EXPERT INTERVIEW

Revolutionizing neurodegenerative disease research with brain-on-chip technology

The complexity of the human brain creates significant challenges for advancing neurological drug development. Traditional two-dimensional (2D) culture models cannot accurately mimic the true physiology of human tissue. This has led to over- or underestimating cellular behaviors and drug responses in preclinical phases and many subsequent costly candidate failures in clinical trials. A similar situation is seen when relying on animal models, where a drug's success in rodents may not reliably translate to human outcomes.

Organ-on-chips have emerged as a promising alternative, potentially overcoming some of the limitations of other models. Such platforms, which can reproduce key functions of various organs, hold the potential to provide novel insights into diverse physiological events, including mechanisms of disease development and drug response. The neuroscience field is expected to benefit greatly from these innovative tools, specifically in the form of 'brain-on-chips', which could significantly improve the study of neurodegenerative pathologies and help in the search for novel treatments.

Excited by the potential of organ-on-chips, preclinical Contract Research Organization ETAP-Lab is combining its neurotoxin manufacturing capabilities with NETRI's NeuroFluidics technology. This collaboration, namely the BIO-DIAMOND project, aims to develop brain-on-chip models of Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis. When combined with high-content imaging capabilities, ETAP-Lab aspires to capture the intricacies of the human brain *in vitro*. This would enable faster and cost-effective drug development processes and provide novel insights into the development of neurological diseases. In a conversation with ETAP-Lab's CEO, Nicolas Violle, we delved into the details of their advanced cell models and the potential transformative impact on the field of neuroscience.

Meet the expert

Nicolas Violle
Chief Executive Officer
ETAP-Lab

Q One of ETAP-Lab's focus areas is neurodegenerative diseases. Can you explain why this is an important research avenue for you?

A Neurogenerative diseases such as Alzheimer's disease are very challenging to study and, up to now, most trials have failed in the clinic. The reasons for these challenges include the lack of advanced animal models and a limited understanding of the intricate pathology involved. For years, the predominant hypothesis revolved around the role of amyloid-beta plaques in disease development. This prompted widespread efforts to target these plaques. But even with the development of potent antibodies, there were still no discernible benefits for patients.

We now know that at the very early stages of Alzheimer's disease, there is an important amyloid charge in the cerebrospinal fluid (CSF) before the emergence of plaques. This revelation redirected our focus to the early molecular stages where soluble amyloid-beta oligomers start to accumulate, which are extremely toxic to neurons and other cellular components of the human brain. Laboratories are now starting to focus on removing or limiting the toxicity of these oligomers with the hope of modifying the evolution of the disease.

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Q Could you elaborate on the methods ETAP-Lab is utilizing to explore the soluble amyloid-beta oligomer hypothesis?

A Based on this knowledge, we successfully developed a robust method for generating stable and soluble amyloid-beta oligomers from recombinant human amyloid-beta monomers. This was very challenging because these oligomers tend to spontaneously aggregate, so they did not remain soluble. When attempting to stabilize them in a soluble form, they typically stayed as monomers, which are not toxic. We identified a way to keep them stable and soluble in an oligomeric form. Our preparation contains a mixture of trimers, tetramers, and low-molecular-weight oligomers, and remaining monomeric forms of the protein. This is the heart of our technology. We then established that these oligomers indeed display a potent and reproducible toxicity in neurons both *in vitro* and *in vivo* (Figure 1).

In subsequent works, we broadened our scope to Tau oligomers for Alzheimer's disease and alpha-synuclein oligomers for Parkinson's disease. In this context, we successfully devised a method for producing stable misfolded oligomers from recombinant full-length human Tau and alpha-synuclein proteins, respectively. Notably, this accomplishment was realized without resorting to any chemical modification or the aid of helper proteins.

