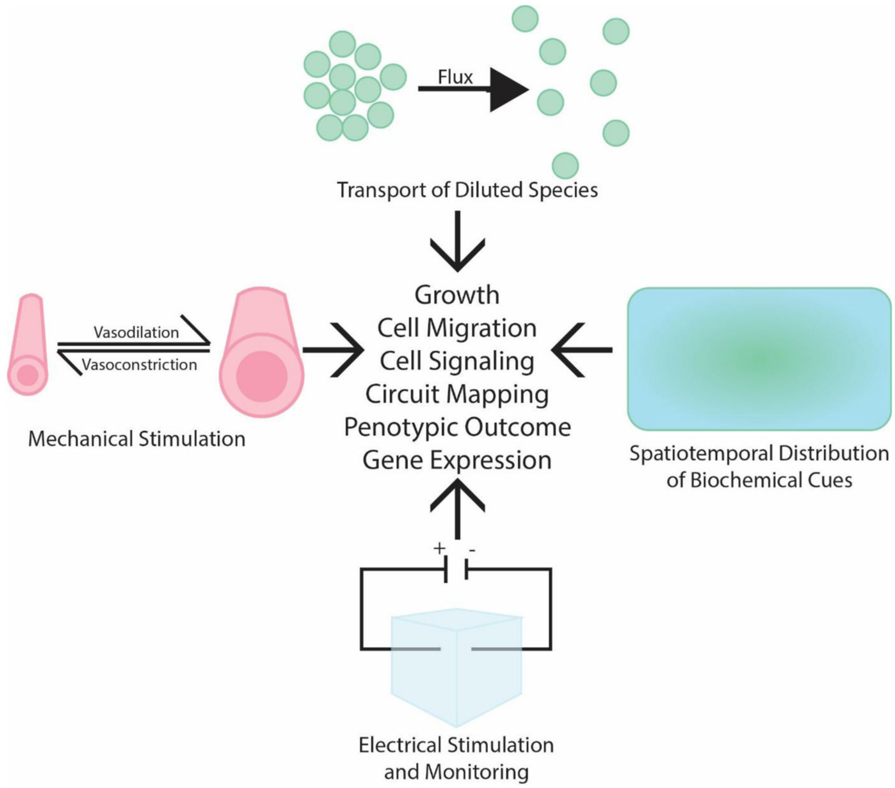

Fig. 2.

Hierarchical design of neural systems on a chip toward control of neural microenvironmental parameters and modeling of higher order trajectories. Control over microenvironmental parameters, such as transport of diluted species, mechanical stimulation, electrical stimulation, and spatiotemporal distribution of biochemical cues, through functional and augmented features, allows NSCs to model higher order trajectories of the human nervous system.

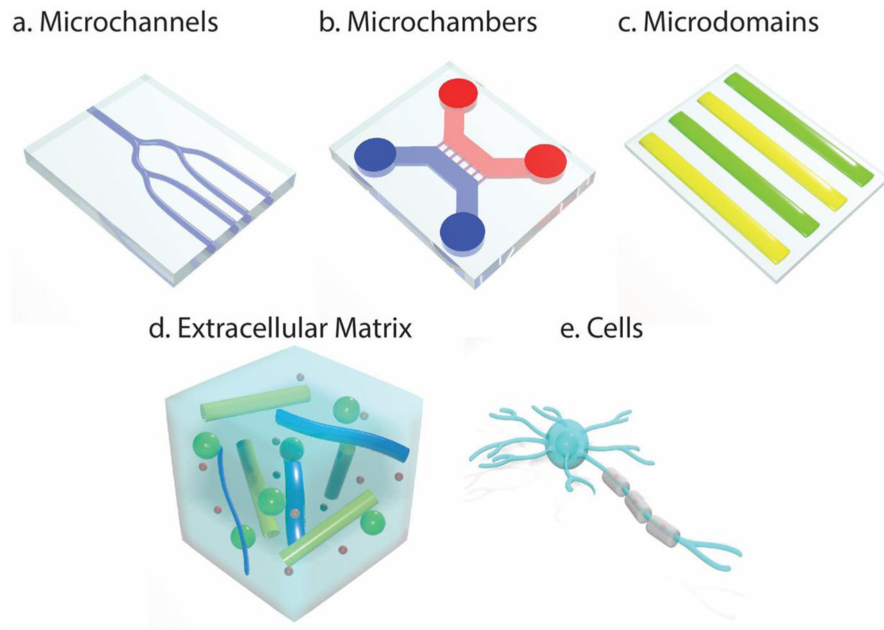

Fig. 3.

The fundamental building blocks of neural systems on a chip include microchannels (**a**), microchambers (**b**), functionalized microdomains (**c**), extracellular matrix (**d**), and cells (**e**).

