3D bioprinting of hiPSC-derived neurons for brain-on-a-chip applications

Corinna Barella[1], Donatella Di Lisa[1], Alberto Lagazzo[2], Giulia Parodi [1], Sergio Martinoia[1], Laura Pastorino[1]

[1]*Department of Informatics, Bioengineering, Robotics and Systems Engineering (DIBRIS), University of Genoa, Genoa, Italy*
[2]*Department of Civil, Chemical and Environmental Engineering (DICCA), University of Genoa, Genoa, Italy*

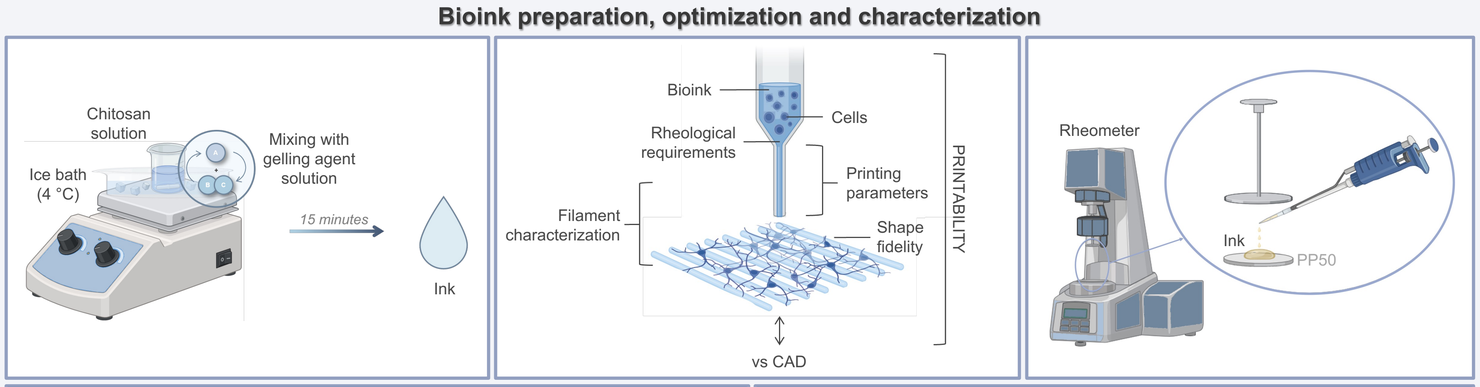
corinna.barella@edu.unige.it

Introduction

The complexity of the human brain poses a substantial challenge in the advancement of central nervous system models. While traditional two-dimensional neuronal cell cultures have long been the gold standard for *in vitro* studies in neurophysiology, recent research has shifted towards cultivating neuronal networks in three-dimensional (3D) structures to create more sophisticated and life-like neuronal models [1]. To address these challenges, 3D bioprinting has emerged as a leading technique for crafting precisely shaped scaffolds aimed at replicating the complexity and cellular diversity of brain tissue *in vitro* [2]. The choice of the biomaterial constituting the bioink is of core importance. Among biopolymeric hydrogels, chitosan-based thermogels have demonstrated optimal characteristics for promoting neuronal growth and the development of functional neural networks derived from human-induced pluripotent stem cells (hiPSC) co-cultured with astrocytes [3]. In this work, a natural hydrogel chitosan-based thermoink formulation was developed and characterized for the 3D bioprinting of compartmentalized neuronal co-cultures with precise control over the distribution of neural cells to mimic the spatial and functional organization of brain tissue *in vivo*, providing a valuable platform for studying neural behaviour and interactions.

