

bioMAT4EYE

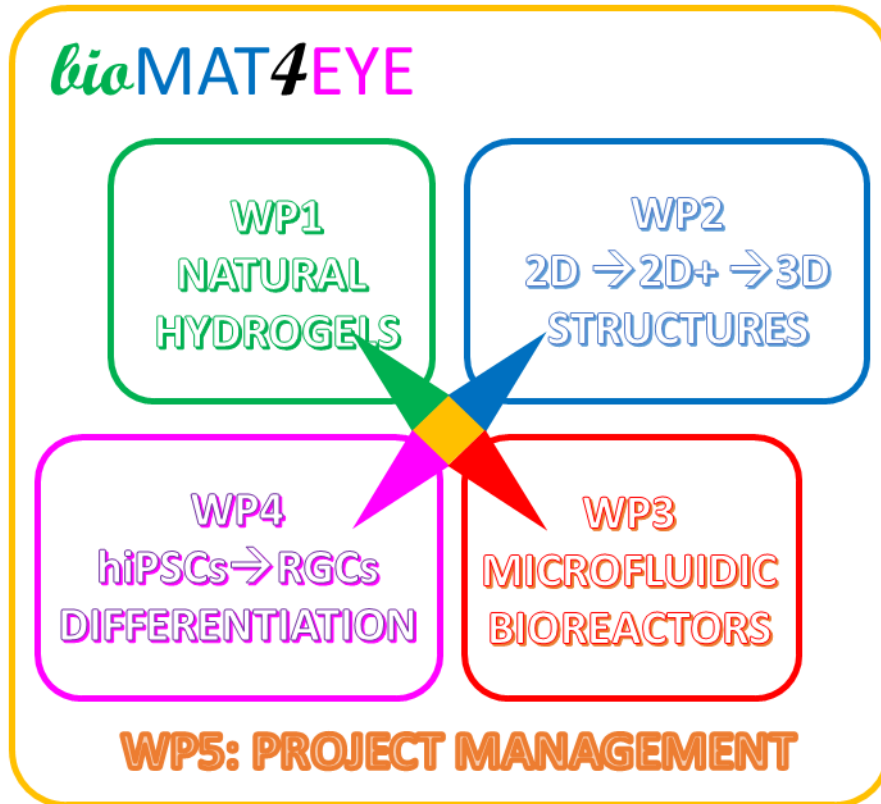
M-ERA.NET Conference "Advanced Materials & Battery Technologies for a Sustainable Future" | 1-2 April 2025 | Dresden, Germany



# Neoteric Biomaterials for hIPSCs Monitored Differentiation to RGCs: Creation, Microfabrication & Microfluidics (bioMAT4EYE)

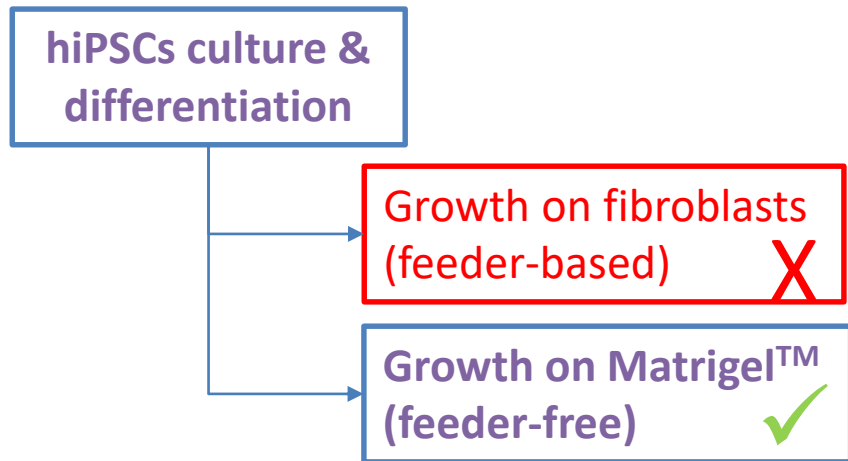


# Outlook



- ❖ The problem
- ❖ The project
- ❖ The consortium
- ❖ WP1: Biopolymers & hydrogels
- ❖ WP2: The 2D-3D structures
- ❖ WP3: The microreactor
- ❖ WP4: the cells
- ❖ Training, diffusion and dissemination

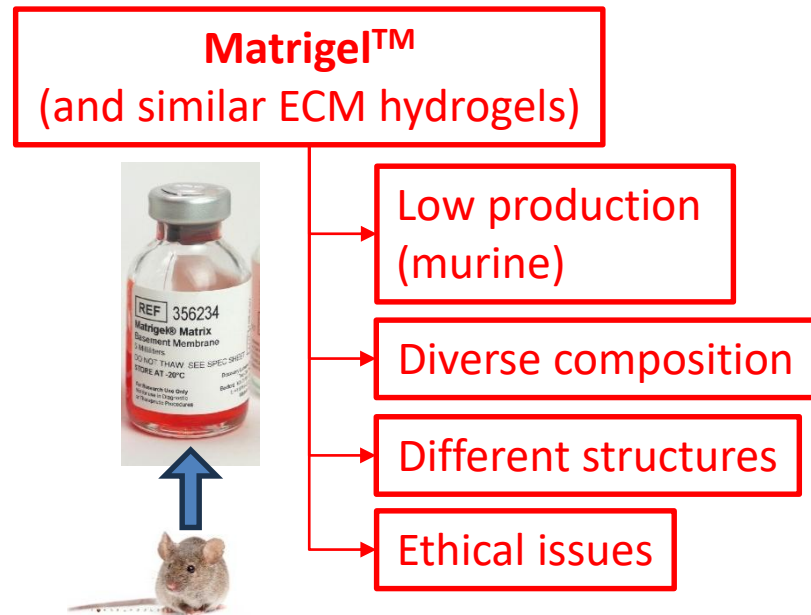
## A personalized biomedicine promise



## Strategy

**bioMAT4EYE** will address Matrigel substitution by **joining** several multidisciplinary research teams with key expertise in **biomaterial production** (fermentation, extraction), physicochemical **modification** and **conformation** in **2D, 2D+ and 3D structures** by diverse technologies, **micro-bioreactor** design, construction and physicochemical control, and **hiPSCs** generation, culture and **monitored differentiation** to **RGCs**. RGCs can be the basis for **cell therapies** for patients with **optic neuropathies** of low prevalence (e.g. LHON, DOA) and **age-related** increasingly prevalent ones (e.g. glaucoma).

