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Microchips and Microbiomes: Engineering the Gut-Brain Connection

Biography:

Karishma Rohatgi is a senior studying Biomedical Engineering at USC's Viterbi School of Engineering, specializing in Molecular and Cellular Biology. She is keen on entering the biotechnology sphere after graduation and exploring the intersection between engineering and biology.

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Introduction:

Picture this: you are sitting in the examination hall, about to take an exam you do not feel prepared for. The proctor makes the classic "if you need to use the bathroom, use it now" announcement, but you don't feel the need to go. The test papers get handed out, and your mind starts simultaneously racing and going blank. Waves of heat creep up your stomach, into your neck, and up to your forehead, throbbing consistently. And now, all of a sudden as if the proctor wished it into existence, you have the dreaded feeling of having to go poo.

This has happened one too many times to me; I am sure you have all felt it too.
Many would not consider the possibility of the nervous system affecting bowel movements, but research shows that the connection is not only real but incredibly powerful.

The link between the brain and bowel movements has long interested engineers and biomedical scientists, and historical evidence shows the exploration of the gut-brain connection dating back to the 18th century [1]. However, with recent advances in bioengineering technology and modeling techniques, scientists have been able to study the connection in greater detail [2]. To develop conclusions about the gut-brain connection, traditional research methods, like non-human animal studies or clinical trials, often either fall short of closely resembling the human body or pose financial and time-related burdens. Enter organ-on-a-chip (OoC) technology: a novel bioengineering technology allowing scientists to replicate human organ functions in the lab. OoCs serve as a powerful and efficient tool for researchers to explore with greater precision, accuracy, and speed than traditional methods. At the most fundamental level, researchers take cells from human organs — such as the brain and the bowel, in this case — and place them onto the chip, allowing them to grow into functional tissue while recreating key aspects of the organ's physiological environment. In the article that follows, OoCs, as applied to studying the gut-brain connection, will be explored in comprehensive detail.

The Gut-Brain Axis

Researchers have recently found a strong connection between the gut microbiome (bacteria and gut cells) and the brain, fittingly called the Gut-Brain Axis (GBA). The GBA is most simply

understood as a network of nerves connecting the brain and gut, sending signals in both directions [2]. This connection is so profound that scientists have termed the gut the "second brain," referring to its complex neural network as the enteric nervous system (ENS) [2]. The GBA could explain why people feel the urge to poop when they are nervous, such as before an important exam. This connection works the other way around, too, where gut issues such as bloating, indigestion, and stomach pain can all impact our mental health [3]. In fact, around ⅓ of patients with Irritable Bowel Syndrome (IBS) are diagnosed with anxiety and depression, so the GBA is not only present but also powerful [4]. To understand how the GBA is modeled, we first need to learn about the physiology of the GBA, such as the ENS and the central neural pathway, termed the vagus nerve, through which the GBA operates.

The Enteric Nervous System and The Vagus Nerve

The ENS is comprised of two layers of 100 million nerve cells, each lining the gastrointestinal tract from the esophagus to the rectum [3]. It controls intestinal functions such as motility, secretion, digestion, and absorption [5]. Although it is considered a separate nervous system from the brain, it is in constant communication with it via neurotransmitters (signaling molecules), sending and receiving information between them regarding the state of the gut and brain [6]. It follows, then, that disruption of the ENS does not just cause stomach aches and bowel issues — due to the GBA, it can also cause brain fog, anxiety, fatigue, and contribute to neurodegenerative diseases like Alzheimer's and Parkinson's [7].

The vagus nerve connects the ENS to the brain. Its long body begins at the brainstem and spans to the key functional organs, such as the heart, lungs, and finally ends at the gut [8]. It can be

understood as a two-way highway, where each “car” is a neurotransmitter that travels from the brainstem to the gut or vice versa, with exits branching off to the different organs in the body. The vagus nerve is understood as the primary bridge between the brain and the gut.

Traditional Modeling Technologies of the GBA

By now, you may have become conscious of just how complicated the GBA is. Yet, it is only a recently understood phenomenon, meaning we have only discovered the tip of the iceberg. Studying it is an even bigger challenge as it spans multiple regions of the internal human body and can only be done using humans themselves or by creating other models of it. However, traditional models like non-human animal models or human clinical trials pose several limitations.