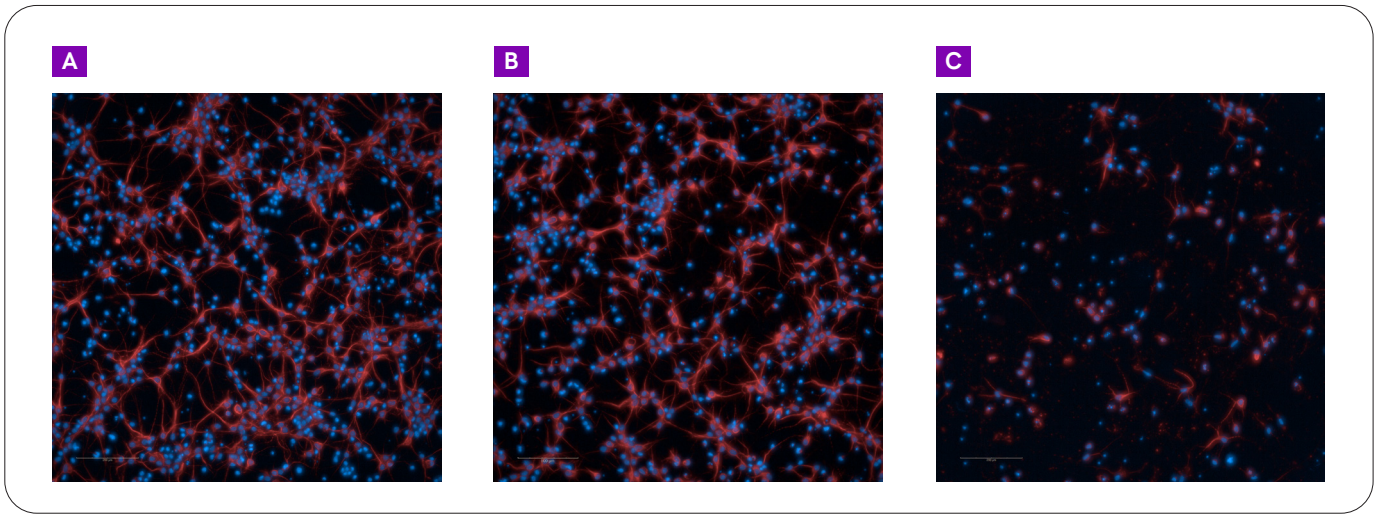

Figure 1: Neurotoxic effect of amyloid-beta oligomers (AβO) on primary neurons. Primary rat neurons were treated with vehicle (A), 1µM AβO (B), or 3µM AβO (C). To visualize morphological changes, cells were labeled with MAP2 (red) and DAPI (blue) and imaged on the Operetta CLS system in confocal mode using the 20x water immersion objective.

Q What advanced *in vitro* cell models are being utilized in your research?

A In collaboration with our partner NETRI, we are developing new models of neurodegenerative diseases using oligomers on brain-on-chip devices. These devices are really interesting because they allow you to reconstitute key features of the human brain, particularly its compartmentalization. Our approach enables the cultivation of human cells within a compartmentalized structure with microfluidic control, mimicking cerebral architecture. By manipulating the nature of cells cultured in different environments and combining these cell culture scenarios with our neurotoxins, we aim to establish study models for neurodegenerative diseases using human cells. This type of cell culture allows us to closely monitor events such as neurotoxic spreading, which is a fundamental mechanism in the pathology (Figure 1). We know there is a demand from pharmaceutical companies to do this kind of study, and currently, the only alternative involves animal experimentation.