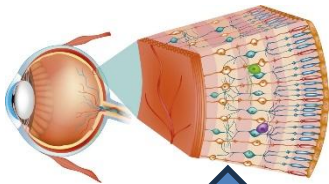
## Posing a problem



# The general structure of the project

A Hierarchical Approach for hiPSCs differentiation to RGCs by combining Biomaterials, Surface Chemistry and Microfluidics (*bioMAT4EYE*)

Retina: Optic neuropathies (DOA, LHON, glaucoma)



**RGCs**

Retinal Ganglion Cells

**MATRIGEL**

**hiPSCs**

human induced Pluripotent Stem Cells

**WP1  
NATURAL  
HYDROGELS**

Pululano  
Quitosano  
Alginato

**WP4  
hiPSCs → RGCs  
DIFFERENTIATION**

**WP3  
MICROFLUIDIC  
BIOREACTORS**

**WP2  
2D → 2D+ → 3D  
STRUCTURES**

Project structure: the consortium

8 PARTNERS, 5 COUNTRIES



**KU LEUVEN** 

**WP1&2**

**PFI**   
 PART OF RI-SE **WP1&2**  


  
 UNIVERSIDAD COMPLUTENSE MADRID **WP1&2**  
  
**WP5**  
  
 Instituto de Investigación Hospital 12 de Octubre **WP1&4**  
  
**WP2&3**

  
 UNIVERSITÄT LEIPZIG **WP3&4**



  
 University of Ljubljana **WP3&4**



In bold: WP leader

**WP5: UCM**      **WP1: KU Leuven**      **WP1: PFI-RISE**  
**WP3: Ljubljana University (LU)**      **WP4: i+12**

**WP1  
NATURAL  
HYDROGELS**

KUL, UCM,  
RISE PFI, i+12

T1.1. Hydrogel production and modification

➤ **Exopolysaccharides: alginate, pullulan, chitosan, xanthan gum**

T1.2. Hydrogel physicochemical characterization

➤ **HPLC-SEC, viscosity, SEM, NMR, FTIR, Rheology...**

T1.3. Cytocompatibility assessment

➤ **hiPSCs, RPCs adhesión & proliferation**

**T1.1**

UCM → Bacterial alginate: *Azotobacter vinelandii*

RISE-PFI / Chitinor → Chitosan

**T1.2**

KU Leuven → Pullulan: *Aerobasidium pullulans*

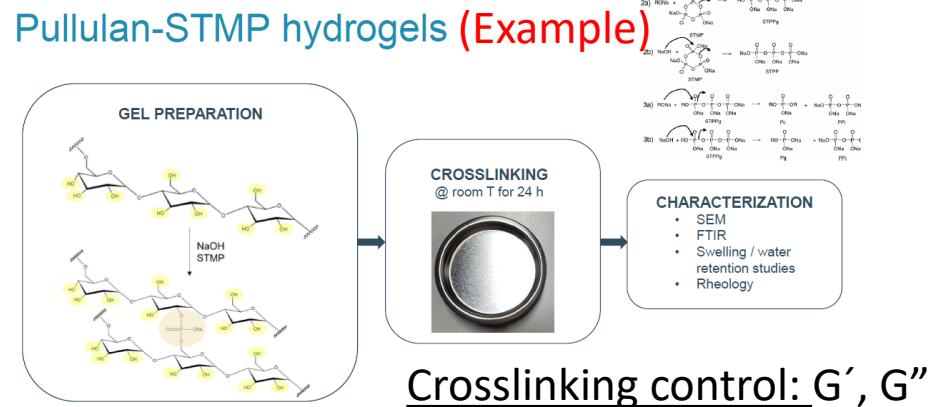
Acid extraction & purification

1) **BioProcess variables:** carbon source, nitrogen source, %oxygen, temperature, time...



- Molecular weight
- Ratio G/M (alginate)
- Acetylation grade
- Melanin (pullulan)
- Protein content
- Phenolics & antioxidants

2) **Crosslinking:** STMP, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Fe<sup>3+</sup>, chitosan, NaOH, UV...



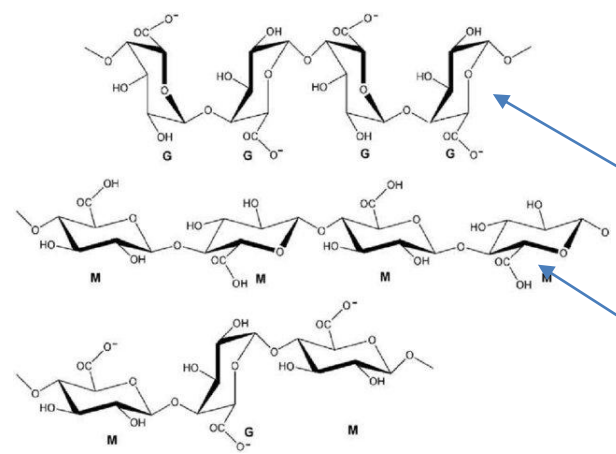
T1.1  
T1.2

Bioprocess examples: bacterial alginate

X  
Xanthan gum →  
*Xanthomonas campestris* (plant pathogen)

↓

✓ High MW /pure alginate →  
*Azotobacter vinelandii* ATCC 9046

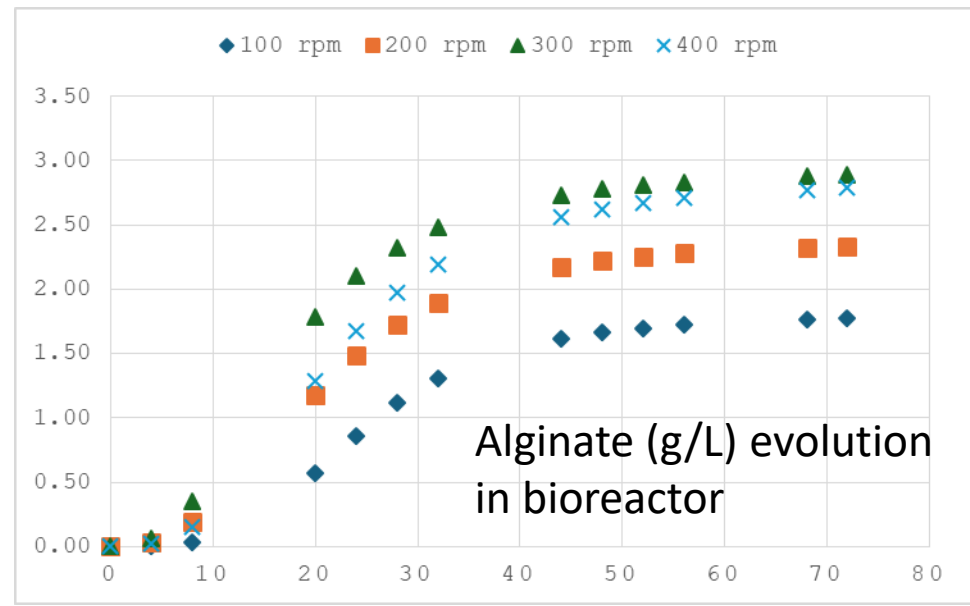


Guluronic acid

Mannuronic acid

Process variables

- Stirring speed  
(O<sub>2</sub>, N<sub>2</sub> transfer)
- Carbon source  
(sac, glu, potato waste)
- Nitrogen source  
(yeast extract, N<sub>2</sub>)
- Inoculum build-up  
(1, 2, 3 inocula stages)
- Process time  
(34 to 72 h)



Property affected

- Molecular weight  
(MW, PDI,  $\mu_a$ )
- Acetylation degree
- Mannuronic / Guluronic ratio
- Alginate concentration

T1.1  
T1.2

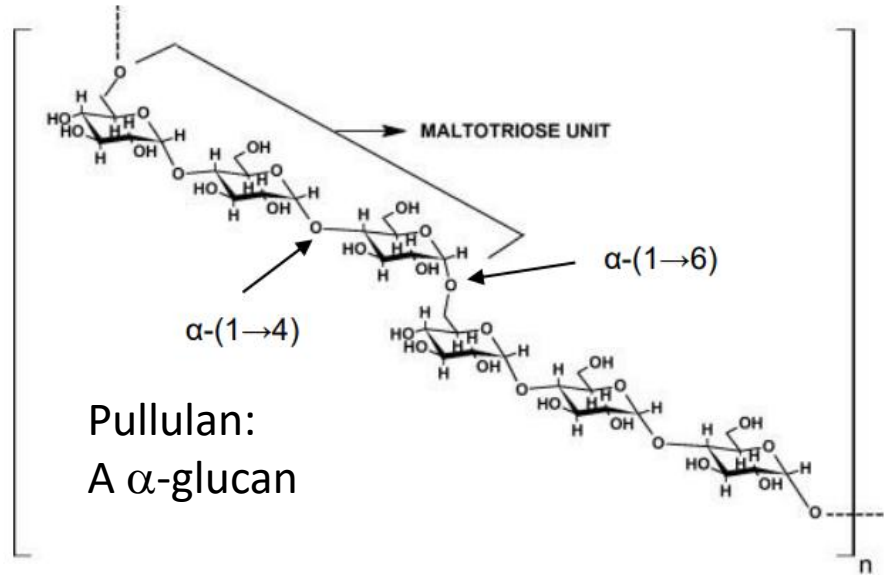
Bioprocess examples: pullulan (*Aerobasidium pullulans*)