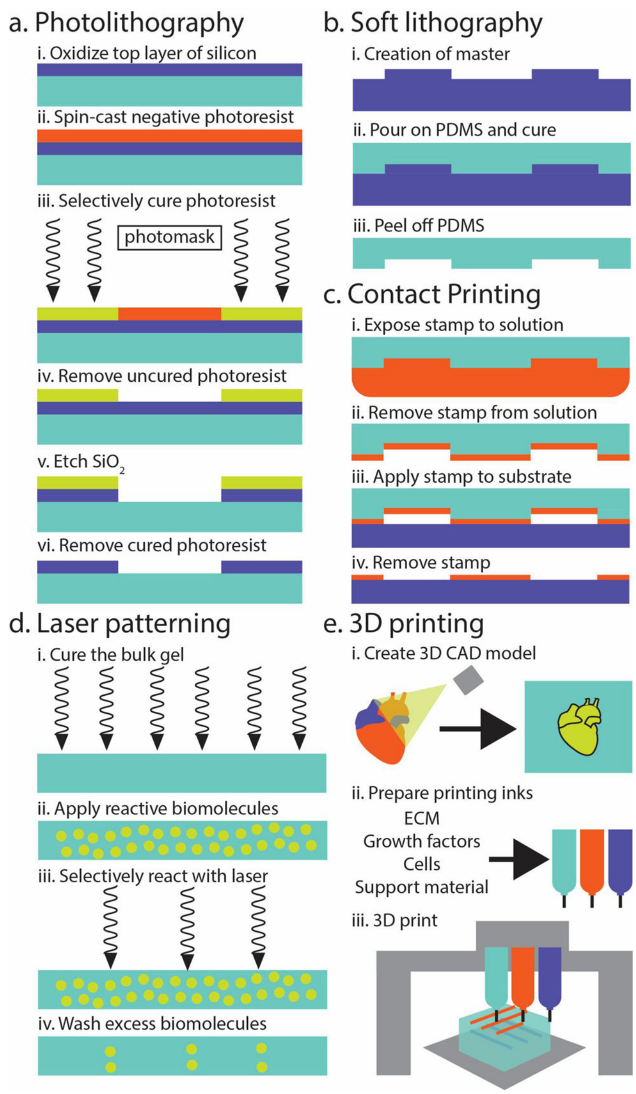

Fig. 4.

Schematics of the five commonly used neural system on a chip manufacturing techniques: photolithography (**a**), soft lithography (**b**), contact printing (**c**), laser patterning (**d**), and 3D printing (**e**).

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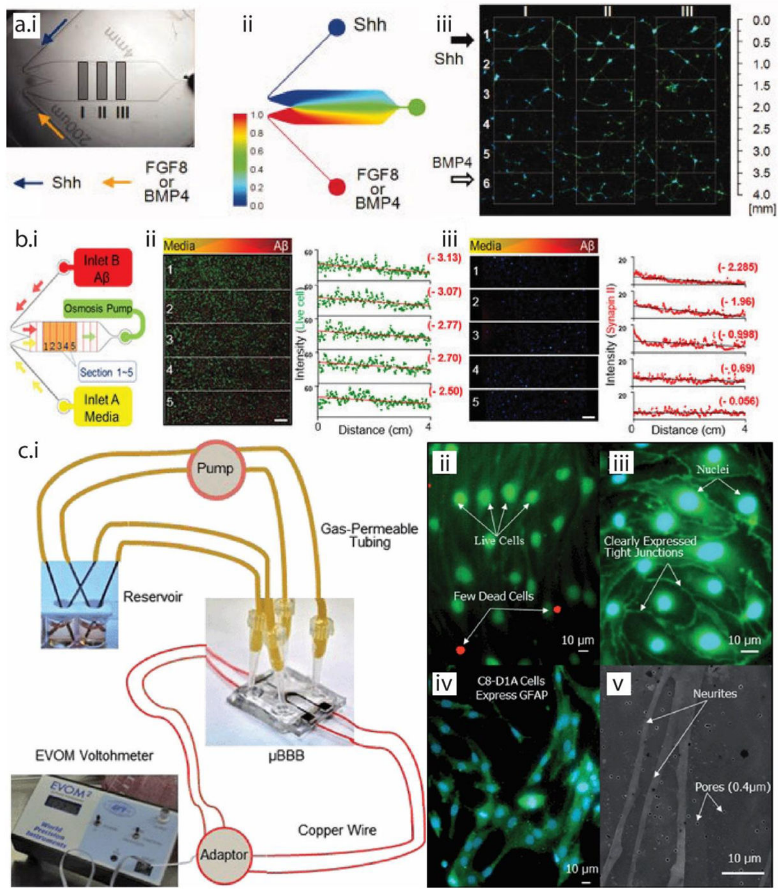

Fig. 5.