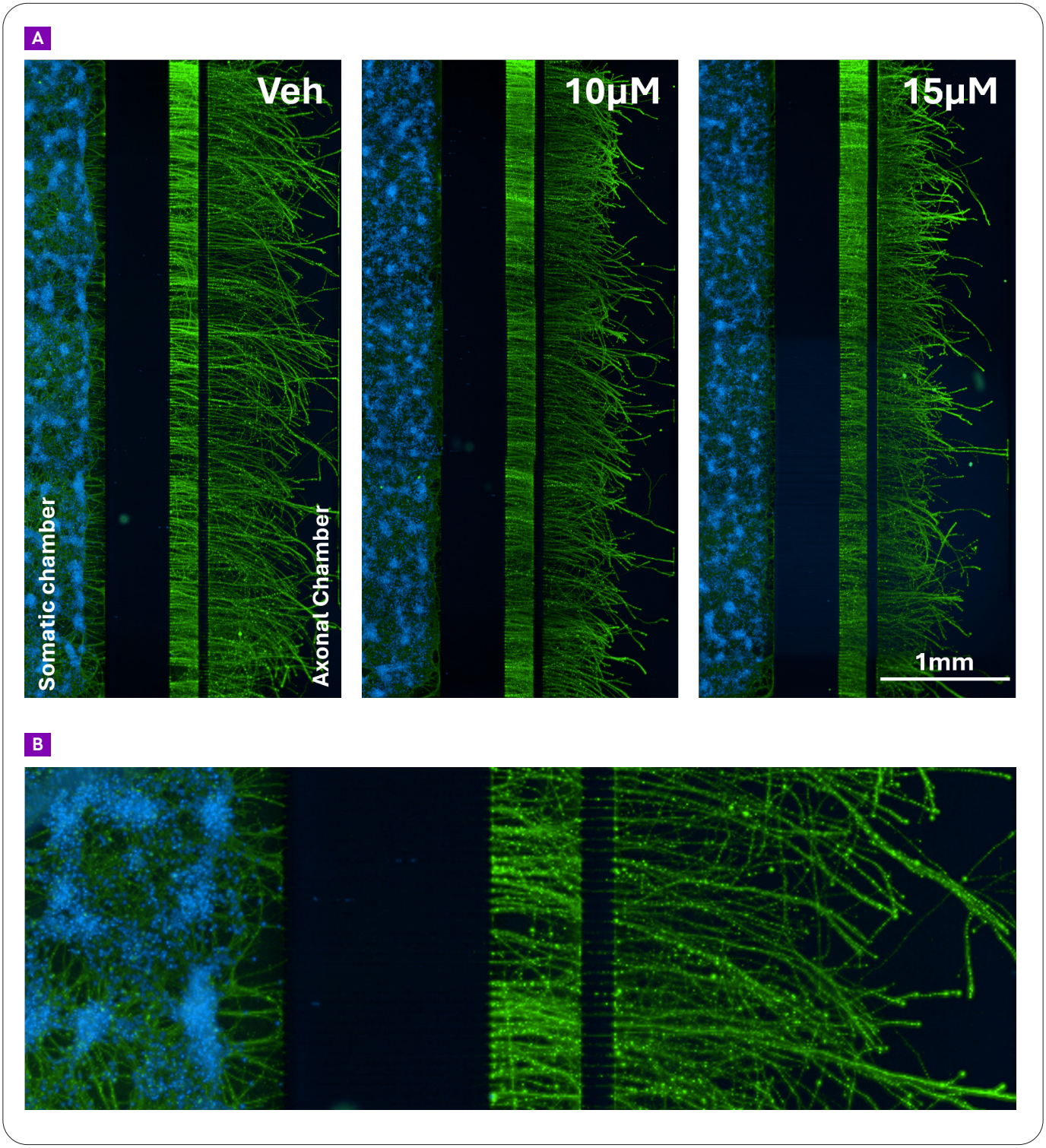

Figure 2: Development of neurotoxic spreading assay in NETRI NeuroFluidics devices. Primary rat neurons were grown in DuaLink Shift NeoBento™ to allow compartmentalization of cells and synapses and labeled with MAP2 and DAPI. Images were acquired on the Operetta CLS system in confocal mode using the 10x objective. A) Based on the increase in AβO concentration in the axonal chamber, ETAP-Lab was able to demonstrate a decrease in the number of nuclei in the somatic chamber and in the fragmentation of axons in the axonal chamber (overview images). B) Detailed view of healthy neurons in the vehicle control.

Q Which advanced analysis technologies are employed to facilitate this research?

A For this project, our primary tool is the Operetta CLS™ high-content analysis system that enables rapid acquisition of complex images and high-content data controlled by the simple and powerful Harmony high-content analysis software. A huge advantage of the Operetta is limiting potential bias, as the process of image acquisition will be identical from one well to another, preventing issues and saving valuable time. It allows us to easily visualize and analyze changes in cell and nuclear morphology as well as cell viability, which are commonly employed in the study and treatment of human diseases.

In this work specifically, we use the Operetta CLS to determine cellular responses to neurotoxins implicated in neurodegenerative diseases and pharmacological treatments. Some notable examples of its integration into our work include:

- **The development of reliable cell viability assays.** This is fundamentally important for efficient drug neurotoxicity screening and to establish the mechanisms of drug action.
- **The development of reliable neurite outgrowth assays.** Again, this is an extremely important tool in drug discovery and neuroscientific research.
- **The development of reliable assays to measure synaptic loss.** Synapses are one of the earliest elements affected in several neurodegenerative diseases and so identifying chemical or biological modulators of synaptic density contributes to the understanding of pathophysiological mechanisms and the identification of new therapeutic targets.
- **The development of neurotoxic spreading assays.** Using the Netri NeuroFluidics devices, we can intoxicate the presynaptic neurons, post-synaptic neurons, or synapses with oligomers and study neurotoxic spreading. This situation would allow us to study a lot of things and potentially test new medications.

