Executive Summary

The anatomical complexity of human central nervous system (CNS) encompasses its cellular and extracellular diversity, multi-scale heterogeneity, and structural anisotropy. These features are fundamental for the regulation of many physiological processes that enable bodily functions such as sensation, perception, memory, and movement. Therefore, a deeper understanding of CNS physiology and pathology requires a comprehensive analysis of its unique structure-function relationships. In this scenario, bioengineered *in vitro* models represent effective tools for investigating neural development and functioning, as well as delineating the biological mechanisms involved in the onset and progression of neurological disorders. Indeed, these systems hold great promise to recapitulate CNS intricacy, while offering the right balance between physiological relevance and usability compared to currently used preclinical models. The main purpose of this PhD project is the design of bioengineered neural tissue models, by combining the use of neural stem cells (NSCs) and cell-instructive substrates. Different biofabrication strategies have been explored to model the neural microenvironment with increased biomimicry, leading to the optimization of *in vitro* systems here classified according to their manufacturing method.

Specifically, an electrospun scaffold-based model was developed through the realization of a biomimetic culture system, designed to guide the growth and maturation of NSCs under *in vivo*-like stimuli. Electrospinning technique was employed to fabricate a polycaprolactone/polyaniline (PCL/PANI) nanofibrous scaffold, having electroactive properties and ECM-like morphology. The use of a rotating drum collector in the electrospinning setup allowed for the deposition of fibers in an aligned configuration. *In vitro* experiments were performed by culturing NSCs (NE-4C cell line) on the nanofibrous mats to investigate the effect of scaffold physicochemical properties on neural cell behaviour. According to the results, the fabricated mats exhibited distinct electroconductivity, defect-free fibers, and anisotropic morphology and mechanical response. *In vitro* tests confirmed the capability of PCL/PANI mats to support NSC attachment and growth, enabling the formation of a uniform cell monolayer showing characteristic epithelial-like morphology. The phenotypic characterization demonstrated that NSCs were able to generate mature neurons and astroglial cells after two weeks from differentiation induction, underlining the ability of nanofibers to steer neuronal differentiation rather than standard 2D culture. The aligned topography of electrospun mats was

shown to affect the arrangement of NSC-derived neurons, providing contact guidance for cell elongation in a preferential direction. The functional maturity of the neural network was then demonstrated by live cell imaging analysis of calcium flux. Therefore, the electrospun scaffold-based model was proved to recapitulate some of the essential features of the native microenvironment, including the electroactive properties, multicellularity, and anisotropic fiber-shaped assembly. Due to these characteristics, the model provides useful insight into the efficacy of fiber-mediated topographical patterning in directing neural cell fate and organization.

Additionally, with the intention to more accurately reproduce neural tissue 3D microarchitecture, the potential of additive manufacturing technologies has been exploited to develop 3D bioprinted models. Living neural-like constructs were fabricated through an extrusion-based approach, by encapsulating NSCs (NE-4C cell line) within a gelatin methacryloyl (GelMA) bioink. Rheological analyses showed that the formulated hydrogel had fast crosslinking under cell-friendly conditions and shear-thinning behaviour, confirming its suitability as extrudable bioink. *In vitro* studies revealed high cell survival after the printing process and progressive growth of encapsulated cells in 3D bioprinted constructs. In addition, protein expression analyses demonstrated successful neural differentiation into mature neuronal and astroglial phenotypes after three weeks from induction. Based on these findings, a multi-scaffold assembly approach was optimized to integrate directional guidance cues within the 3D bioprinted system. 3D bioprinted hybrid constructs were fabricated by positioning the NSC-laden GelMA bioink onto an aligned microfibrous structure. The latter was produced by melt electrowriting technology and designed to have aligned topography and interconnected porosity to guide neural cell organization in a 3D fashion. According to the results, complete cell colonization of the constructs was achieved, with cells extensively infiltrating across the microfibrous structure and preferentially arranging according to fiber orientation. Live calcium imaging revealed spontaneous neuron signalling after three weeks of differentiation culture, indicating the formation of a functionally aligned neural network. Finally, the feasibility of using this manufacturing approach for the assembly of more complex cytoarchitecture was preliminarily assessed by the establishment of a 3D co-culture system comprising NE-4C cells and motor neuron-like cells (NSC-34 cell line). To conclude, the here described results underlined the ability of microfibrous substrates and bioartificial matrices to promote neural cell maturation within an *in vivo*-like 3D environment, thus offering new opportunities for the creation of functional bioengineered neural tissues.