Highlights of microfluidic neural systems on a chip (NSCs). **a**) Microfluidic NSC for studying differentiation of neural progenitor cells under the influence of chemical gradients. *i*) Schematic of a Shh/FGF8 or Shh/BMP4 gradient microfluidic device; *ii*) visualization of gradient; and *iii*) immunoassay of TuJ1 to quantify cell clusters and neurite bundles in the device. Reprinted with permission.[56] Copyright John Wiley & Sons 2009. **b**) Microfluidic NSC for Alzheimer's disease studies. *i*) Schematic of β-amyloid gradient device; *ii*) live/dead assays of sections 1–5 with intensity plots and slopes shown on the right; and *iii*) imaging of synapsin-ii distribution. Reprinted with permission.[85] Copyright Nature Publishing Group 2013. **c**) Microfluidic NSC for modeling the blood-brain barrier (BBB). *i*) Schematic of the BBB; *ii*) live/dead stain of endothelial cells; *iii*) immunoassay of tight junction ZO-1 in endothelial cells; *iv*) immunoassay of GFAP in astrocytes; and *v*) environmental scanning electron micrograph of astrocytes. Reprinted with permission.[39] Copyright Royal Society of Chemistry 2012.

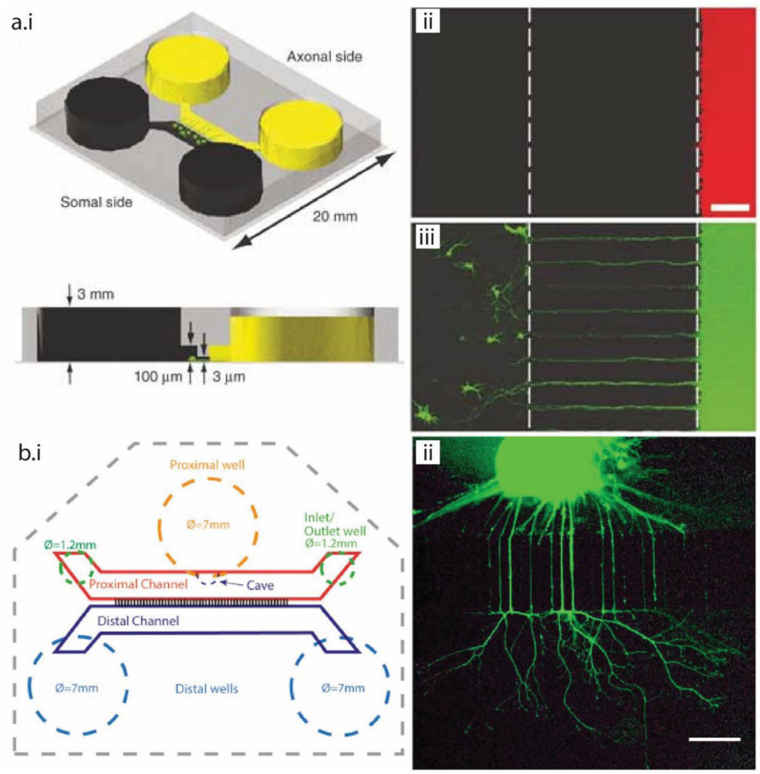

Fig. 6.

Highlights of compartmentalized neural systems on a chip (NSCs). **a**) Compartmentalized NSC for central nervous system axonal injury, regeneration and transport. *i*) Prismatic and cross-sectional views of a somal – axonal compartmentalized NSC; *ii*) demonstration of fluidic isolation with Texas Red dye, scale bar is 100 µm; and *iii*) application of Green Cell Tracker to the axonal side with backtracked identification of neurons in the somal chamber. Reprinted with permission.[22] Copyright Nature Publishing Group 2005. **b**) Compartmentalized NSC for the neuromuscular junction. *i*) Schematic of a compartmentalized NSC for development of NMJs; *ii*) spinal cord motoneurons plated in the proximal channel extend axons into the distal channel to contact myotubes. Reprinted with permission.[71] Copyright Elsevier 2016.