By combining high-throughput screening methods and automated microscopy in a microplate format, we can assess the neurotrophic, neuroprotective, or neurotoxic effects of numerous chemical compounds in a controlled, reproducible, and automated manner, ensuring accurate and consistent conclusions.

Q Where do you see the future of these cell models and what excites you the most?

A Well, when we look at the current situation, there's a growing public outcry— and rightly so— pushing to reduce animal experimentation. While we hear a lot about alternatives, truth be told, they're not quite ready yet. Also, in the realm of neurodegenerative diseases and neurology in general, clinical trials have seen many failures, probably linked to overly simplified models, likely due to the complexity and lack of understanding of the diseases.

The translational aspect of these models is crucial, and, in my opinion, it's what will help us break through and bring new drugs to market. The models we're developing will enable us to better select molecules and truly predict their actions in patients. So, that's what excites me the most about the future of these cell models— the potential to revolutionize drug development and, ultimately, make a real impact on patient outcomes.

About ETAP-Lab

ETAP-Lab is a Contract Research Organization (CRO) with three decades of expertise in designing preclinical studies with high translational value for the pharmaceutical industry and biotechnology companies. The company's proficiency extends to dermatological, cardiovascular, and neurological diseases. In dermatology, they offer tailored preclinical study designs, including animal models for inflammatory diseases such as psoriasis and atopic dermatitis (eczema). Additionally, studies are conducted on skin grafts and substitutes through a microsurgery approach. Concerning cardiovascular and pulmonary diseases, the company provides preclinical study services, including models of heart failure, pulmonary arterial hypertension, and myocardial ischemia.

In the field of neurology, ETAP-Lab offers specialized preclinical research services on stroke and neurodegenerative diseases. With more than 10 models of ischemic and hemorrhagic stroke and a state-of-the art imaging platform, ETAP-Lab provides a unique expertise in the field. Regarding neurodegenerative diseases, ETAP-Lab also offers *in vitro* and *in vivo* services, with particular expertise in exclusive toxin manufacturing. These toxins, identified as highly toxic protein forms, play a crucial role in these conditions. It is in this context that the BIO-DIAMOND project, in collaboration with partner NETRI, aims to develop brain-on-chip devices. The goal is to accelerate the development of drugs for neurodegenerative diseases by enabling more predictive efficacy measurements.

About the expert

Nicolas Violle

Nicolas Violle, holder of a PhD in neuroscience obtained in 2008, commenced his career at ETAP-Lab as a project manager specializing in preclinical stroke research. This experience enabled him to collaborate with major pharmaceutical groups, thereby strengthening his understanding of the sector. In 2014, Nicolas took a significant entrepreneurial step by taking over the leadership of ETAP-Lab as CEO. Concurrently, he enhanced his business management skills by earning an MBA. Under his guidance, ETAP-Lab underwent a substantial evolution.

In 2017, in partnership with the University of Caen and Professor Denis Vivien, Nicolas founded STROK@ LLIANCE, an extension of ETAP-Lab. This collaboration led to the creation of the first CRO entirely dedicated to preclinical stroke research. His strategic vision also resulted in the establishment, in 2019, of a specialized in vitro laboratory for neurodegenerative diseases.

In 2023, ETAP-Lab experienced a major acceleration marked by the construction of a new building in Caen, the launch of the BIO-DIAMOND and BRYOFLAM research projects both granted by France 2030, and the introduction of an immunohistochemistry analysis and bioassays service. Simultaneously, Nicolas initiated the design of another building in Nancy and successfully orchestrated external growth with the acquisition of SYNCROSOME, another CRO specializing in cardiovascular research.

With only seven employees in 2014, Nicolas guided ETAP-Lab through a phase of constant growth, now boasting over 40 employees. His ability to spearhead innovative initiatives and foster successful partnerships has undeniably contributed to the success and increasing recognition of ETAP-Lab in the preclinical research field.

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