In Vivo Animal Models

An animal model is a non-human species (rats, dogs, pigs, monkeys, etc.) that can mimic certain aspects of human biological processes [9]. *In vivo* animal models are experiments conducted within living animals to model common bodily functions shared between humans and animals [9]. These models are particularly useful for studying whole-body responses and systemic interactions [10]. Scientists have studied the GBA through in vivo animal models such as germ-free mice [11].

For instance, researchers from Kyoto University in Japan studied the association between autism and gastrointestinal (GI) disorders by using a CHD8 heterozygous knockout mouse model [12]. This model type involves mice that were engineered to have only one CHD8 gene, a gene

strongly linked to autism in humans [12]. After several weeks of study, they found that these autistic mice had GI abnormalities, further supporting their hypothesis that brain disorders impact gut health via the GBA [12].

However, while in vivo models can give insight into the general interaction of the gut-brain axis, they fail to "recapitulate human conditions" and complexities due to physiological differences and inconsistencies in the gut microbiome between the two species [13]. Further, in vivo models are inadequate due to the time-consuming and labor-intensive processes, as well as certain ethical considerations that arise with animal testing [13].

Human Clinical Trials:

Scientists have also attempted to study the gut-brain axis through clinical trials with human subjects [14]. Human clinical trials are research studies in which humans are subjected to medical interventions — such as new drugs, treatments, and devices — to gauge their efficacy, safety, and potential side effects [14].

For example, researchers at King's College London conducted a clinical trial using 36 pairs of twins (72 individuals) over 60 years old to explore the impact of prebiotics for gut health on improved memory [15]. One twin would be given the prebiotic and the other would be given a placebo [15]. They found that the group of twins who were given the prebiotic had a higher cognitive ability [15]. Using human clinical trials to test the mechanisms of the GBA can be a useful tool for understanding its intricacies: the data comes directly from humans, unlike animal models, which may produce different results due to differences in physiology.

Yet, these studies are often slowed down by complicated regulations, long approval times, and high costs. Due to this, it is difficult for scientists to get meaningful data quickly or efficiently from clinical trials, making them, while appropriate and informative in some contexts, ineffective models for studying the GBA [14].

Bridging the Gap of Traditional Modeling Technologies: OoCs

Accordingly, we need an all-encompassing solution to address the issues with the models presented above. Thankfully, new advances in engineering have created an impressive solution to this problem: organ-on-a-chip modeling. OoC models are USB-sized microfluidic devices that allow for the analysis of human-derived cell cultures that mimic the internal and external environment of several human organs in the lab (in vitro) [16]. So far, organs such as the lungs, intestines, liver, kidneys, and heart have been successfully replicated on OoCs [17]. More recently, though, multi-organ-on-chips (multi-OoCs) have been created, which researchers use to study in-depth communication systems between organs in the human body, such as the GBA in our case [17]. So that bowel movement caused by your anxiousness before an exam? Scientists can now simulate that exact gut-brain signal on a chip using real human cells. But to fully understand OoCs in the context of the GBA, it is important to familiarize yourself with the basic components of OoCs.

The Structure and Makeup of OoCs

Each OoC is a small, transparent chip made from a soft polymer material called PDMS [17]. Inside, it contains tiny (1-100μm), fluid-filled channels called microfluidic channels. These

channels are lined with human cells dispensed into them [17]. For reference, these channels are roughly the same diameter as human hair [18]. The chip also contains several blood vessel layers lined with human tissue (endothelial) cells to mimic organ boundaries [16]. Once the OoC has had enough time to replicate and expand these human cells from their initial transplantation, it begins to mirror the natural movements of the organ it intends to simulate [16].