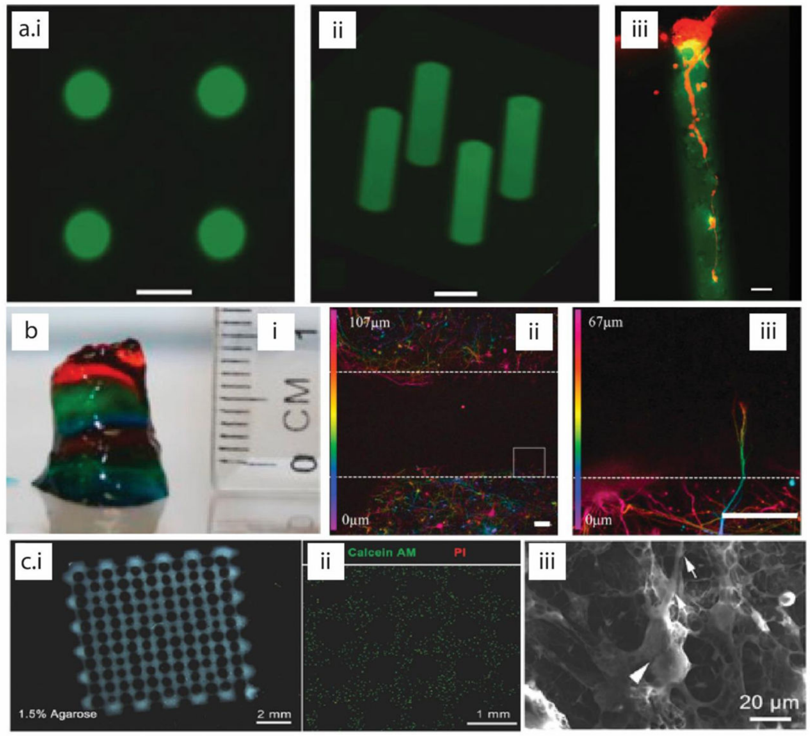

Fig. 7.

Examples of hydrogel neural systems on a chip (NSCs). **a**) Hydrogel NSC containing photolabile properties for study of 3D cellular migration. Top down (*i*) and prismatic (*ii*) images of fluorescently labeled oligopeptide channels within a 3D hydrogel – scale bars are 200 μm; and *iii*) primary rat dorsal ganglia growing exclusively within a GRGDS peptide modified column – scale bar is 100 μm. Reprinted with permission.[35] Copyright Nature Publishing Group 2004. **b**) Hydrogel NSC for modeling cortical neuron outgrowth in brain-like environments. *i*) 3D printed layered brain-like structure; *ii*) confocal image of neurons after 5 days – scale bar is 100 μm; and *iii*) magnified image of area inside square showing axonal projection into the cell-free gel – scale bar is 100 μm. Reprinted with permission.[50] Copyright Elsevier 2015. **c**) Hydrogel NSC for study of neural progenitor cell differentiation. *i*) Neural progenitor cell-laden 3D printed porous hydrogel structure; *ii*) live/dead assay of hydrogel construct; and *iii*) scanning electron micrograph of a neuron in the 3D structure with the arrows indicating the soma and axon, respectively. Reprinted with permission.[81] Copyright John Wiley & Sons 2016.

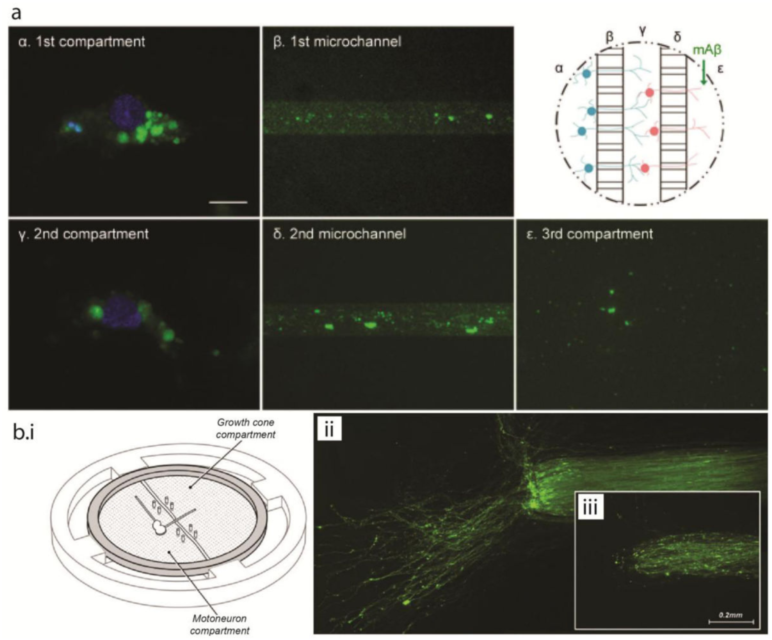

Fig. 8.