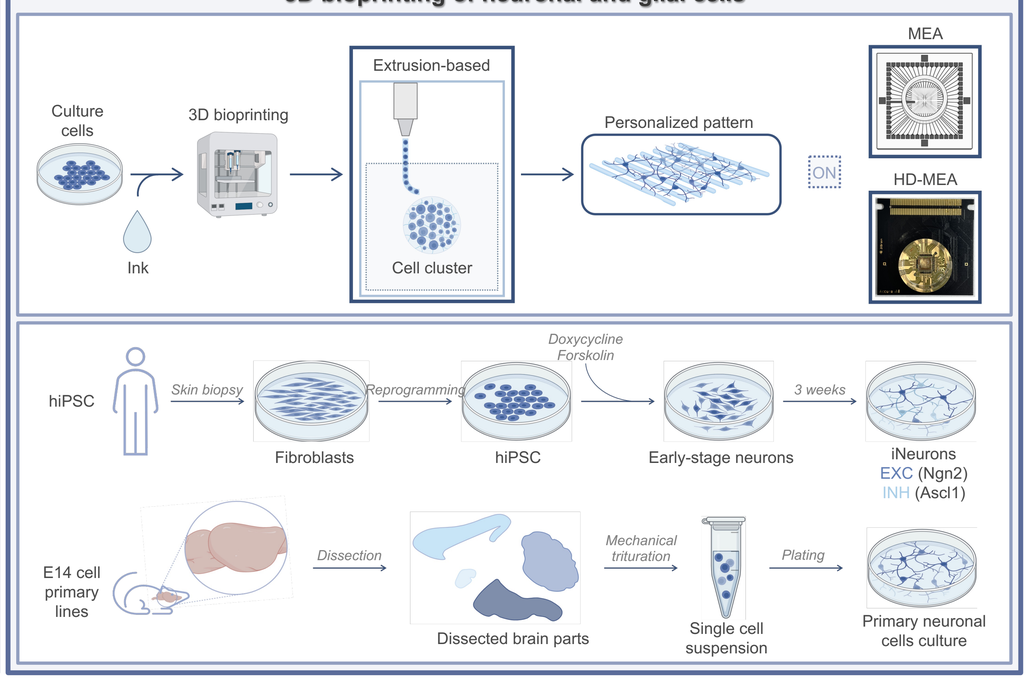
Materials and Methods

Bioink preparation, optimization and characterization



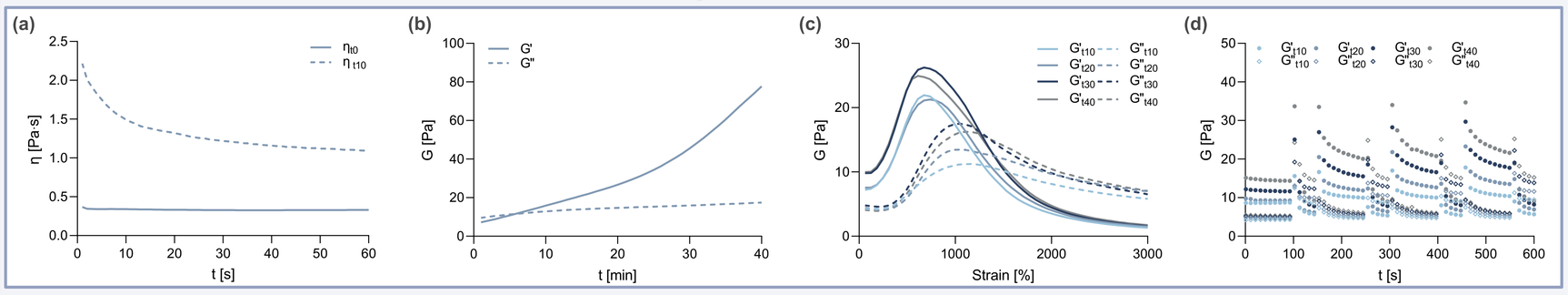
(a) Morphological characterization of the freeze-dried chitosan-based thermoink by SEM at magnifications x200 (left), x500 (middle), and x1000 (right); scale bar: 30 µm. **(b)** Representative inverted-optical images of filaments printed with a 27 G nozzle, used to assess filament diameter, post-printing shape fidelity, and uniformity factor; scale bar: 200 µm.

