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the frequency of senescent β cells in human islets was increased during aging, and this proportion appeared to be increased further in T2D. Notably, β cell senescence in T1D and T2D showed many distinctive features which await further investigation.

Together, these two studies highlight the significance of β cell senescence in the etiology of both T1D and T2D, and suggest senolytic therapy as a common strategy for treating these diseases [6,7]. In the future, it may be essential to further delineate the origins, underlying mechanisms, and downstream effectors of senescent β cells, as well as their heterogeneity, in the pathophysiology of diabetes. It will be of interest to investigate whether other pancreatic cell types become senescent and also contribute to diabetes. In addition, potent senolytic agents with increased tissue specificity and decreased side effects are anticipated to promote these exciting findings into translational medicine.

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References

1. Bakhti, M. *et al.* (2019) Modelling the endocrine pancreas in health and disease. *Nat. Rev. Endocrinol.* 15, 155–171
2. Tian, Y. *et al.* (2018) The interplay between noncoding RNAs and insulin in diabetes. *Cancer Lett.* 419, 53–63
3. Liston, A. *et al.* (2017) Beta-cell fragility as a common underlying risk factor in type 1 and type 2 diabetes. *Trends Mol. Med.* 23, 181–194
4. Tian, Y. *et al.* (2018) New ADCY3 variants dance in obesity etiology. *Trends Endocrinol. Metab.* 29, 361–363
5. Hernandez-Segura, A. *et al.* (2018) Hallmarks of cellular senescence. *Trends Cell Biol.* 28, 436–453
6. Thompson, P.J. *et al.* (2019) Targeted elimination of senescent beta cells prevents type 1 diabetes. *Cell Metab.* 29, 1045–1060
7. Aguayo-Mazzucato, C. *et al.* (2019) Acceleration of β cell aging determines diabetes and senolysis improves disease outcomes. *Cell Metab.* 30, 129–142
8. Aguayo-Mazzucato, C. *et al.* (2017) β Cell aging markers have heterogeneous distribution and are induced by insulin resistance. *Cell Metab.* 25, 898–910
9. Gurau, F. *et al.* (2018) Anti-senescence compounds: a potential nutraceutical approach to healthy aging. *Ageing Res. Rev.* 46, 14–31
10. Palmer, A.K. *et al.* (2019) Targeting senescent cells alleviates obesity-induced metabolic dysfunction. *Aging Cell* 18, e12950

Forum

An Organ-On-A-Chip Engineered Platform to Study the Microbiota–Gut–Brain Axis in Neurodegeneration

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After decades of research, the etiology of neurodegenerative disorders such as Alzheimer's or Parkinson's disease is still mostly unknown. Recent findings indicate that the microorganisms in the human gut might be involved in neurodegenerative pathways. Here, we discuss an innovative groundbreaking bioengineering approach that could make a difference in this intriguing scenario.

A Novel Hypothesis: The Microbiota–Gut–Brain Axis

The two most common neurodegenerative disorders have a very long history. The first reported case of Alzheimer's disease (AD) was in 1906, while Parkinson's disease (PD) was described in 1817. Since then, intensive research has provided plenty of clinical, genetic, and molecular evidence of AD and PD signs, mechanisms, and druggable targets, leading to the discovery of pharmacological therapies, unfortunately only symptomatic. AD and PD are prevalent disorders with heavy societal costs. In the USA alone, 5.8 million Americans are currently affected by AD[i] and ~1 million are living with PD[ii]. The sum of global healthcare costs of AD, other dementias, and PD is estimated to be more than US$300 billion per year. The scientific community has seen many failures in large clinical trials addressing neurodegenerative disorders, and novel drug discovery strategies are mandatory [1]. Based on this scenario, many researchers began to think that most accepted current hypotheses for AD and/or PD pathogenesis are probably only part of the answer, and that we need fresh viewpoints.

A promising novel hypothesis for AD and/or PD etiology looks at the pathogenic role of intestinal microbiota in brain neurodegeneration. The microbiota comprises a complex community of microorganisms: it is dynamic and tunable according to age, diet, and medication. It is composed of metabolically active bacteria, viruses, and fungi, and can also be found in the mouth and nose, from which there are multiple ways to reach the central nervous system (CNS), for instance through cranial nerves (particularly the olfactory nerve), promoting subacute and long-lasting inflammatory responses potentially relevant for AD and/or PD pathogenesis [2].