Applications highlights of neural systems on a chip (NSCs) for modeling neurological diseases, disorders and injuries. **a**) A compartmentalized NSC for studying long-distance transport of β-amyloid for better understanding Alzheimer's disease, with a schematic showing three compartments and fluorescent images the transport of fluorescein isothiocyanate – tagged β-amyloid monomer across all three compartments. Reprinted with permission.[65] Copyright John Wiley & Sons 2014. **b**) A compartmentalized NSC for studying peripheral nerve repair. *i*) Schematic of a compartmentalized NSC for manipulating, injuring, or treating isolated neurites; *ii*) regeneration of an untreated axonal injury; and *iii*) degeneration following the same axonal injury with the application of Nocodazole. Scale bar is 0.2 mm. Reprinted with permission.[78] Copyright Elsevier 2014.

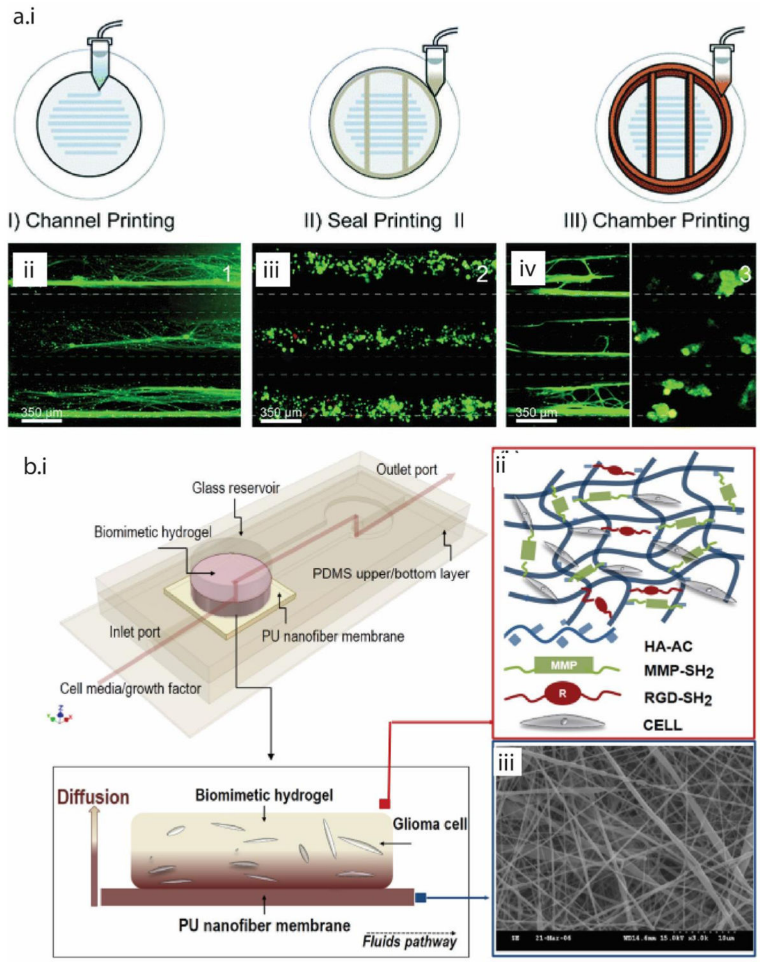

Fig. 9.

Highlights of emerging areas and future directions for neural systems on a chip (NSCs). **a**) 3D printed NSCs. *i)* Schematic of 3D printing process for a compartmentalized NSC; *ii*) three parallel microchannels with neurons and axons shown in the first chamber; *iii*) axons from the first chamber associated with self-assembled Schwann cells within the second chamber; and *iv*) axon termini from the first and second chamber interacting with epithelial cells in the third chamber. Reprinted with permission.[21] Copyright Royal Society of Chemistry 2015. **b**) Hydrogel NSC for modeling brain tumors. *i*) Prismatic and side view schematics of a microfluidic chip coupled with a glioma-laden hydrogel; *ii*) structure of the biomimetic hydrogel; and *iii*) a SEM image of the electrospun fiber separating the microfluidic channel from the hydrogel. Reprinted with permission.[25] Copyright John Wiley & Sons 2014.

Table 1

Summary of neural systems on a chip. Abbreviations: Hippocampal Neuron (HN), Cortical Neuron (CN), Motoneuron (MN), Neural Progenitor (NP), Schwann Cells (SC), Epithelial Cells (EC), Thalamic Neuron (TN), Peripheral Neuron (PN), Myoblast (MB), Astrocyte (AC), Oligodendrocyte (OC), Striatal Neuron (SN), Spinal Neuron (SPN), Myocyte (MC), Peripheral nervous system (PNS), Central Nervous System (CNS), External Tissue (EXT), Midbrain Dopaminergic Neuron (mDAN), Microglia (MG), Microelectrode Array (MEA), Polydimethylsiloxane (PDMS), Polycaprolactone (PCL), Polycarbonate (PC), Poly(ether imide) (PEI), Polytetrafluoroethylene (PTFE), Poly(ethylene glycol) diacrylate (PEGDA). Asterisk (*) indicates photolithography was also used.