Process variables

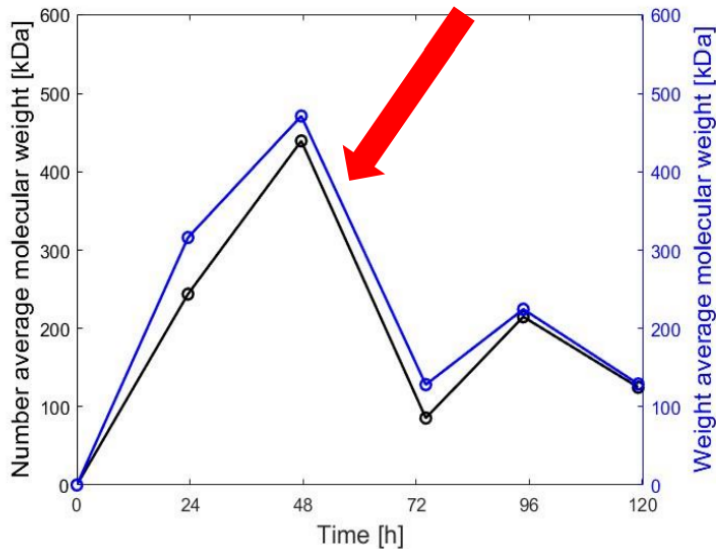
Carbon source (sac, glu)      Aeration flow (1→2 v.v.m.)  
 Nitrate concentration (0.6, 1.0 g/L)      Process time (48 to 120 h)

Property affected

Melanin production (from day 3)  
 Pullulan production (40→70 g/L)



Pullulanase



| Day | $M_N$ [kDa] | $M_W$ [kDa] | PDI [-] |
|-----|-------------|-------------|---------|
| 0   | 0           | 0           | -       |
| 1   | 316.093     | 243.888     | 1.296   |
| 2   | 471.238     | 439.448     | 1.072   |
| 3   | 128.157     | 85.560      | 1.498   |
| 4   | 224.510     | 214.857     | 1.045   |
| 5   | 128.987     | 124.758     | 1.034   |



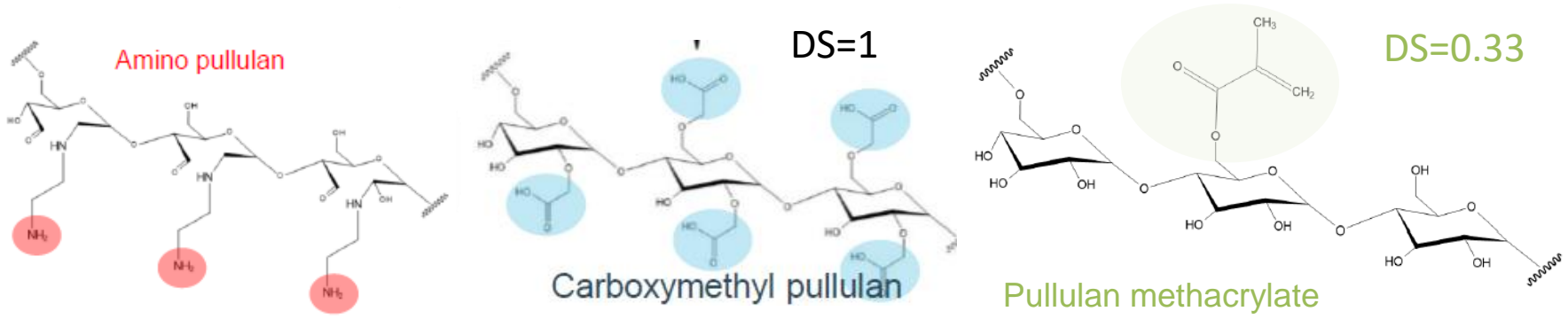
T1.1

T1.2

### 3) Chemical modification

UCM and RISE-PFI → Hydrogels based on chitosan-alginate (or xanthan gum)

KULeuven → chemical modifications of pullulan –metacrylated, carboxymethylated, amino-

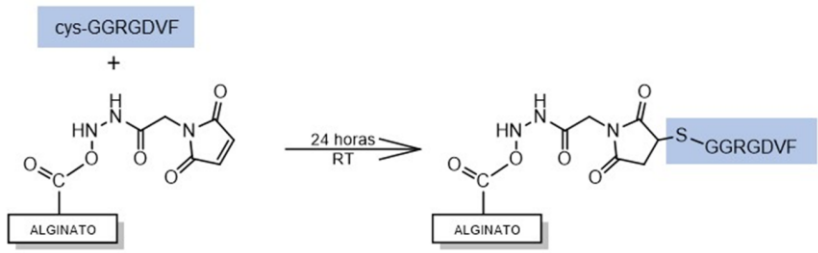


### 4) RGD-peptide (from integrins) grafting: a) Carbodiimide → R-NH<sub>2</sub> b) Maleimide → R-SH

UCM → alginate

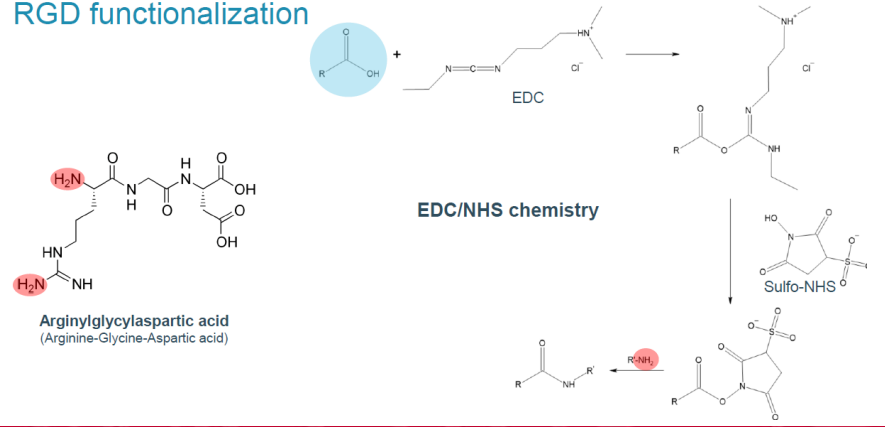
UCM, KUL, RISE PFI → alginate, pullulan, chitosan