This functional relation between the intestinal microbiota and the brain, referred to as the 'microbiota–gut–brain axis', shifts the trigger of AD and/or PD from the CNS to the body periphery, and

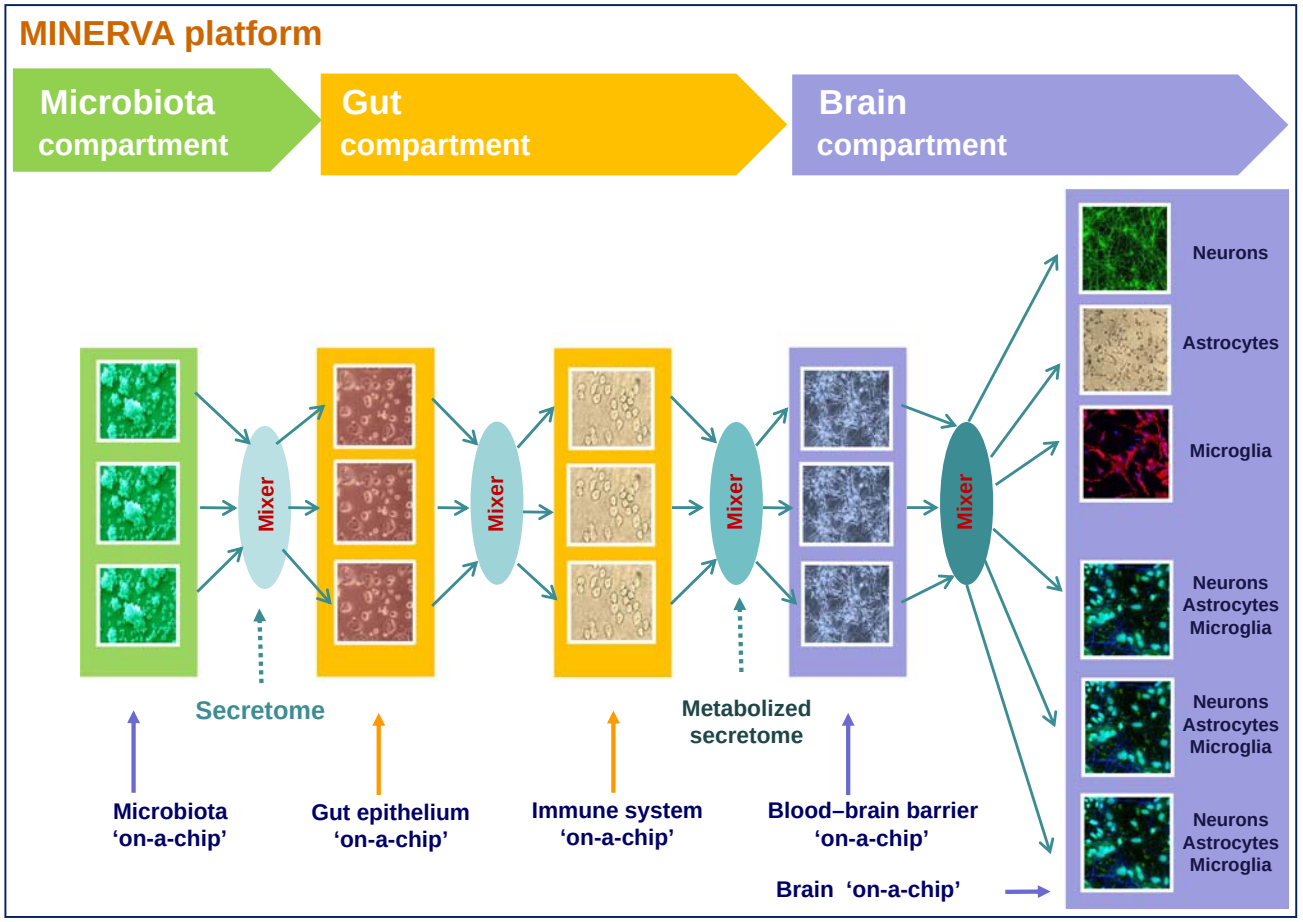

Figure 1. MINERVA (MIcrobiota-Gut-BraiN EngineeRed platform to eVAluate intestinal microflora impact on brain functionality).

For a Figure360 author presentation of Figure 1, see the figure legend at https://doi.org/10.1016/j.molmed.2019.07.006.