3D bioprinting of neuronal and glial cells



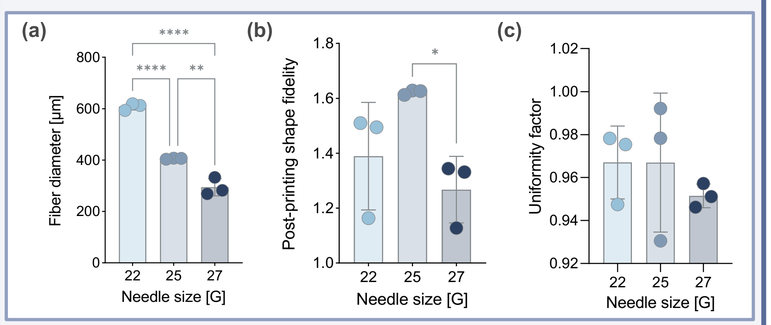
Results

Rheological characterization



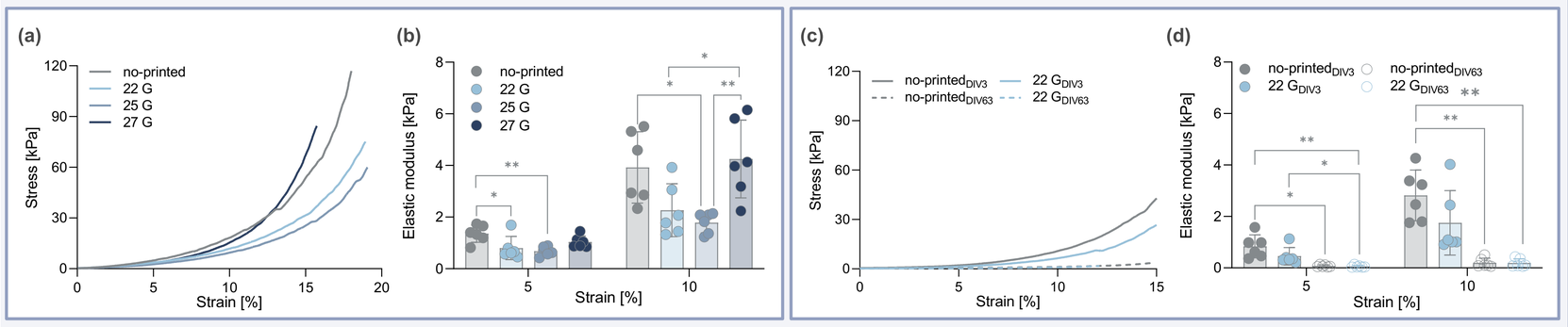
(a) Viscosity curves measured immediately after the preparation of the chitosan-based thermoink solution (t_0) and after the gelation time determined from the gelation kinetics analysis (t_{10}). **(b)** Gelation kinetics of the chitosan-based thermoink measured at t_0. **(c)** Yield stress profiles measured at 10, 20, 30, and 40 minutes after pre-gelation. **(d)** Oscillatory strain sweep tests performed at increasing pre-gelation times (0-40 minutes).