Maleimide-based cystein containing peptide grafting



Tested by <sup>1</sup>H-NMR: 100% grafting

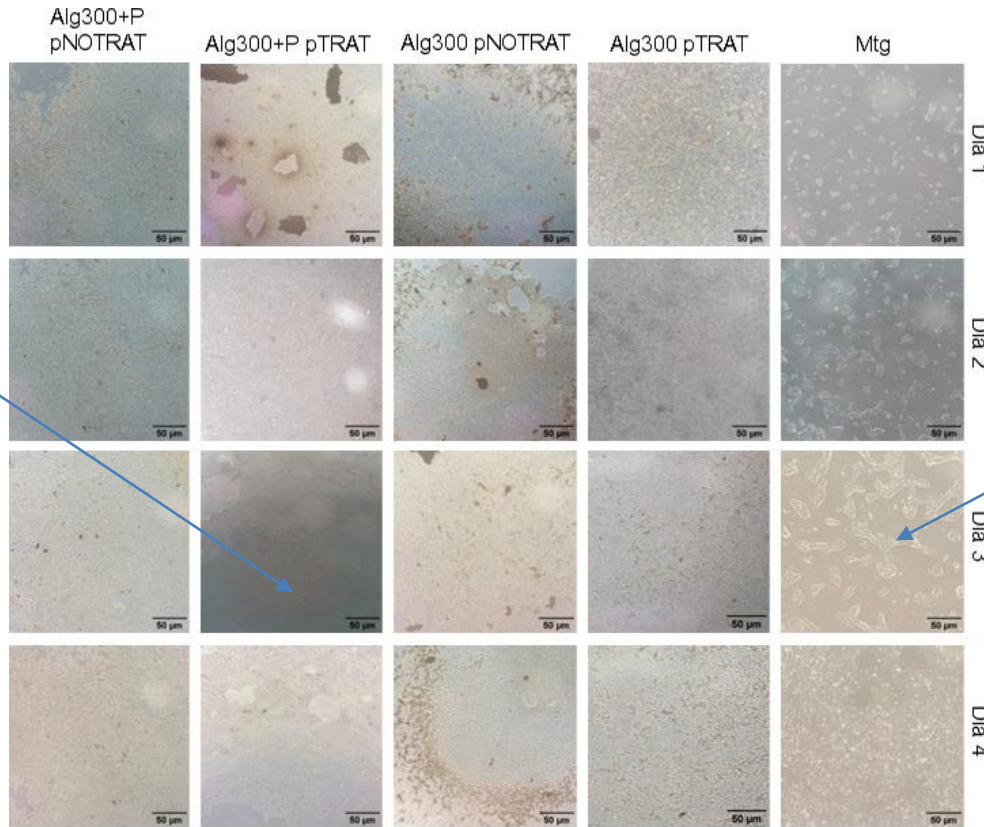
RGD functionalization



hiPSCs + alginate

Example: hiPSC culture trial with alginate 300 rpm - bioreactor

Partners involved  
UCM, i+12



Strange structures on the surface

hiPSCs clumps only in Matrigel

It seems that there are holes in the surface → Ca<sup>2+</sup> ion exchange? → need for a more stable crosslinking or surface activation. **Alginate citotoxicity? Structure degradation? Surface degradation due to culture media?**

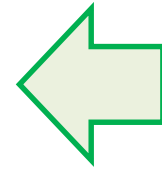
hRPCs+neat pullulan (STMP crosslinked)

Correct adhesion and proliferation

Partners involved  
KUL, i+12

| Sample                           | Day -1 (w/o cells) | Day 1 | Day 4 | Day 7 | Day 10 |
|----------------------------------|--------------------|-------|-------|-------|--------|
| Matrigel control                 |                    |       |       |       |        |
| Hydrogel coating 6 wt% pullulan  |                    |       |       |       |        |
| Hydrogel coating 8 wt% pullulan  |                    |       |       |       |        |
| Hydrogel coating 10 wt% pullulan |                    |       |       |       |        |

Best condition?



# A first joined scientific paper: to *Macromolecular Bioscience*

T1.3

## RPCs + chitosan-RGD

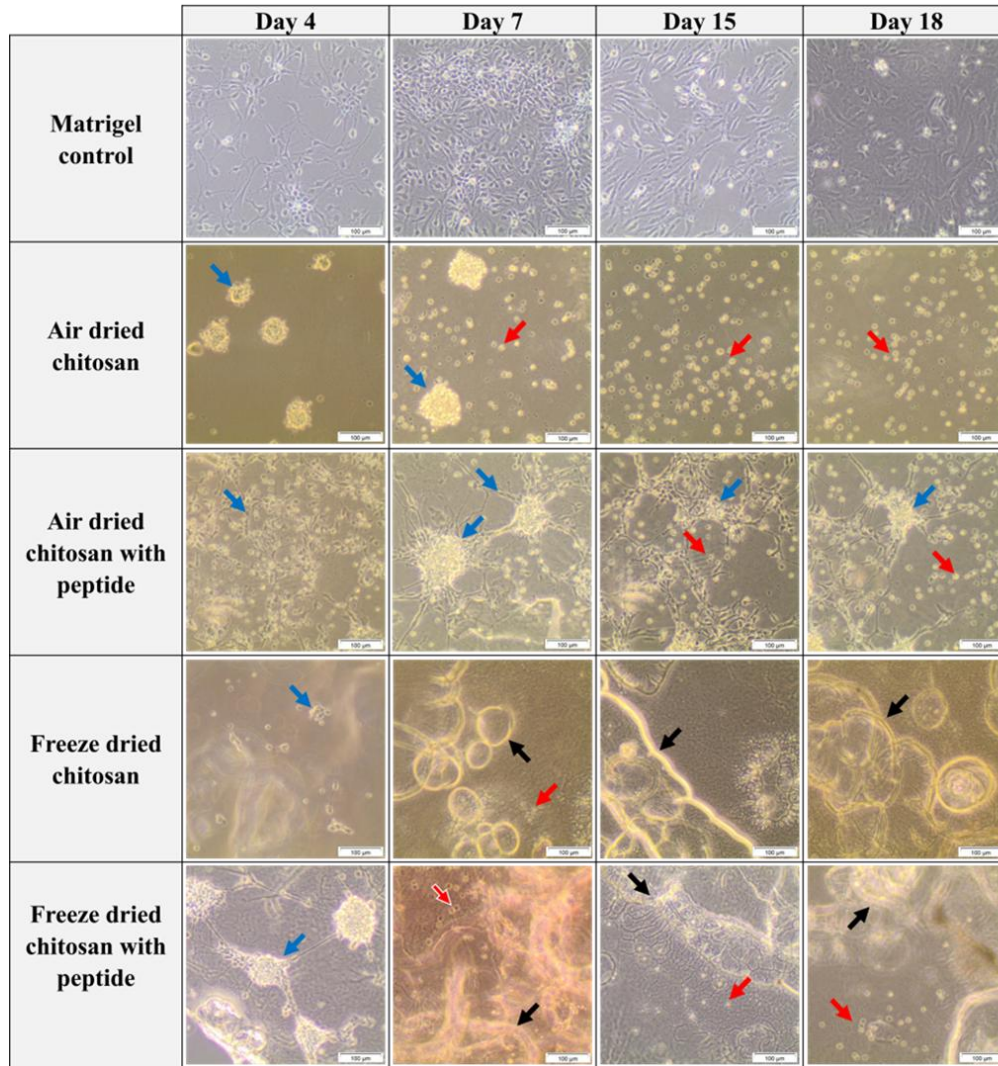
### Correct adhesion and proliferation

**Cell culture results of hRPCs seeded on Matrigel, air- and freeze-dried chitosan films with and without peptide.**

Each experimental condition was tested in three independent replicates. Representative images were obtained with brightfield microscopy (10x objective). In this figure the biomaterial (**black arrows**), live (**blue arrows**) and dead cells (**red arrows**) are shown.

Matrigel sample: hRPCs are visualized as individual cells; Air-dried chitosan film: a few cells with a rounded shape can be seen; Air-dried chitosan with peptide film: the cells have axons and group together forming bigger structures; Freeze-dried chitosan film: a few live cells are deposited on the sample; Freeze-dried chitosan film with peptide: cells group together and progressively detach from the biomaterial. Scale bar: 100  $\mu$ m.