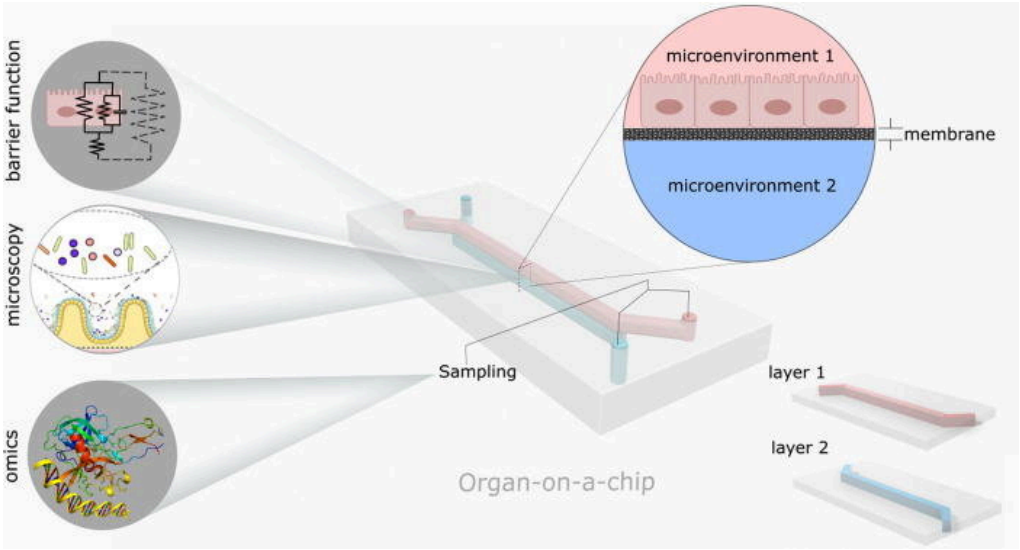

Figure 1. Organ-on-a-chip of the human lung. Details the relative organization and makeup of the chip [1]

Take a look at the top right of the graphic above. There is an artificial microfluidic channel labeled as the 'membrane,' and the human endothelial cells are stacked on top of the membrane (square-shaped boxes) as if it were the tissue boundary of an organ. 'Microenvironment 1' includes the specific cell types and their composition *outside* of the "organ," and 'Microenvironment 2' represents the specific cell types and their composition surrounding the *inside* of the "organ". OoCs allow researchers to utilize microfluidic technology to mimic organ

boundaries and recreate distinct microenvironments on either side of these channels, enabling the study of how the organ interacts with its internal and external environments [16].

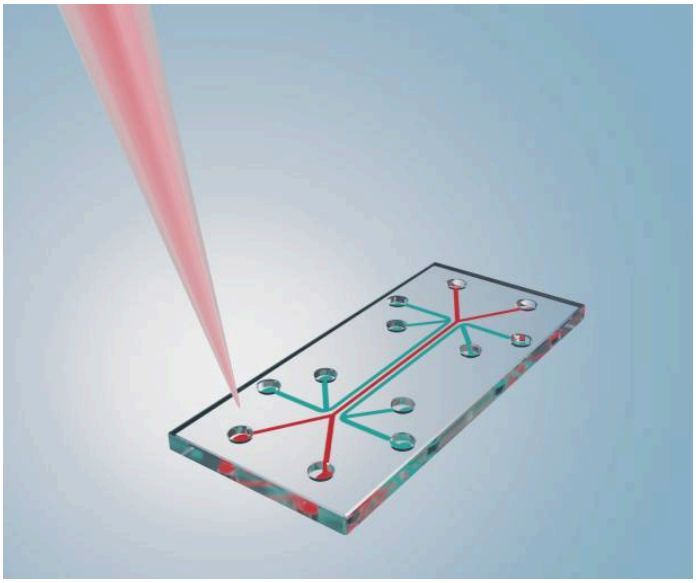

Figure 2. Another example of an OoC. Has a snowflake-like microfluidic structure on both ends [2]

The snowflake-shaped wells at the end of the microfluidic channels are where researchers first transfer the human-derived cells. These cells then flow from the wells through the microfluidic channels to the different areas along the chip, similar to how cells circulate throughout the human body within organs.

Physical Basis of OoCs

But how do we get cells to move without our circulatory system pushing cells to move throughout our bodies? On a microscopic level, OoCs are engineered using physical principles like fluid shear force and concentration gradients to simulate the flow of bodily fluids and mimic organ behavior.

