Application to be sent to Claire Mendoza and Clemence Grosnit:
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Deadline: March 1st, 2024

EUR CARe PhD program pre-proposal

(2 pages maximum)

PhD Director:
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PhD Director affiliation:
Toulouse NeuroImaging Center (ToNIC) - Inserm

PhD co-Director:
Juliette FITREMANN - juliette.fitremann@cnrs.fr

PhD co-Director affiliation:
SOFTMAT - Chimie des colloides, polymeres et assemblages complexes - CNRS

Research project title:
Multi-scale Therapy in Brain Regeneration: Glia in the Peripheral Nervous System and 3D hydrogels as an Innovative Strategy

Research program abstract (max 500 words):
Cell-based therapies offer significant potential for regenerating injured nerve tissue after a stroke. The ideal strategy should lean to well-defined criteria: 1) ease of access of the cell source; 2) long-term safety; 3) long-term survival; 4) anatomical and functional integration into the host tissue; 5) reproducibility. Nerve tissue exists not only in the central nervous system but also in the periphery. The enteric nervous system in the gastrointestinal tract represents a highly organized network of neurons and glia. Enteric glia are intriguing due to their well-established neurotrophic role and remarkable plasticity in response to inflammation or injury. Recent data have revealed a neurogenic role for enteric glia, allowing them to transdifferentiate and give rise to neurons. This presents a significant advantage compared to other compartments of the nervous system where neurogenesis is limited.

Today, strategies exclusively centered on cells have revealed their limitations, marked by high mortality rates and low regenerative capacity. It is imperative not only to focus on cells but also to consider their supporting structures. The extracellular matrix stands out as an indispensable component of brain parenchyma. Adhering to the regulatory and safety standards set by the French health authorities for Innovative Therapy Medicines (*ANSM - https://ansm.sante.fr/documents/reference/bonnes-pratiques-de-fabrication-des-medicaments-de-therapie-innovante*), brain extracellular matrix substitutes often take the form of synthetic hydrogels. However, most hydrogels are not permissive to cell colonization and cells grow only at the surface. Consequently, we have collaborated with the SOFTMAT laboratory (Fitremann J, CNRS – co-Director for this project) to develop innovative synthetic supramolecular hydrogels made of N-alkyl-D-galactonamides (*Chalard et al., 2018; Hacene et al., 2022*). Our results have shown that in vitro, human neural stem cells, primary enteric glia or primary dermal fibroblasts (*Chalard et al. 2018; Hacene et al., 2022; Fitremann et al., in preparation*) developed as a 3D network of cell clusters. Cells colonize well inside the gel and are guided by the gel fibers. Such architectures are likely to favour the imbrication of enteric glia with neurons and the circulation of fluids.

This project centers around an innovative and multi-level approach dedicated to achieving brain repair following stroke. Our goal is to introduce a safer and more effective strategy compared to existing ones.

Specifically, we plan to develop a strategy using autologous enteric glia combined with 3D hydrogels, leveraging a validated a preclinical stroke model (rat).
The assessment of our project's efficacy will be based on two key milestones:
1/ The quality of brain tissue regeneration after the implantation of an autologous enteric glia graft structured by the 3D hydrogels;
2/ Functional recovery achieved through the anatomical and functional integration of the graft enteric glia/3D hydrogel.

The verification methods include the following: in vitro labelling of enteric glia using CRISPR/Cas9 technology (GFP tagging); in vivo MRI and post-mortem histology for evaluating tissue regeneration and the integration of grafted enteric glia within the host; real-time monitoring of the in vivo evolution of enteric glia/3D hydrogel graft (MRI); and behavioural tests to evaluate functional recovery following the therapeutic intervention.

Describe in 50 words max for each how this project fits the 3 defining criteria of the CARe graduate programme:

1) **Relation to CARe topics of Cancer, Ageing and/or Rejuvenation**
   Our project focuses on brain regeneration (*Rejuvenation*) applicable to stroke, a pathology affecting an elderly population (*Ageing*).

2) **Multidisciplinary aspect**
   This project is at the interface of neurobiology (*biology*) and 3D-printed biomaterials (*chemistry*).

3) **International and/or industrial aspect(s)**
   - PhD Director Carla CIRILLO has ongoing international collaborations with 1/ Prof Hideki ENOMOTO (Kobe University, Japan) to identify an enteric glia subpopulation with neurogenic potential; and 2/ Dr Conor MCCANN (UCL, UK) to evaluate the regenerative potential of enteric glia in a model of intestinal neuropathological loss.
   - PhD co-Director Juliette FITREMANN is currently working with Jenny Malmström (Auckland, New Zealand) on gelatin-methacrylate / N-alkyl-D-galactonamide composites for the culture of cardiac cells. Also, the method of synthesis of three N-alkyl-D-galactonamides has been licensed to Innov'Orga (France), making these molecules commercially available.

5 keywords in line with EUR CARe

Regeneration, cell therapy/enteric glia, stroke, 3D implant.

5 references of the teams, highlighting the co-signatory students:

1. Colitti N, Desmoulin F, Le Friec A, Labriji W, Robert L, Michaux A, Conchou F, Cirillo C*, Loubinoux I*. Long-term intranasal nerve growth factor treatment favors neuron formation in de novo brain tissue. *Frontiers in Cellular Neuroscience* 2022. PMID: 35928573
2. Hacene S, Le Friec A, Desmoulin F, Robert L, Colitti N, Fitremann J, Loubinoux I, Cirillo C. Present and future avenues of cell-based therapy for brain injury: The enteric nervous system as a potential cell source. *Brain Pathol*, 2022. PMID: 35773942
3. Andriamiseza F, Bordignon D, Payré B, Vaysse L, Fitremann J. 3D printing of biocompatible low molecular weight gels: Imbricated structures with sacrificial and persistent N-alkyl-d-galactonamides. *J Colloid Interface Sci*. 2022. PMID: 35276518
4. Accardo A, Cirillo C, ..., Loubinoux I. Interfacing cells with microengineered scaffolds for neural tissue reconstruction. *Brain Res Bull* 2019. PMID 31348979
5. Chalard A, Vaysse L, Joseph P, Malaquin L, Souleille S, Lonetti B, Sol JC, Loubinoux I, Fitremann J. Simple Synthetic Molecular Hydrogels from Self-Assembling Alkylgalactonamides as Scaffold for 3D Neuronal Cell Growth. *ACS Appl Mater Interfaces* 2018. PMID: 29757611