Under review in *Macromolecular Bioscience*  
Partners involved: RISE-PFI, i+12, UCM



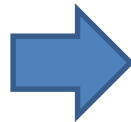
**WP2**  
2D → 2D+ → 3D  
STRUCTURES

RISE PFI, UCM,  
KUL, CHITINOR,  
RGM3D, RISE  
PFI, i+12

- T2.1. Formulations of biomaterials for 3D printing
  - Chitosan and its mixtures with alginate, pullulan...
- T2.2. 2D+ constructs
  - Thermal nanoimprint lithography, **femtosecond laser ablation**...for physical guidance of axons (differentiation).
- T2.3. 3D constructs
  - **RGM 3D printing unit basic & modifications, 3D scaffolds, microbioreactors**...

**T2.1**

Chitinor



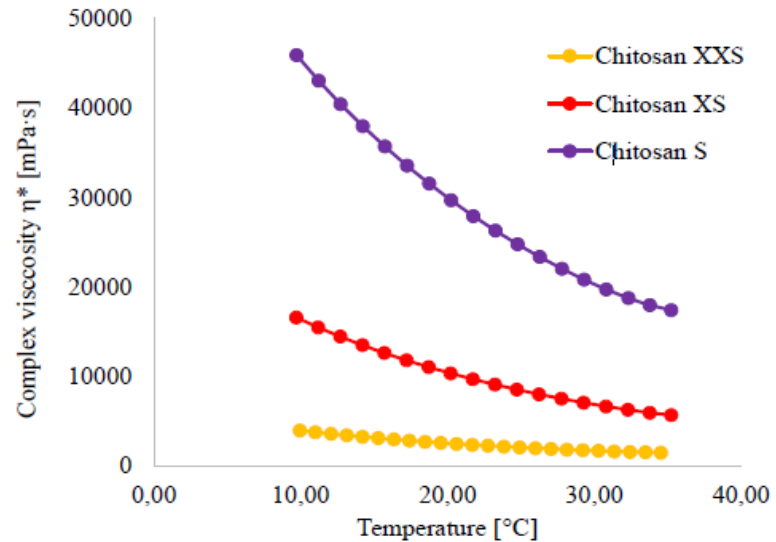
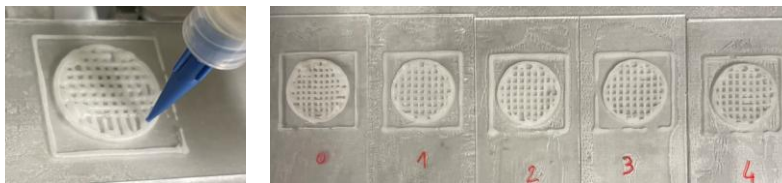
Several chitosan samples



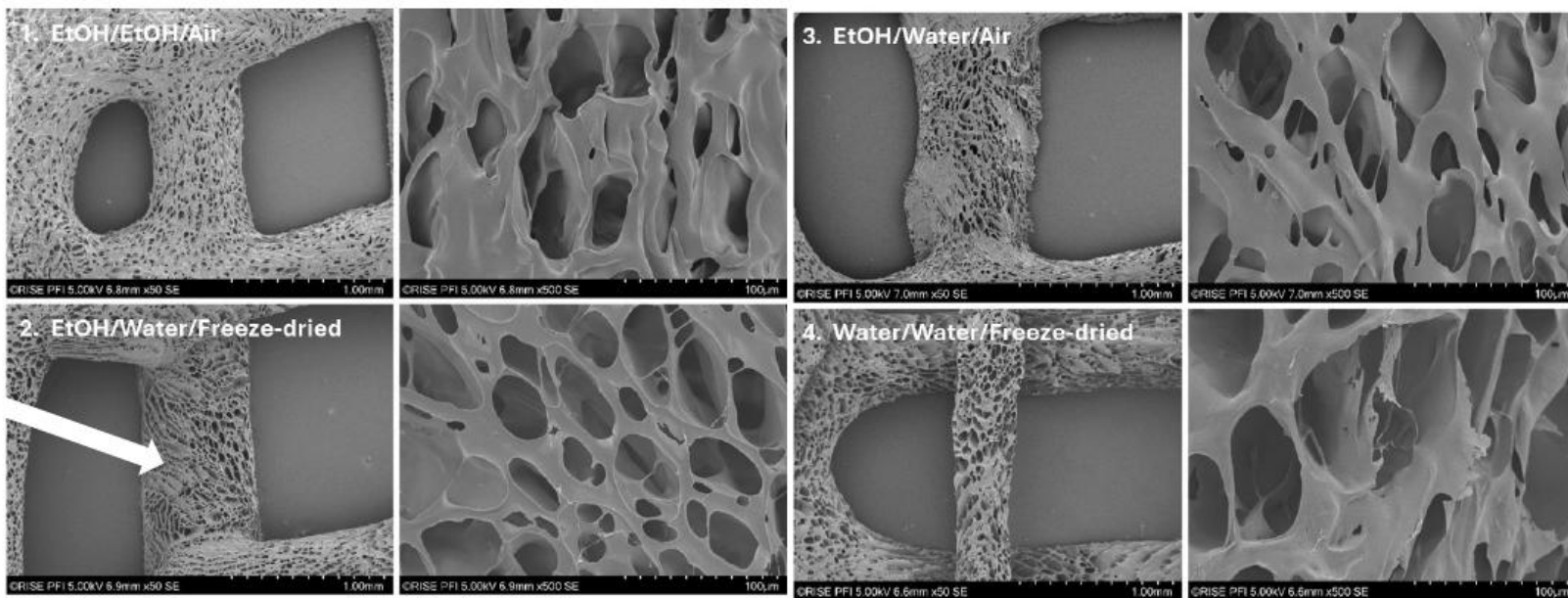
RISE-PFI

Ink formulation & rheology  
Scaffold characterisation

| Ink          | Chitosan Mw |
|--------------|-------------|
| Chitosan S   | 340         |
| Chitosan S   | 340         |
| Chitosan XS  | 201         |
| Chitosan XS  | 201         |
| Chitosan XXS | 99          |
| Chitosan XXS | 99          |



T2.1



T2.2

UCM → femtosecond laser ablation of PS plates

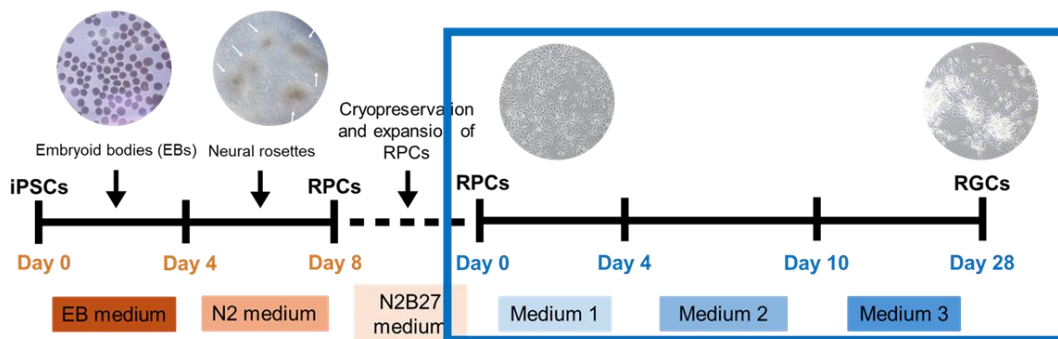
Control runs: Laminin + Poly-D-lysine

i+12 → Morphology of texturized wells

→ RPCs differentiation to RGCs

Differentiation of RPCs to RGCs

p35 with a 1 cm<sup>2</sup> area using a femtosecond laser

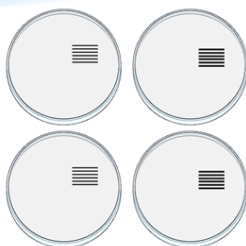
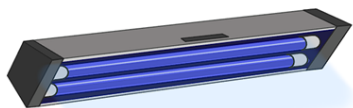


| Plate  | Groove size (μm) | Groove spacing (μm) |
|--------|------------------|---------------------|
| 100 mW | 58±6             | 320±40              |
| 200 mW | 120±10           | 250±20              |
| 30 mW  | 28±4             | 56±3                |
| 50 mW  | 51±3             | 18±5                |