Fluid Shear Force:

Cells in our body are rarely static. Conventional cell culture models only promote cell expansion in one location, but to mimic the dynamic nature of cells, OoCs need pumps of their own. Such technology is called micro-pump perfusion [19]. These micro-pumps essentially push fluid through the chip, mimicking cell duties such as the delivery of nutrients and collection of waste products as they move around the human organs [19].

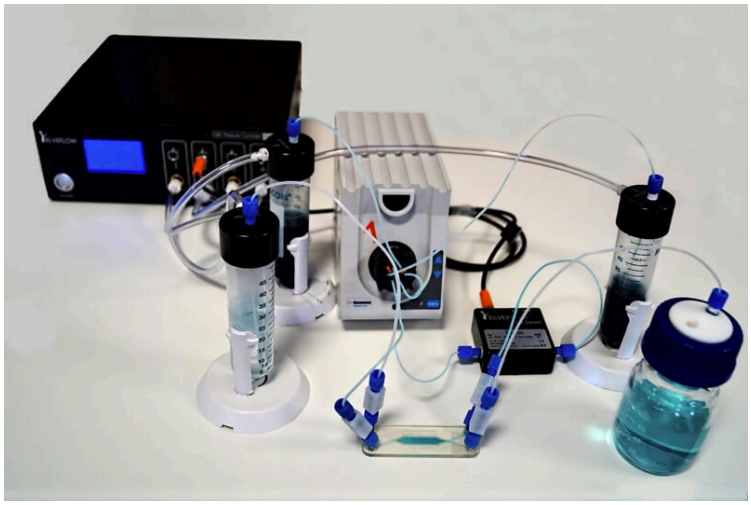

Figure 3. Micro-pump perfusion in action. 4 pumps (in blue) are attached to the OoC, pumping liquid through it [3].

Concentration Gradient:

All cells move depending on a defined concentration gradient, either moving from an area of high density to low density or depending on biochemical signals to direct their flow [20].

Concentration gradients are a common biological phenomenon that allows our bodies to remain relatively stable, or in homeostasis [21]. Microfluidic technology successfully mimics this ordered diffusion of cells based on their concentration gradient, using micro-valves and micro-pumps to alter flow velocity and achieve biochemical concentration gradients necessary to accurately mimic cells inside and outside the organ(s) of interest [22].

GBA Modeling Through Multi-OoCs

Now that we have more insight into how OoCs work, we can specifically discuss OoC modeling for the GBA. Since the GBA involves the nervous system and the gut, the GBA-on-a-Chip is a multi-OoC model. The GBA-on-a-Chip involves culturing both gut epithelial tissue cells and neuronal cells on the same chip and observing the cross-talk between those cells via microfluidic channels connecting the two "organs" [23]. An example of the GBA-on-a-Chip is shown below, where the specific signal molecule being observed between the gut cell and neuronal cell is an exosome.

