MOLECULAR GELS FROM ALKYLGALACTONAMIDES: FROM NEURONAL CELL CULTURE to 3D PRINTING

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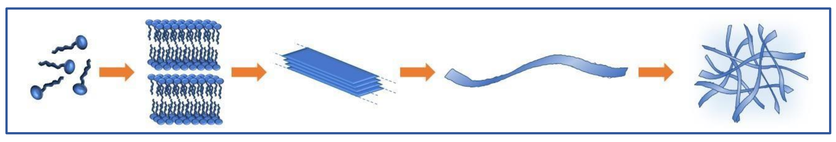
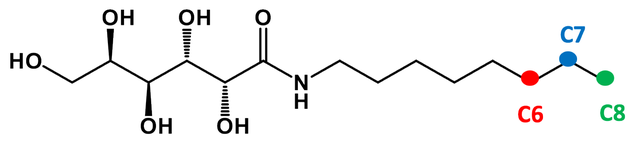
MOLECULAR HYDROGELS: NON–POLYMER HYDROGELS



Formed by the self-assembly of small molecules



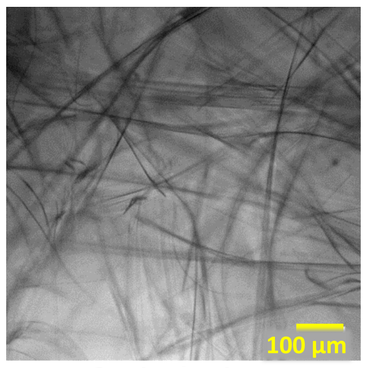
N-hexyl-galactonamide (Gal-C6)
N-heptyl-galactonamide (Gal-C7)
N-octyl-galactonamide (Gal-C8)

0,5 wt% in water
110°C

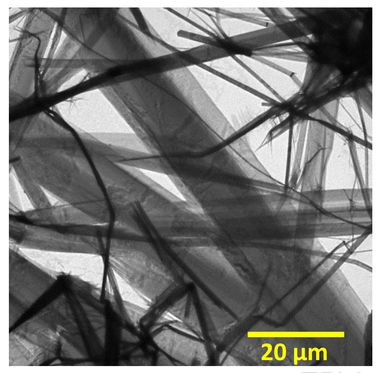
Slow cooling to RT

MICROSCOPIC CHARACTERIZATION

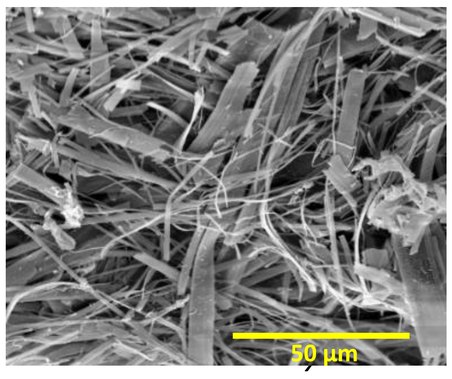
Hydrogels of N-Heptyl-D-Galactonamide (Gal-C7)



Optical microscopy



TEM



- Long fibers thanks to slow cooling → cohesive network
- Very large ribbons and mesh
- Heterogeneous fiber size: large, narrow, straight, flexible…
- **Gal-C7** and **Gal-C8** → Fibers 5 times wider compared with **Gal-C6**

MECHANICAL CHARACTERIZATION

Compression tests

Modulus Y_0 :

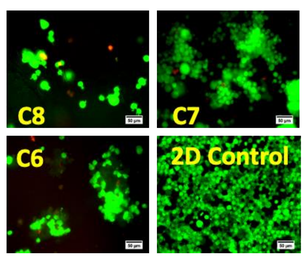
Gal-C6	150	kPa
Gal-C7	15	kPa
Gal-C8	25	kPa

X 10

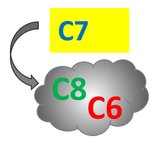
- Elastic modulus of the brain ≈ 0,1 to 1 kPa
- Very soft hydrogels are needed for differenciation into neurons