MINERVA is a multiorgan-on-a-chip platform that could be used to address the role of the microbiota in neurodegeneration. MINERVA combines five miniaturized sensorized, optically accessible organ-on-a-chip devices, serially connected through a microfluidic pipeline: a 2D culture system to model intestinal microbiota (green); the gut compartment (yellow), where a 2D culture system for the gut epithelium is coupled to a suspended cell culture system to model parts of the immune system; a 2D culture system to model the BBB; and different options to use to model the brain (purple). The whole platform is designed in triplicate, apart from the last device, where up to six independent culture chambers are provided, with mixers to balance culture media composition before the next passage. The 'MICROBIOTA compartment' features one miniaturized microfluidic device, hosting microbial cultures. The soluble molecules of the microbiota, collectively called the 'secretome', will flow to the next compartment, as occurs in the human body, such as for the passage of microbiota-secreted neurotoxins. The 'GUT compartment' hosts two organs-on-a-chip devices modeling the gut epithelium and the strictly related immune system cells, such as macrophages and lymphocytes. The 'BRAIN compartment' recapitulates the BBB and the main brain cells relevant for neurodegeneration, such as neurons, astrocytes ,and microglia that can be cultured individually or in co-culture, in 3D condition because of the presence of an hydrogel matrix in which the cells are embedded.

this different perspective has an impact on therapeutic strategies. Human studies are accumulating fast: they support an imbalance of microbiota bacterial strains towards a proinflammatory profile in patients with AD and/or PD and suggest selected strains involved in AD and/or PD pathology or drug response. For instance, *Porphyromonas gingivalis*, a strain commonly present in the oral cavity, was suggested to have a role in AD through a well-described mechanism that involves toxic proteases called gingipains [3], while the gut strains *Enterococcus faecalis* and *Eggerthella lenta* have been associated with the decreased bioavailability of the PD medication levodopa [4]. The importance of the gut microbiota for AD and/or PD pathology is still not fully clear, but our understanding is rapidly expanding to other CNS disorders.

The Technological Tools: Current Solutions from Organs-on-a-Chip Devices

The role of microbiota in neurodegeneration is not easily addressable because

there is a gap that keeps researchers from clarifying potential microbiota–neurodegeneration mechanisms: the lack of a comprehensive *in vitro* model of the microbiota–gut–brain axis.

Bioengineering research is focusing on advanced technological devices and 3D engineered models to improve the reliability of current *in vitro* tools [5,6]. The new frontier of bioengineering research uses microfluidic systems, called 'organs-on-a-chip', that combine lab-on-a-chip technology with 3D organotypic culture. Organs-on-a-chip are miniaturized bioreactors of millimetric dimensions, with continuous perfusion of the culture medium. Technically, they present a compromise between the need for a very thin culture chamber, <1 mm thick, thus imageable in fluorescence diagnostics as in lab-on-a-chip platforms, and the need to culture and harvest enough cells for quantitative biological assays, as in 3D organotypic culture. Organs-on-a-chip can not only mimic minimal functional aspects of physiopathological states in tissues and organs, but also help to evaluate the effects of therapeutic agents, lowering costs, and improving throughput compared with animal models, with the advantage of reduced ethical concerns.

Organs-on-a-chip have already served as models of several organs and tissues of the body, mimicking the transport of agents through microanatomical interfaces, such as the blood–brain barrier (BBB), and even for intestine microbiota culturing [6].

Recently, a microfluidic device optimized for high-resolution imaging of live 3D cell models (MOAB) was successfully developed [7] and tested to investigate the effect of therapeutics in pathological conditions, such as muscular dystrophy [8], metastatic colonization [9], and neurodegeneration [10].

To investigate the microbiota–neurodegeneration hypothesis, the main engineering issue lies in the need to interface individual organs-on-a-chip devices to model the passage of microbiota-secreted neurotoxins through different body systems and microanatomical barriers, shifting from an organ-on-a-chip approach for modeling AD and/or PD and other neurodegenerative disorders [11] to a more complex, comprehensive 'multiorgan-on-a-chip' strategy.