T2.2 i+12 → RPCs differentiation to RGCs

### Plate coating

- Day -2: texturized plates were sterilized using UV light for 30 minutes
- Day -1: 1 mL Poly-D-Lysine O/N RT
- Day 0: 1 mL laminin 2 h 37°C



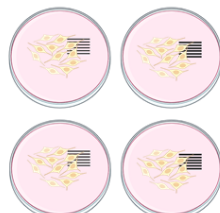
### Cell seeding

- Cell detachment with accutase
- Cell counting (Neubauer's chamber)
- $0,83 \cdot 10^6$  RPCs/p35
- Incubate at 37°C and CO<sub>2</sub> 5%

$0,83 \cdot 10^6$  RPCs



Control  
(Conventional culture)



Texturized plates  
(2D plus culture in 1 cm<sup>2</sup> area)

### Cell culture

- **Medium 1** → Days 0-3: N2B27 + DAPT (4 μM).
- **Medium 2** → Days 4-9: N2B27 + DAPT + BDNF (50 ng/ml).
- **Medium 3** → Days 10-28: N2B27 + BDNF
- Brightfield microscopy to visualize cell culture



RPCs



RGCs

Day 0 Day 4 Day 10 Day 28

Medium 1

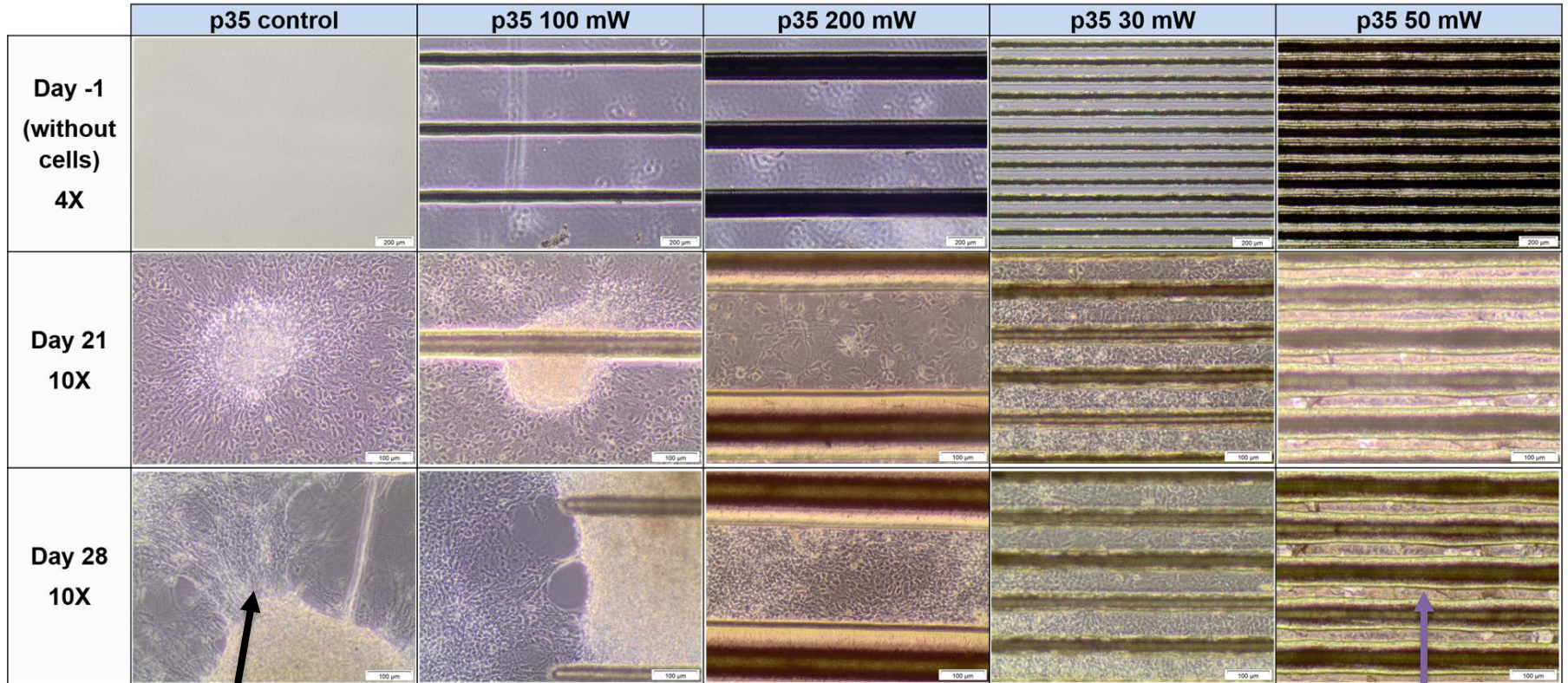
Medium 2

Medium 3



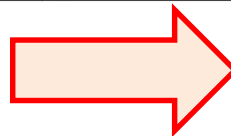
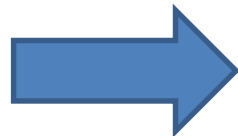
T2.2

i+12 → RPCs differentiation to RGCs



2D flat surfaces  
RGCs clumps & axons

Higher  
texturization



Higher surface  
cytotoxicity ?

2D+ Groove textures surfaces

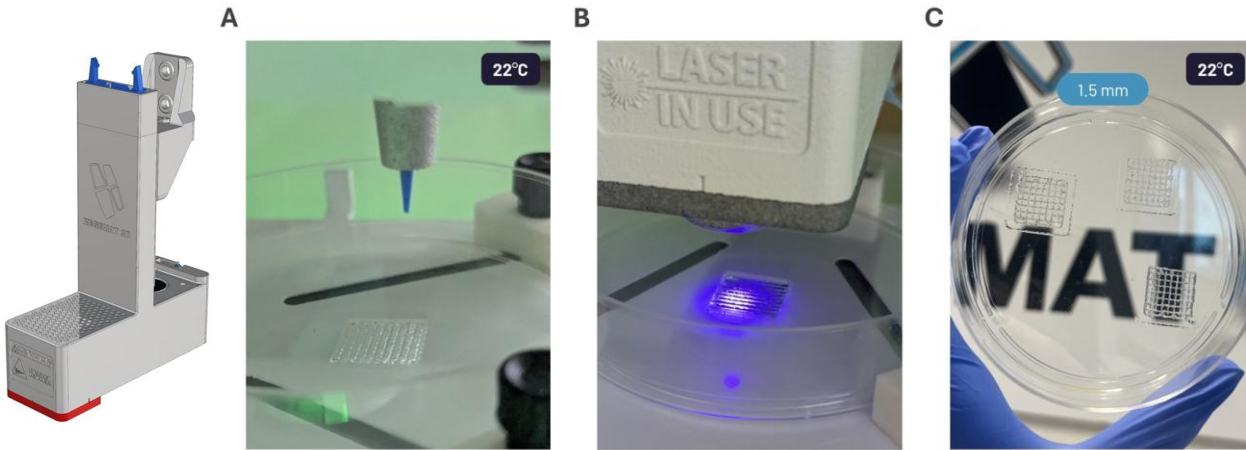
RPC expansion between grooves → axon elongation