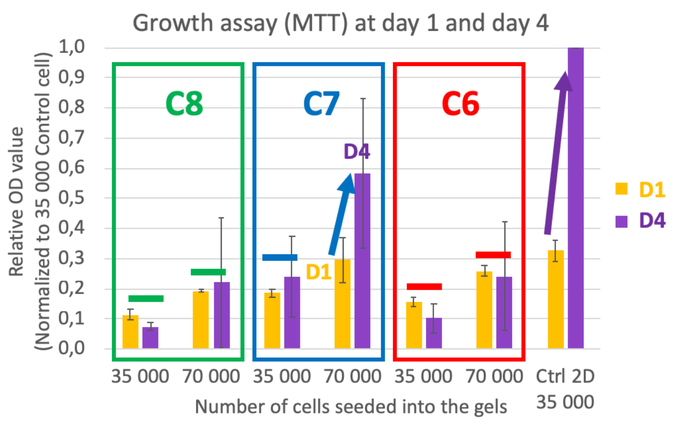
DEAD-LIVE AND GROWTH OF NEURO-2A ON THE GELS

Dead/live test at day 7



1-carbon difference



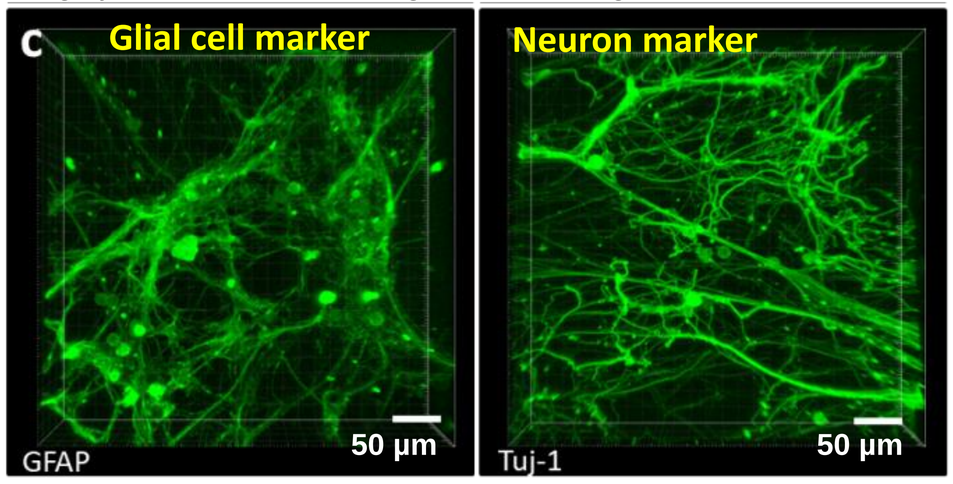


Best results on Gal-C7 – N-Heptyl-D-Galactonamide

ADULT HUMAN NEURAL STEM CELLS

Patient biopsies → Neurospheres → Cell culture on hydrogel → Embedding in polyacrylamide → Immunostaining steps → Confocal microscopy

In this case, the confocal microscopy imaging revealed only the cells
The gel fibers are dissolved during immunostaining



7 days

- Dense network of both neurons and glial cells
- Numerous neurites
- Bundles of straight and very long neurites
- Neurites followed the gel fibers.
- Heterogenous network of fibers → diversity of the cells morphology
- Cell differenciation depend on rigidity at the microscale and neurite growth is mechanosensitive

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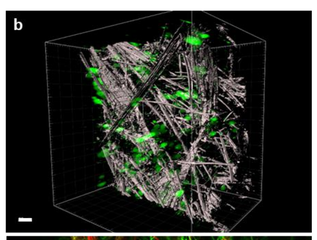
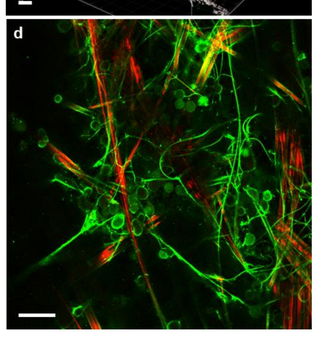
3D PENETRATION OF CELLS IN THE GEL