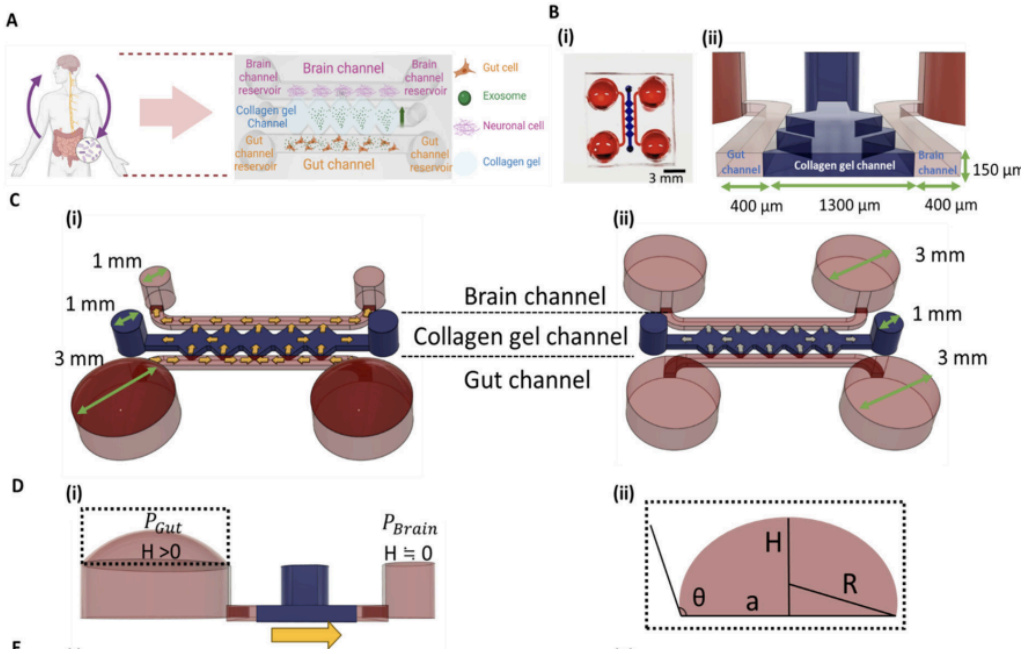

Figure 4. GBA-on-a-Chip example. Details the organization of the GBA on the chip, as well as details surrounding the physics of the channel and the measurement specifications [4]

If we focus our attention on (A) in Figure 4, we can see the organization of the brain and gut organ channels, as well as the exosome that is communicating with the brain and gut, shown by the green arrow [23]. In this model, the brain and gut "organ" channels are separated by a collagen gel channel. This collagen gel channel mimics the extracellular environment surrounding cells, allowing for more realistic cell behavior and interactions between the two "organs" [23]. The exosomes travel through this collagen gel channel and act as the main signalling vehicle, facilitating communication between the gut and brain regions [23]. Thus, this movement replicates how molecular signals usually travel within the human GBA [23]. By observing this signaling movement between the simulated brain and gut, researchers can learn more about the human GBA without relying on human trials. The GBA-on-a-Chip then makes studying the human GBA both simple and highly informative.

Next Generation Multi-OoC: The MINERVA Platform

In November 2024, researchers designed a GBA multi-OoC system that simulated bidirectional communication between the gut and the brain [24]. It was designed to explore the role the gut microbiome has in neurodegenerative diseases like Alzheimer's [24]. The MINERVA multi-OoC chip has three compartments: the microbiota, the gut, and the brain [24]. Figure 5 below details the specific signaling characteristics between the three compartments.