Cell Types	Compartmentalization	Tissue	Design	Manufacturing	Materials	Length	Application	Ref.
CN	Somal - axonal	Brain	Compartmentalized/Hydrogel	Soft lithography	PDMS, Matrigel	cm	Function	67
CN	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	cm	Function	103
CN, HN	CN - HN	Brain	Compartmentalized	Soft lithography	PDMS	cm	Disease	77
CN	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	mm	Disease/Injury	63
MN, MB	MB, axonal - somal	PNS/EXT	Compartmentalized	Soft lithography	PDMS	mm	Function	71
HN, SPN, SC, EC	Somal - axonal, SC - EC	Brain	Compartmentalized	3D printing	PCL	cm	Function	24
Neurons	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	cm	Function	23
SCGN, EC	Somal - axonal, EC	PNS/EXT	Compartmentalized	Soft lithography	PDMS	mm	Disease	69
mDAN	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	mm	Disease	104
HN	Somal - axonal	Brain	Compartmentalized	Contact Printing	PDMS	μm	Function	29
Neurons	None	Brain/PNS	Compartmentalized	Soft lithography	PDMS	mm	Function	64
CN, OC	Somal - axonal, glia	Brain	Compartmentalized	Soft lithography	PDMS	mm	Function	105
CN, OC, AC	Somal - axonal, glia	Brain	Compartmentalized	Soft lithography	PDMS	mm	Function	75
Neurons	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	mm	Function	62
CN, SN	CN - SN	Brain	Compartmentalized	Soft lithography	PDMS	cm	Function	70
EC, NP	EC - NP - EC	Brain/EXT	Compartmentalized	Soft lithography	PDMS, Collagen	mm	Function	73
PN, MN	MN and PN - PN	PNS	Compartmentalized	Soft lithography	PDMS, PC	cm	Injury	78
CN	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	mm	Disease	65
MN, AC, OC, MG, MC	glia and MN - MC	PNS/EXT	Compartmentalized	Soft lithography	PDMS	mm	Disease	28
Glioma, CN	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	cm	Disease	68
CN, HN	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	cm	Injury	22

Cell Types	Compartmentalization	Tissue	Design	Manufacturing	Materials	Length	Application	Ref.
HN	Somal - axonal	Brain	Compartmentalized	Soft lithography	PDMS	cm	Function	66
MN, MB	MB, axonal - somal, MB	PNS/EXT	Compartmentalized	Soft lithography	PDMS	mm	Function	106
MN, MB	MN - MB	PNS/EXT	Compartmentalized	Soft lithography	PDMS	mm	Function	107
HN, CN	HN - CN	Brain	Compartmentalized	Soft lithography	PDMS	mm	Disease	72
MN, myocytes	MN myocyte	PNS/EXT	Compartmentalized	Photolithography	Silicon	μm	Function	93
CN,TN	CN - TN	Brain	Compartmentalized/MEA	Soft lithography*	PDMS, Silicon, PEI	mm	Function	54
CN,TN	CN - TN	Brain	Compartmentalized/MEA	Soft lithography*	PDMS, Silicon, PEI	mm	Function	53
HN	Somal axonal	Brain	Compartmentalized/MEA	Laser Patterning*	Agarose, Silicon	mm	Function	108
HN	Somal axonal	Brain	Compartmentalized/MEA	Laser Patterning*	Agarose, Silicon	mm	Function	109
HN	Somal axonal	Brain	Compartmentalized/MEA	Laser Patterning*	Agarose, Silicon	mm	Function	110
HN, glia	Cell isolation	Brain	Compartmentalized/MEA	Laser Patterning*	Collagen, Silicon	mm	Function	111
NP	None	Brain	Hydrogel	3D printing	Alginate, Agarose	mm	Disease	81
Glioma, HN	None	Brain	Hydrogel	Contact printing	Acrylamide Hydrogel	mm	Function	33
CN, AC	None	Brain	Hydrogel	Soft lithography	PDMS, Matrigel	cm	Function	80
CN	None	Brain	Hydrogel	3D printing	Gellan Gum-RGD	mm	Injury	50
SPN	None	PNS	Hydrogel	Laser patterning	Agarose	mm	Function	35
SPN	None	PNS	Hydrogel	Laser patterning	PEGDA, Agarose	mm	Function	112
SPN	None	PNS	Hydrogel	Laser patterning	PEGDA, Agarose	mm	Injury	113
SPN	None	PNS	Hydrogel	Laser patterning	PEGDA, Agarose	mm	Injury/Disease	114
CN	None	Brain	Hydrogel	Manual assembly	Collagen, Silk protein	mm	Injury	115
CN	None	Brain	MEA	Microfabrication	Silicon	mm	Function	116
HN	None	Brain	MEA	Microfabrication	Silicon	mm	Function	117
CN	None	Brain	MEA	Microfabrication	Silicon	mm	Function	118
HN	None	Brain	MEA	Microfabrication	Silicon	mm	Function	119
Retzius cell	None	Brain	MEA	Microfabrication	Silicon	μm	Function	120
SPN	None	Brain	MEA	Microfabrication	ITO-coated Glass	mm	Function	121
Hippocampal slice	None	Brain	MEA	Microfabrication	Silicon	mm	Injury	122
Hippocampal slice	None	Brain	MEA	Microfabrication	Silicon	mm	Function	123