Fiber characterization



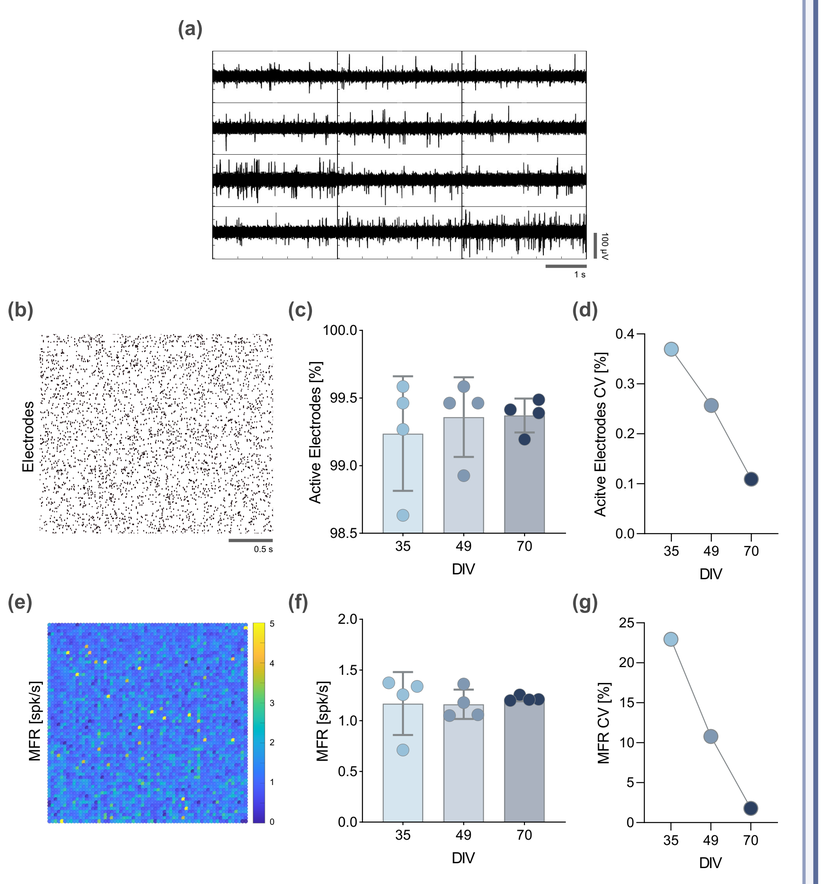
Average filament diameter **(a)**, post-printing shape fidelity **(b)**, and uniformity factor **(c)** of constructs printed with 22 G, 25 G, and 27 G nozzles.

Mechanical characterization



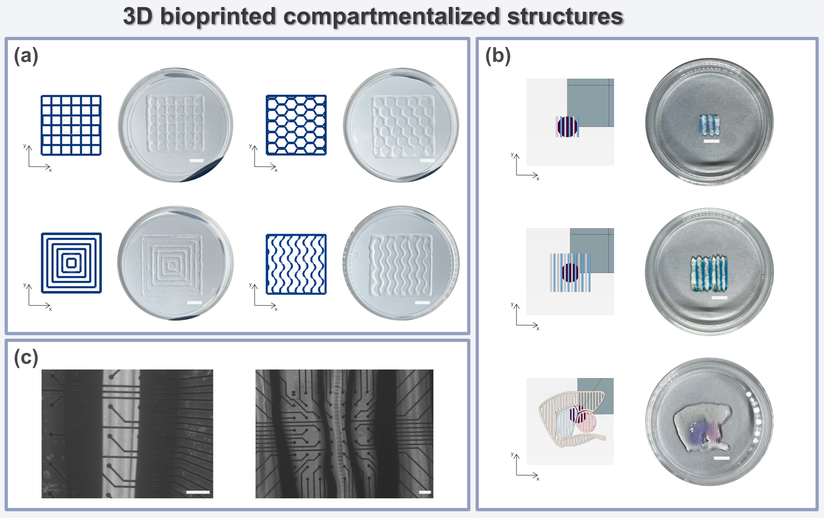
Representative stress-strain curves of acellular ink samples **(a)** and cell-laden constructs **(c)**, with the corresponding elastic modulus values shown in **(b)** and **(d)**.