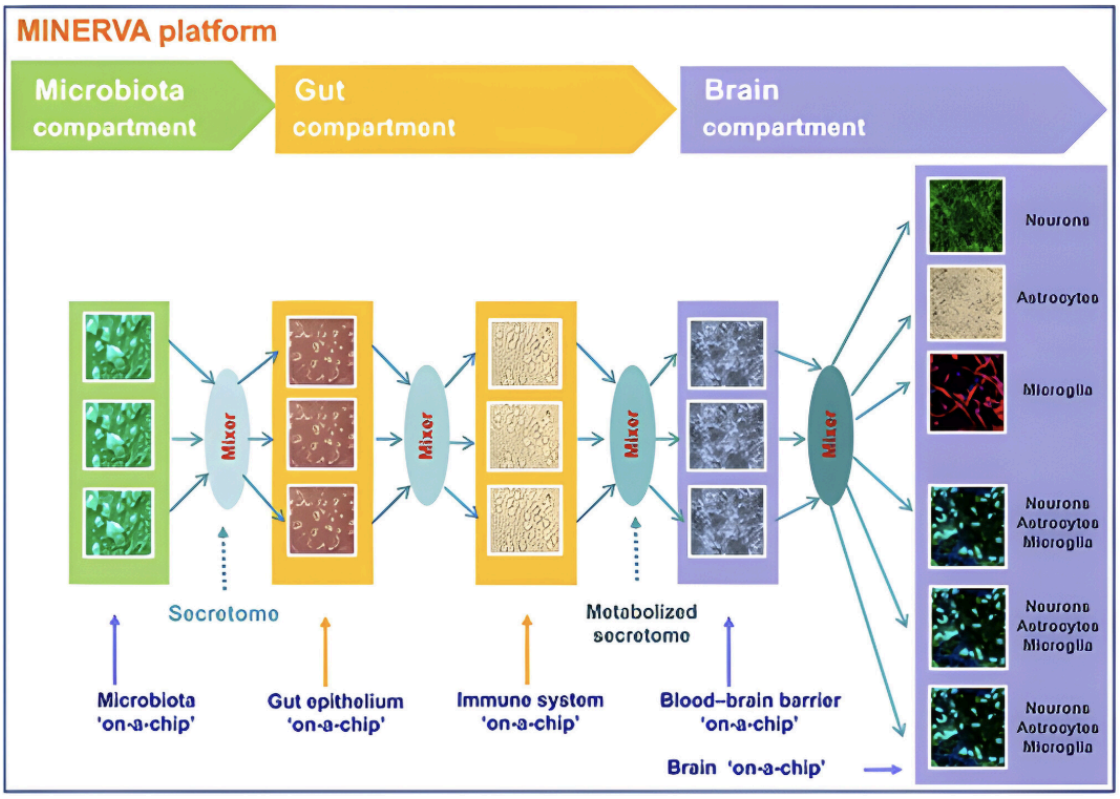

Figure 5. MINERVA Platform compartmental organization and workflow. (Note: The brain compartment has a blood-brain barrier chip and a brain chip.) [5].

The microbiota compartment produces cells that secrete soluble products, which then go through microfluidic channels to the gut compartment, containing epithelial tissue cells and immune cells [24]. The brain compartment is separated by a blood-brain barrier (BBB), which mimics the selective permeability of the actual human BBB, allowing only certain molecules from the gut compartment to cross into the brain compartment [24]. The brain contains neurons that are directly involved in neurodegenerative diseases like Alzheimer's [24]. In this way, the MINERVA platform is an innovative in vitro solution for studying how the gut microbiota interacts and impacts the progression of Alzheimer's. Figure 6 below shows how the actual MINERVA chip looks.

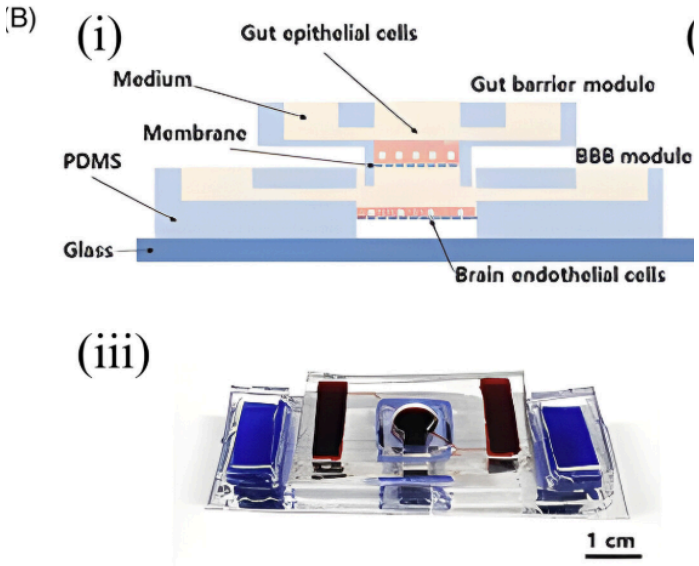

Figure 6. MINERVA Platform real-life model. Demonstrates how the gut, brain, and BBB are situated on the chip [5].

To illustrate the effectiveness of the MINERVA platform as a model for investigating the GBA, researchers studied the pharmacokinetics (drug pathway and interactions) of Donepezil through a multi-OoC system composed of five MINERVA chips like the ones shown in Figures 5 and 6 [25]. Donepezil is an FDA-approved drug commonly used to treat symptoms of dementia and Alzheimer's Disease, such as memory loss and thinking ability [25]. They investigated Donepezil's ingestion, its transport through the GBA, and its eventual delivery to the brain [25]. This study aimed to demonstrate how accurate the MINERVA platform was in simulating the GBA, and the researchers found that it is indeed accurate [25]. After 24 hours of introducing Donepezil to the multi-OoC system through the intestinal chip (the first in the sequence), the system demonstrated efficacy in transport of the drug through the GBA, as measurable concentrations of Donepezil were present in the neuronal MINERVA chip (the last in the

sequence). The five-chip organization is shown below, with each chip simulating parts of the GBA from the intestinal cells to the neurons in the brain [25].