**T2.3** REGEMAT 3D printing unit basic & modifications

**RGM3D → 2023: Novel printing head with in-situ photopolymerization**

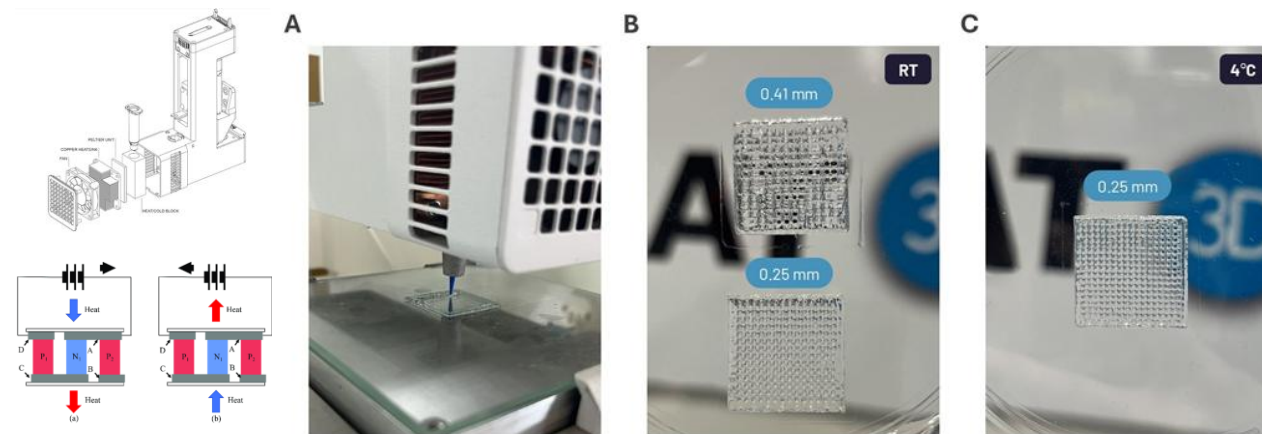


(A) GelMA printing process using a 0.41 mm nozzle on a Petri Dish at 22°C.

(B) Photopolymerization process of the scaffold via irradiation with 405 nm UV light.

(C) GelMA scaffolds with a pore size of 1.5 mm after printing and photocuring.

**RGM3D → 2024: Novel printing head with in-situ Peltier-effect temperature precise control**

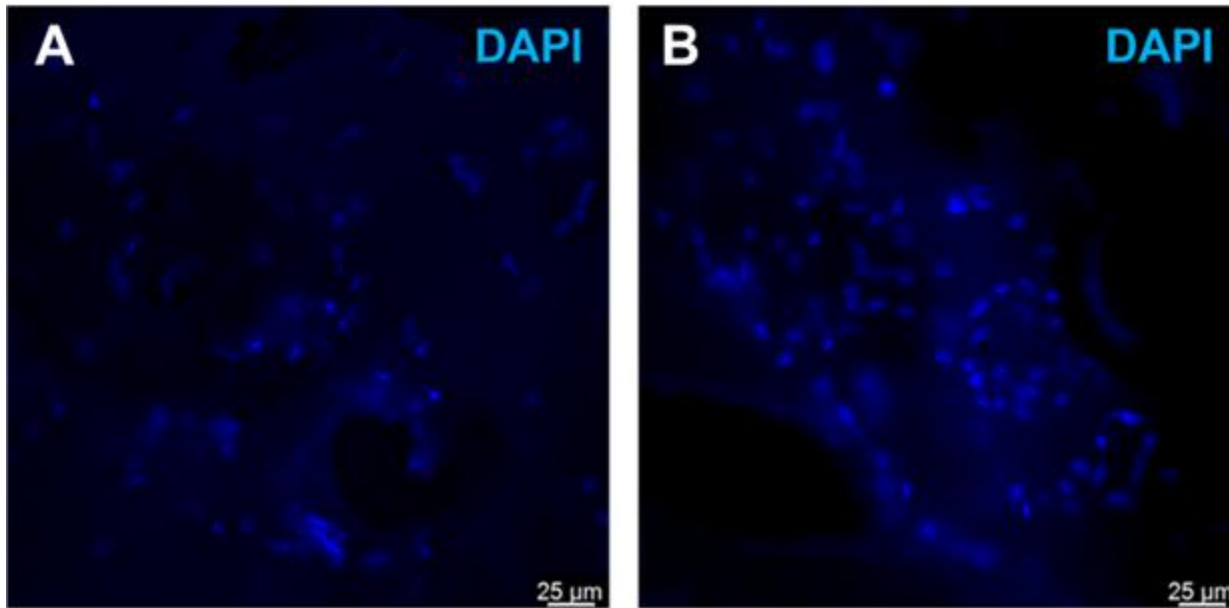


(A) Printing process of the first layer, showcasing the Peltier cooling module and the attached syringe.

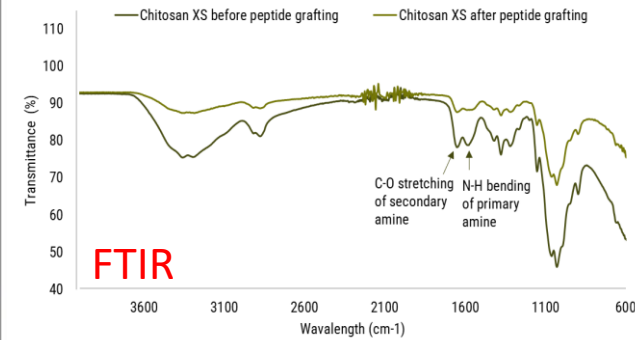
(B) Scaffolds printed at room temperature (RT) with pore sizes of 1 mm and 0.8 mm, using nozzles of 0.41 mm (upper) and 0.25 mm (lower), respectively.

(C) Scaffold with a pore size of 0.8 mm, printed at 4°C using a 0.25 mm nozzle.

A first joined scientific paper: to *Macromolecular Bioscience*

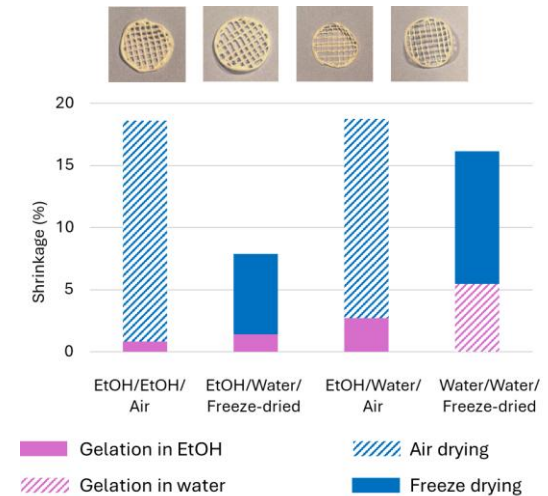


**RPCs + chitosan-RGD**  
**Apparent correct adhesion and proliferation**



**Immunocytochemistry analysis to evaluate the presence of hRPCs on the scaffolds at day 6 of cell culture.** Representative confocal images with 40x objective show the nuclei counterstained with DAPI. (A) Chitosan XS scaffold. (B) Chitosan XS scaffold modified with peptides that contain the RGD sequence. Scale bars: 25 μm.