Electrophysiological characterization



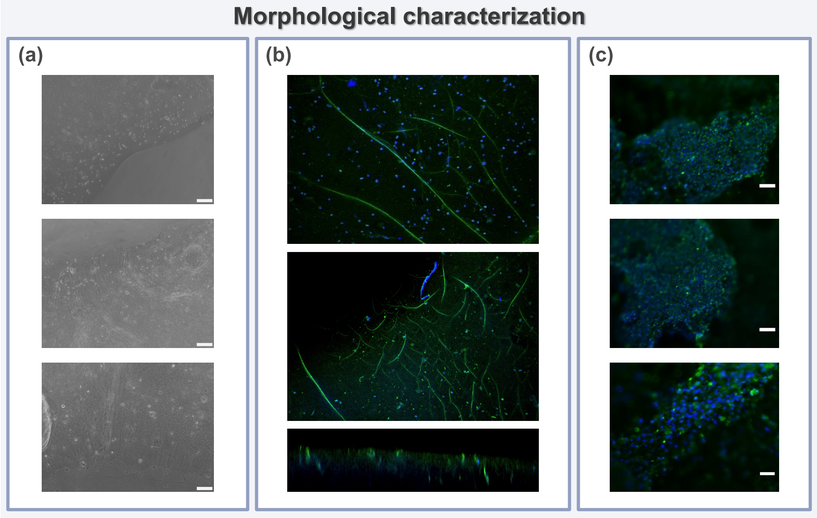
(a) Raw data recorded by few electrodes of a representative culture. **(b)** Representative raster plot of 5 seconds of spontaneous activity, a black dot represents a detected spike. **(c)** Bar plot of the percentage of active electrodes during development. **(d)** Coefficient of variation (CV) of the amount of active electrodes. **(e)** Mean Firing Rate (MFR) map in which the colour bar represents the spiking rate for each channel, identified by each pixel. **(f)** Bar plot of the MFR during time. **(g)** CV of the firing rate.

3D bioprinted compartmentalized structures



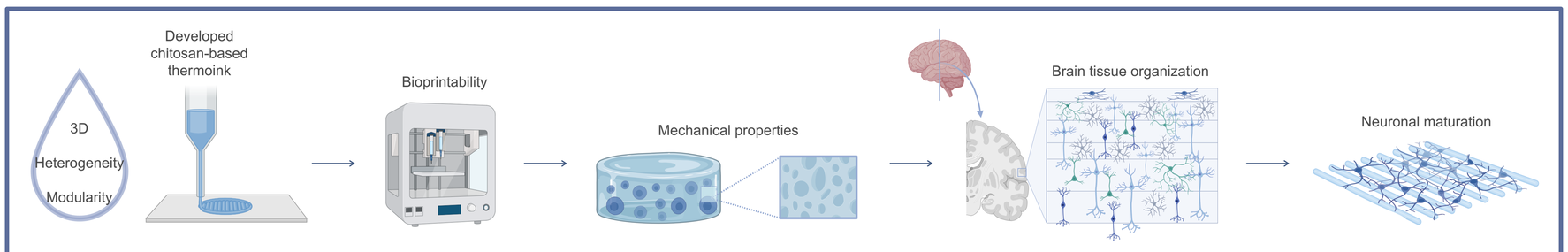
(a) Patterns (20x20x1 mm) bioprinted using 27 G according to different design models: grid pattern (top-left), honeycomb pattern (top-right), concentric square pattern (bottom-left), and gyroid pattern (bottom-right); scale bar: 5 mm. **(b)** Multi-material constructs printed using two/three independent printheads to reproduce the spatial organization of neurons in distinct compartments; scale bar: 5 mm. **(c)** Personalized ink-based pattern printed directly onto microelectrode array (MEA) for electrophysiological applications; scale bar: 100 µm.

Morphological characterization



(a) Brightfield images acquired at DIV1 (top), DIV35 (middle) and DIV49 (bottom); scale bar: 100 µm. **(b)** Maximum intensity projections of confocal z-stacks at DIV35 (top) and DIV49 (middle), showing neuronal networks stained for βIII-tubulin (green) and cell nuclei (DAPI, blue); orthogonal x-z views (bottom); z-stack: 160 µm. **(c)** Fluorescence images stained for βIII-tubulin (green) and DAPI (blue); scale bar: 20 µm.

Conclusion



References

[1] Tarricone G. et al., Tissue-engineered models of the human brain: State-of-the-art analysis and challenges. *Journal of Functional Biomaterials*, 13(3):146, Sep 2022.

[2] Cadena M. et al., 3d bioprinting of neural tissues. *Advanced healthcare materials*, 10(15):2001600, 2021.

[3] Di Lisa D. et al., Long-term *in vitro* culture of 3d brain tissue model based on chitosan thermogel. *Biofabrication*, 2023.