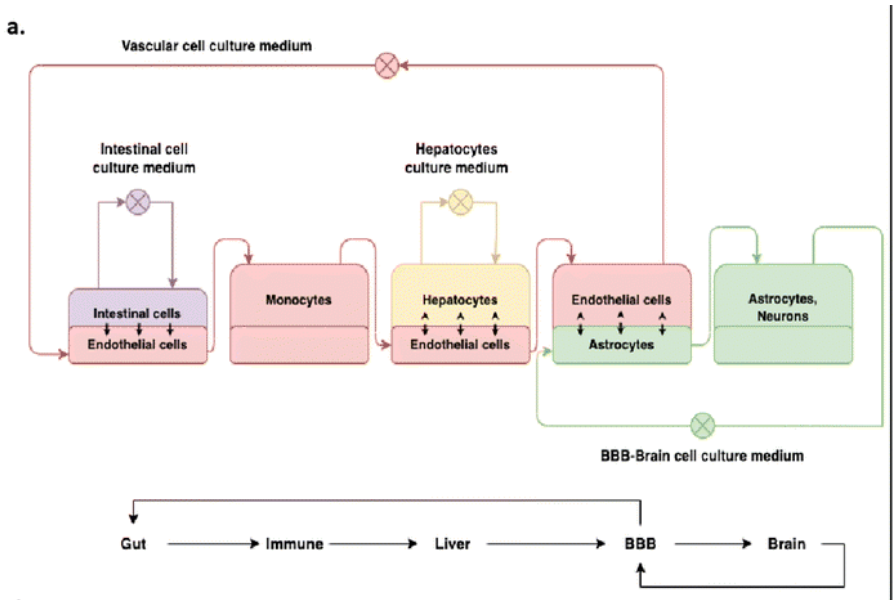

Figure 7: Five MINERVA chips arranged in sequence, simulating the different organs that Donepezil interacts with, including key components of the GBA (intestines and brain) [6].

This proof-of-concept study successfully demonstrated that multi-OoCs like the MINERVA platform are successfully able to recapitulate the human GBA in terms of drug metabolism and targeted delivery to the brain.

Applications and Advantages of GBA-on-a-Chip Technology:

GBA multi-OoCs like the MINERVA platform are useful in studying more than just neurodegenerative disorders like Alzheimer's. They help study the general communication characteristics between the gut and brain, too. Beyond that, simulating the GBA *in vitro* provides increased scope for personalized medicine opportunities in treating neurological disorders. It also

helps reduce the ethical concerns that come with traditional methods like *in vivo* animal testing and human clinical trials.

Personalized Medicine:

In order to model human physiological and biochemical characteristics on a chip, OoCs still require human cell extraction. So, replicating a patient's exact cell composition inside and around the target organ on the OoC will allow the replication of an individual's unique physiology and functions [26]. This will allow scientists to conduct safer personalized disease modeling and drug testing because it will enable them to test the safety and efficacy of a drug on the specific cellular environment of the patient before ever administering it to the patient themselves [26]. Applying personalized medicine to the GBA via the GBA-on-a-chip will be even more practical, as each individual is found to have a unique microbiotic makeup [27]. By mimicking a patient's specific gut microbiome on the chip, physicians can test whether or not the drug might work for that particular patient.

Interestingly, researchers have found that medications used to treat neuroinflammation, a common symptom in patients with Alzheimer's, act differently among patients due to their differing gut microbiota [28]. For instance, they found that when patients were given the antibiotic Minocycline to reduce inflammation, patients exhibited different severities of effects of the drug despite all having Alzheimer's [29]. For this reason, the “one-size-fits-all” approach to treating neurodegenerative disorders like Alzheimer's is flawed because of a patient’s unique gut microbiota and reactions to drugs. To address this, a more personalized approach is needed, such as using an OoC. A specific patient’s cells can be implanted onto a chip to create an OoC

that mimics their gut microbiome and brain. This OoC will then allow clinicians to test whether a specific drug, like Minocycline, is an effective therapy based on the patient's unique microbiome.