Cell Types	Compartmentalization	Tissue	Design	Manufacturing	Materials	Length	Application	Ref.
Hippocampal slice	None	Brain	MEA	Microfabrication	Silicon	mm	Function	124
CN	None	Brain	MEA	Microfabrication	Silicon	µm	Disease	125
SPN	None	Brain	MEA	Microfabrication	Silicon	mm	Function	126
Hippocampal slice	None	Brain	MEA	Microfabrication	Silicon	um	Function	127
EC, AC, MG, CN	AC,MG, CN - EC	Brain/EXT	Microfluidic	Soft lithography	PDMS, PC	cm	Function	128
HN, AC	HN - glia	Brain	Microfluidic	Soft lithography	PDMS	cm	Disease/Injury	55
CN	None	Brain	Microfluidic	Soft lithography	PDMS, Glass	µm	Function	129
Medullary slice	None	Brain	Microfluidic	Soft lithography	PDMS	cm	Function	58
EC, AC	EC - glia	Brain/EXT	Microfluidic	Laser patterning	PDMS, PC	mm	Disease	39
CN	None	Brain	Microfluidic	Soft lithography	PDMS	mm	Disease	85
MN	None	PNS/EXT	Microfluidic	Soft lithography	PDMS	mm	Function	130
NP	None	Brain	Microfluidic	Soft lithography	PDMS	mm	Function	26
MN	None	PNS	Microfluidic	Soft lithography	PDMS	mm	Injury	89
EC	None	Brain/EXT	Microfluidic	Soft lithography	PDMS	cm	Function	40
CN	Somal - axonal	Brain	Microfluidic	Injection molding	PDMS	cm	Disease	84
HN, OC, MG, AC	HN - glia	Brain	Microfluidic	Soft lithography	PDMS	cm	Disease	131
NP	None	Brain	Microfluidic	Soft lithography	PDMS	cm	Function	56
NP	None	Brain	Microfluidic	Soft lithography	PDMS	cm	Disease	43
EC	None	Brain/EXT	Microfluidic	Soft lithography	PDMS	µm	Function	41
Hippocampal slice	None	Brain	Microfluidic	Soft lithography	PDMS, PTFE, Silicon	cm	Function	42
CN	None	Brain	Microfluidic	Soft lithography	PDMS	mm	Injury	132
EC, AC	None	Brain/EXT	Microfluidic	Soft lithography	PDMS	mm	Function	94
SC	None	PNS	Microfluidic	Soft lithography	PDMS, Matrigel	mm	Injury	90
Glioma	None	Brain	Microfluidic/Hydrogel	Soft lithography*	PDMS, Hyaluronic Acid	cm	Function	25
CN	None	Brain	Microfluidic/MEA	Soft lithography	Silicon, PDMS	mm	Disease	51
CN	None	Brain	Microfluidic/MEA	Soft lithography*	Silicon, PDMS	mm	Function	44
HN	None	Brain	Microfluidic/MEA	Soft lithography*	Silicon, PDMS	cm	Function	52
AC, EC	AC - EC	Brain	Microfluidic	Soft lithography	PDMS	mm	Function	59

Table 2

Summary of microfluidic neural systems on a chip. Abbreviations: Endothelial Cell (EC), Neural Progenitor Cell (NP), Extracellular Matrix (ECM)