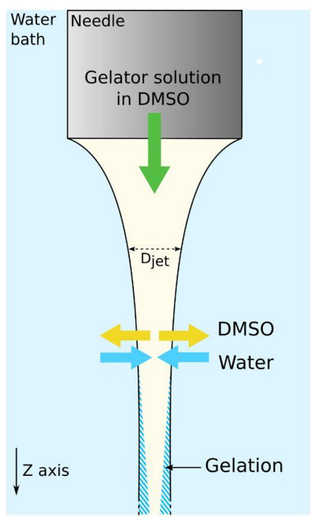
- Confocal microscopy reflection + fluo
- GFP modified Neuro2A cells
- From z stacks → up to 200 µm depth

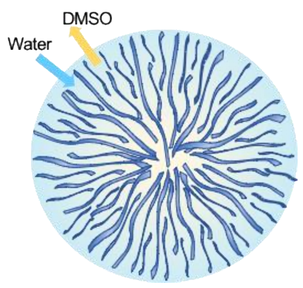


- Confocal microscopy
  reflection mode (red)+ fluo mode (green)
- GFP modified Neuro2A cells
- Thin fibers are revealed by polylysine-FITC

Scale bar = 50 µm

WET-SPINNING



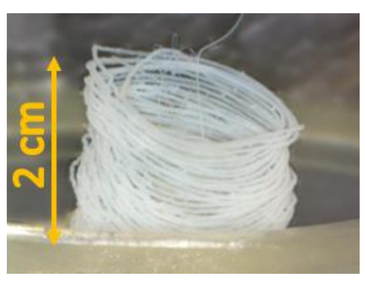


- **Radial self-assembly** of the fibers
- **Self-assembly in spirals** at high flow rates
- Fiber width around 150 nm
- Monodisperses

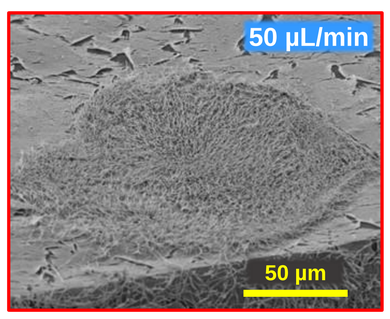
3D-PRINTING

LAAS CNRS

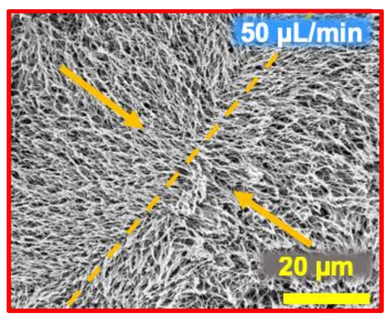
Gel filaments
97 wt% water
*3 wt% **Gal-C7***



Cross section



Longitudinal section



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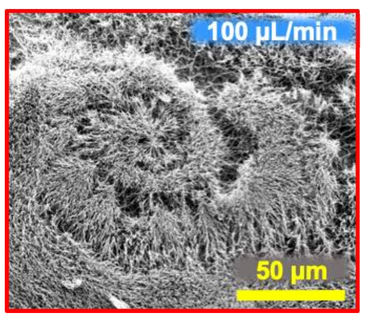
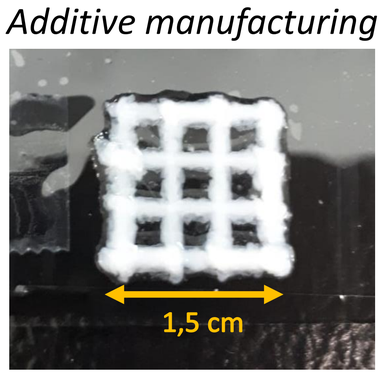


Additive manufacturing



REFERENCES: (1) « Simple Synthetic Molecular Hydrogels from Self-Assembling Alkylgalactonamides as Scaffold for 3D Neuronal Cell Growth », *ACS Appl. Mater. Interfaces* **2018**, *10* (20), 17004
(2) " Wet spinning and radial self-assembly of a carbohydrate low molecular weight gelator into well organized hydrogel filaments", *Nanoscale*, **2019**, DOI:10.1039/C9NR02727K

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