Similarly, personalized drug testing and treatment through OoC technology is warranted for patients with Parkinson's disease, given the differing gut microbiota from patient to patient [28]. Levodopa, the primary drug given to Parkinson's patients to help with body movement and coordination, can be rendered ineffective if certain gut microbes are present in high concentrations in the patient's gut [30]. Researchers from the University of California, San Francisco, found that if a patient has high levels of microbes like *Enterococcus faecalis* and *Eggerthella lenta,* Levodopa might get metabolized by the gut before it ever reaches the brain [30]. As a result, patients with high levels of these microbes might receive very little therapeutic benefit from Levodopa [30]. Here, too, OoCs can step in as a means of personalized treatment: a patient's gut cells can be implanted into an OoC, and Levodopa can be added to see how much of the drug is broken down by their microbiome. This allows doctors to determine whether enough of the drug will reach the brain to have a significant therapeutic effect.

In neurodegenerative disorders, such as Alzheimer's Disease and Parkinson's Disease, personalized treatment is possible with the use of OoCs. Before prescribing any treatments, clinicians could grow a patient's unique gut-brain system on OoC and test how their body responds to a certain drug, avoiding the unnecessary use of treatments that may ultimately be ineffective and pose unnecessary risks to the patient.

Ethical Benefits of GBA-on-a-Chip:

Using GBA-on-a-Chip to explore the GBA in-depth is an exemplary solution given its ethical benefits. It successfully reduces the need for both animal models and human clinical trials, explored in greater detail below.

GBA studies often involve the use of in vivo animal models such as mice, as aforementioned, but in vivo animal models have come under scrutiny for the extreme torture animals face when having to undergo lab testing [31]. Using GBA-on-a-Chip for GBA-related experiments and studies can then reduce the amount of animal testing being conducted, decreasing the unnecessary torture animals face from animal testing. Additionally, animal testing is further unwarranted as 95% of drugs tested on animals fail when evaluated on humans [32].

GBA-on-a-Chip reduces the need for human clinical trials because it mimics the biochemical and physiological environments of the human GBA and thus does not require human testing for GBA medications, drugs, and medical devices. GBA-on-a-Chip also minimizes the regulatory burden and length of human clinical trials. For instance, researchers comparing the use of liver-on-a-chip experiments versus human clinical trials for several liver drugs showed a 42-month decrease and a 93.5% cost decrease when using the OoCs [33]. Accordingly, GBA multi-OoCs can be a cheaper and faster alternative to human trials for studying GBA-specific diseases.

The Future: AI-Powered GBA Chip Analysis

The future holds immense potential for GBA-on-a-Chip, as OoCs applied to the GBA are fairly new. One such technological advancement to GBA-on-a-Chip is the integration of Artificial Intelligence (AI) to automate the data collection and analysis of OoCs. When using multi-OoCs

like GBA-on-a-Chip, the data processing and constant monitoring can be burdensome due to the multiple organ modeling [34]. However, AI can help track changes in real time, spot patterns in signaling between the compartments, and predict when these signals might happen again, giving helpful insights into how the gut and brain are connected [34]. The AI model would essentially act as a 24/7 lab assistant, reading the chip live, catching even the most minute biochemical signals, and predicting when the gut or brain might trigger these signals.

Conclusion:

It seems that the “nervous poop” before a big exam was not just in our heads. It was your gut and brain *literally* communicating with one another. “Going with your gut,” then, is not just figurative language; it is backed by scientific evidence proving the existence of the GBA. What is more exciting is how advancements in bioengineering allow us to study this relatively recent phenomenon more deeply than ever before. Technologies like OoCs permit researchers to study the GBA and develop personalized medicine unique to each individual's gut microbiota without the need for human/animal testing. Bioengineering solutions such as these are transforming medicine, making it safer, more personalized, and more precise. OoCs are just one example of how engineering and biology coalesce to answer some of the body’s most fascinating mysteries, and in the future, there will be plenty more.

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