Assessing the Impact of Intestinal Microflora on Brain Function

The European Research Council (ERC) funded a project named 'MINERVA' (MIcrobiota-Gut-BraiN EngineeRed platform to eVAluate intestinal microflora impact on brain functionality, ID 724734), which aims to develop the first microbiota–gut–brain engineered multiorgan-on-a-chip platform to evaluate the impact of intestinal microflora on neurodegeneration. MINERVA aims to provide researchers with the first engineered, validated tool representing *in vitro* the connections among the main players of the microbiota–gut–brain axis. The MINERVA platform (Figure 1) relies on five miniaturized, sensorized, optically accessible organ-on-a-chip devices. Each device is hydraulically connected to the next with a microfluidic pipeline, through which culture medium flows under positive pressure. The five organs-on-a-chip are the gut microbiota, the gut epithelium, the immune system, the BBB, and the brain. Each organ-on-a-chip hosts *in vitro* models, where cells are cultured in standard condition and in triplicate for the microbiota, gut epithelium, immune system, and BBB, while the brain device features cultured neurons, astrocytes, and microglia embedded in an hydrogel matrix to obtain a 3D model, individually (single replicate) or in co-culture (triplicate). Once validated, the MINERVA platform will test healthy and AD neurodegenerative scenarios, using complete microbiota from patients with AD compared with healthy donors.

The MINERVA bioengineering approach is potentially a breakthrough: if successful, it will open the way to a new field at the boundary between neuroscience and engineering, where investigation of the causes of neurodegeneration is shifted from the brain to the body periphery. We acknowledge that the MINERVA approach has the intrinsic risk of oversimplification of *in vitro* compared with *in vivo* conditions, neglecting key players such as the vagal nerve, and the neuroendocrine and enteric nervous systems. However, the high grade of versatility of the platform design mitigates these limitations by allowing the future inclusion of new devices once reliable organ-on-a-chip models for those missing biological systems are available.

In the short-term, this state-of-the-art approach will also benefit from the iPSC technology that allows the transformation of terminally differentiated peripheral cells (such as fibroblasts or white blood cells) into other cell types, while retaining the genetic background of the donor [12]. By combining iPSCs and the MINERVA approach, our mid-term vision is to use this platform to improve therapeutic strategies by focusing on the current drug development process and the protocols designed by the regulator, in particular for neurodegenerative disorders, where the patient's genetic profile might make the difference between the success or failure of a new drug.

We are confident that one day soon, a patient-specific *in vitro* platform, such as MINERVA, will enable researchers to tune drug therapeutic strategies following a personalized medicine approach, boosting the likelihood of therapeutic action in applications such as neurodegenerative disorders, where the success rate of novel drugs is disappointingly low.

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Resources

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References

1. Golde, T.E. *et al.* (2018) Alzheimer's disease: the right drug, the right time. *Science* 362, 1250–1251
2. Bell, J.S. *et al.* (2019) From nose to gut: the role of the microbiome in neurological disease. *Neuropathol. Appl. Neurobiol.* 45, 195–215
3. Dominy, S.S. *et al.* (2019) Porphyromonas gingivalis in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.* 5, eaau3333
4. Maini Rekdal, V. *et al.* (2019) Discovery and inhibition of an interspecies gut bacterial pathway for levodopa metabolism. *Science* 364, eaau6323
5. Bathia, S.N. *et al.* (2014) Microfluidic organs-on-chips. *Nat. Biotech.* 32, 760–772
6. Jalili-Firoozinezhad, S. (2019) A complex human gut microbiome cultured in an anaerobic intestine-on-a-chip. *Nat. Biomed. Eng.* 3, 520–531
7. Izzo, L. *et al.* (2019) Influence of the static magnetic field on cell response in a miniaturized optically accessible bioreactor for 3D cell culture. *Biomed. Microdevices* 21, 29
8. Frattini, P. *et al.* (2017) Autologous intramuscular transplantation of engineered satellite cells induces exosome-mediated systemic expression of Fukutin-related protein and rescues disease phenotype in a murine model of limb-girdle muscular dystrophy type 2I. *Hum. Mol. Genet.* 26, 3682–3698
9. Marturano-Kruik, A. *et al.* (2018) Human bone perivascular niche-on-a-chip for studying metastatic colonization. *Proc. Natl. Acad. Sci. U. S. A.* 115, 1256–1261
10. Tunesi, M. *et al.* (2016) Optimization of a 3D dynamic culturing system for *in vitro* modeling of frontotemporal neurodegeneration-relevant pathologic features. *Front. Aging Neurosci.* 8, 146
11. Yi, Y. *et al.* (2015) Central nervous system and its disease models on a chip. *Trends Biotechnol.* 33, 762–776
12. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676