Study	Motivation	Ref.
Neural differentiation in a co-culture with ECs	Developing a modular blood brain barrier model	128
Neuron viability following beta-amyloid insult	Neuroinflammation in different brain regions	55
Perfusion optimization for brain slices	Improving brain-on-chip environment	58
TEER response to histamine exposure	Blood brain barrier model	39
Application of oligomeric amyloid beta to neurons	Alzheimer's disease	85
Neuronal response to behavior and olfactory stimulation	Developing a device for monitoring neural activity	130
Application of growth factor gradients to NPs	Growth factor gradient optimization	26
Monitoring of axonal response to neural injury	*In vivo* monitoring of neural injury	89
Monitoring TEER response to shear	Blood brain barrier model	40
Isolated exposure of neurons to okadic acid	Alzheimer's disease	84
Transfection across isolated microchambers	Developing a model for transfection studies	131
Application of cytokine gradients to NPs	Cytokine concentration optimization	56
Application of shear and amyloid-beta to neurospheroids	Alzheimer's Disease	43
Culturing ECs in astrocyte conditioned media	Blood brain barrier and drug discovery	41
Brain slice survival with local microperfusion	Long-term brain slice studies	42
Application of potassium to induce cortical spreading depression	Brain injury and migraines	132
EC drug permeability in astrocyte-conditioned medium	Developing a platform for modeling drug delivery	94
Applying tacrolimus to regenerating nerves	Nerve regeneration	90
Monitoring glioma ECM remodeling	Brain tumors	25
Action Potential of isolated axons	Drug screening	51
Action potential in a 3D fluidic environment	3D neuronal networks	44
Effect of perfusion on neural networks	3D neuronal networks	52
Development of a new technique for constructing single-cell arrays	High throughput cell characterization	129

Table 3

Summary of compartmentalized neural systems on a chip. Abbreviations: Central Nervous System (CNS), Peripheral Nervous System (PNS), Neuromuscular Junction (NMJ), Glial-derived Neurotrophic Factor (GDNF).

Study	Motivation	Ref.
Variation of matrix crosslink density and orientation	A new platform for drug discovery	67
Application muscimol to a neural network	Synaptic competition	103
Application of beta-amyloid to a cortico-hippocampal network	Synapse die-back, Alzheimer's disease	77
Measurement of excitotoxin induced degradation of axons	Alzheimer's disease and brain injuries	63
Observation neuromuscular junction formation and activity	Development of a NMJ model on a chip	71
Observation of viral transport and gene expression	Demonstrating the application of 3D printing for NSCs	24
N/A	CNS/PNS regeneration	23
Observation of neural infection and neuron to cell infection	Understanding the mechanism neural infection	69
Monitoring movement of labeled mitochondria in axons	Axon degeneration, neurodegenerative diseases	104
Studying neural development in response to gradient cues	Guiding neuron development	29
N/A	Improving brain on chip capabilities	64
Investigation of myelination of axons by isolated oligodendrocytes	*In vitro* modeling of glia/axon interaction	105
Addition of astrocytes to established networks	A new platform for neural co-cultures	75
Local exposure of biomolecular cues to neurons	Understanding axonal growth	62
Stimulation of neural network formation	Neural network construction	70
Differentiation in a vascular microenvironment	Improving brain microenvironment	73
Monitoring nerve regeneration	Nerve regeneration	78
Monitoring amyloid beta transmission in neural networks	Alzheimer's disease	65
Observation of the formation of neuromuscular junctions	Drug screening and motor neuron pathophysiology	28
Application of 3- and 4-repeat tau protein to neural cultures	Alzheimer's disease	68
Isolation of axonal mRNA	Axonal injury and regeneration	22
Imaging of compartmentalized neurons	Synapse visualization and manipulation	66
Observation of the formation of a NMJ	A new NMJ model on a chip	106
Application of GDNF to a neuromuscular co-culture	Development of neuromuscular junctions	107

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Study	**Motivation**	**Ref.**
Recording activity between cortical and thalamic neurons	Cortical and thalamic connectivity	54
Recording activity between cortical and thalamic neurons	Isolating networks in a controlled environment	53
Development and activity of connections between brain slices	Understanding neural pathways	72
Measuring muscular contraction in response to neural stimulation	Drug screening	93
3D neural network patterning control through thermal etching	Studying neuron-glial signaling, drug screening	111
Manipulating neural network connections through thermal etching	Individual-cell electrophysiological monitoring	110
Individual-cell measurements of a controlled neural network	A new platform for neural network research	109
Monitoring spontaneous firing among spatially controlled networks	Investigating neural network function	108

Table 4

Summary of hydrogel neural systems on a chip. Abbreviations: Traumatic Brain Injury (TBI).

Study	Motivation	Ref.
Characterization of 3D printed neural structures	*In vitro* drug screening and disease modeling	81
Characterizing the properties of neurons in 3D culture	Developing biomimetic and relevant tissue models	80
Printing and imaging of a layered brain like structure	Traumatic brain injury and disease modeling	50
Growing neurons in a peptide patterned medium	Directing cell growth in three dimensions	35
Monitoring neurite outgrowth in response to guidance cues	Understanding nerve regeneration processes	113
Development of *in vivo* 3D neurite outgrowth assay	Drug screening	114
Effect of TBI on a modular 3D brain model	Response to TBI	115
Dual-hydrogel system for cell culture with protein gradients	Developing a 3D microenvironment with molecular patterns	112