Under review in *Macromolecular Bioscience*  
 Partners involved: RISE-PFI, i+12, UCM



WP3  
MICROFLUIDIC  
BIOREACTORS

UL, ULEI, UCM,  
RISE PFI, i+12,  
RGM3D

T3.1. Fluid-dynamic analysis in microreactors for cell culture and differentiation

➤ Fluid-dynamic models for flow analysis...microreactor construction & control testing with Matrigel

T3.2. Physical gradient monitoring and control

➤ Sensors, gradients, pH, T, shear stress...

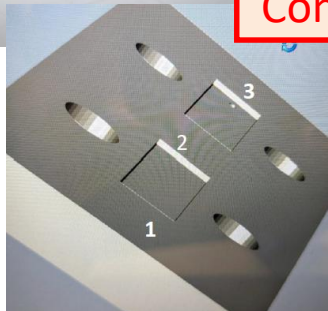
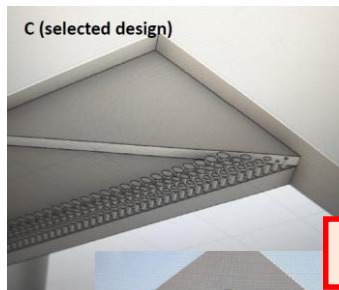
T3.3. Chemical gradient monitoring and control

➤ Chemical sensors for oxygen, CO<sub>2</sub>, validation of models...

T3.1

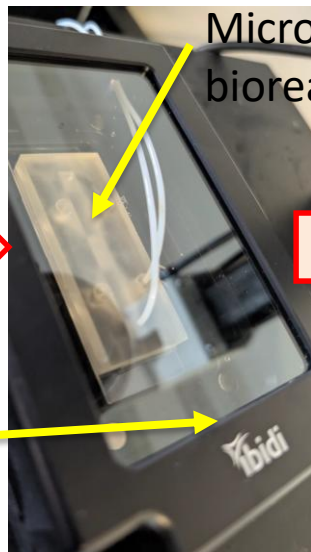
LU → microreactor design & construction  
→ RPC store, thawing & expansion

i+12 → UL team training in Madrid  
→ RPC creation & delivery



Construct

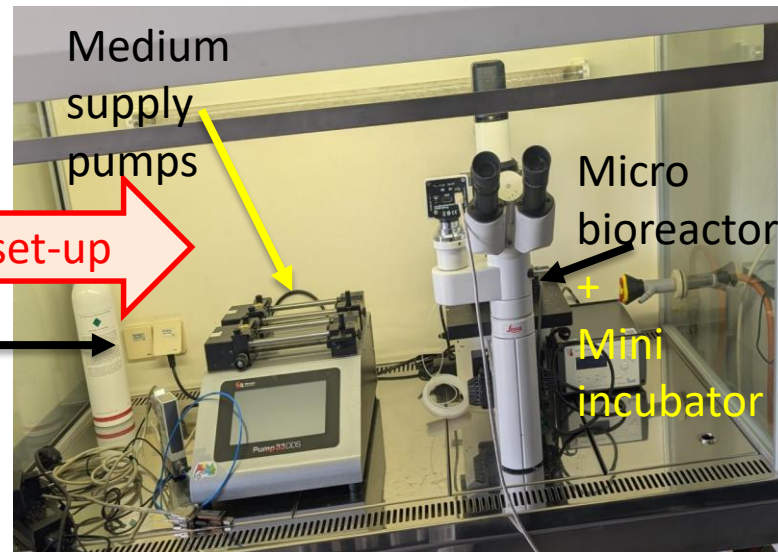
Mini incubator



Micro bioreactor

The set-up

Gas mixture



Medium supply pumps

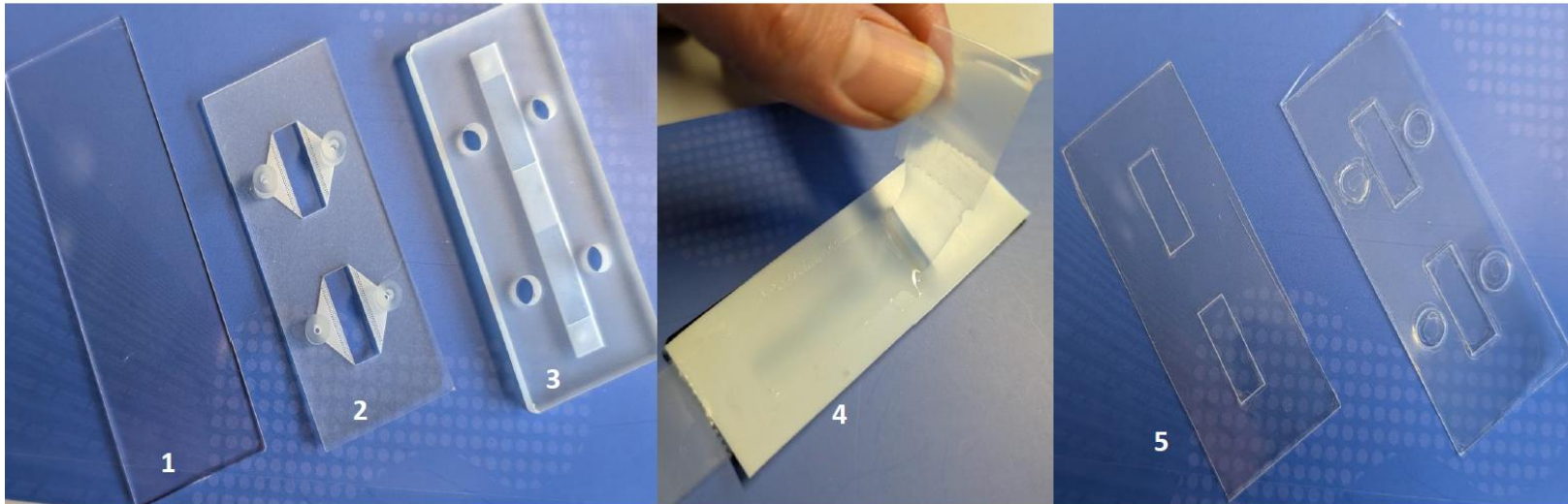
Micro bioreactor

Mini incubator

T3.1

LU → microreactor design & construction  
→ RPC store, thawing & expansion

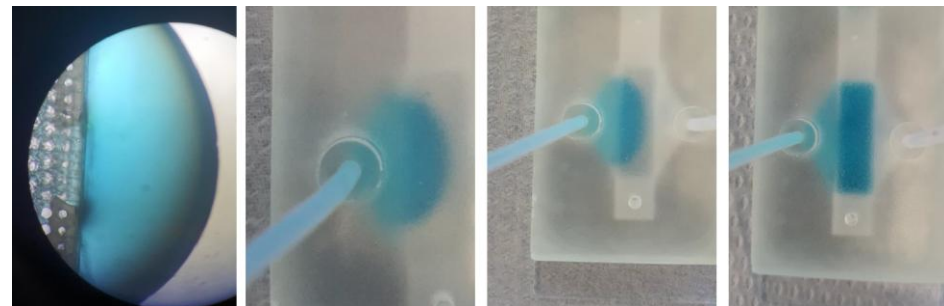
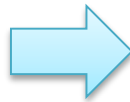
i+12 → UL team training in Madrid  
→ RPC creation & delivery



**Layer Structure (Bottom to Top):**

1. PMMA Base
2. 3D-Printed Cell Culture Chambers & Media Channels
3. 3D-Printed Gas Channel Layer
4. PDMS (Gas-Permeable Layer between Layers 2 & 3)
5. Double-Adhesive Printed Films for Layer Attachment

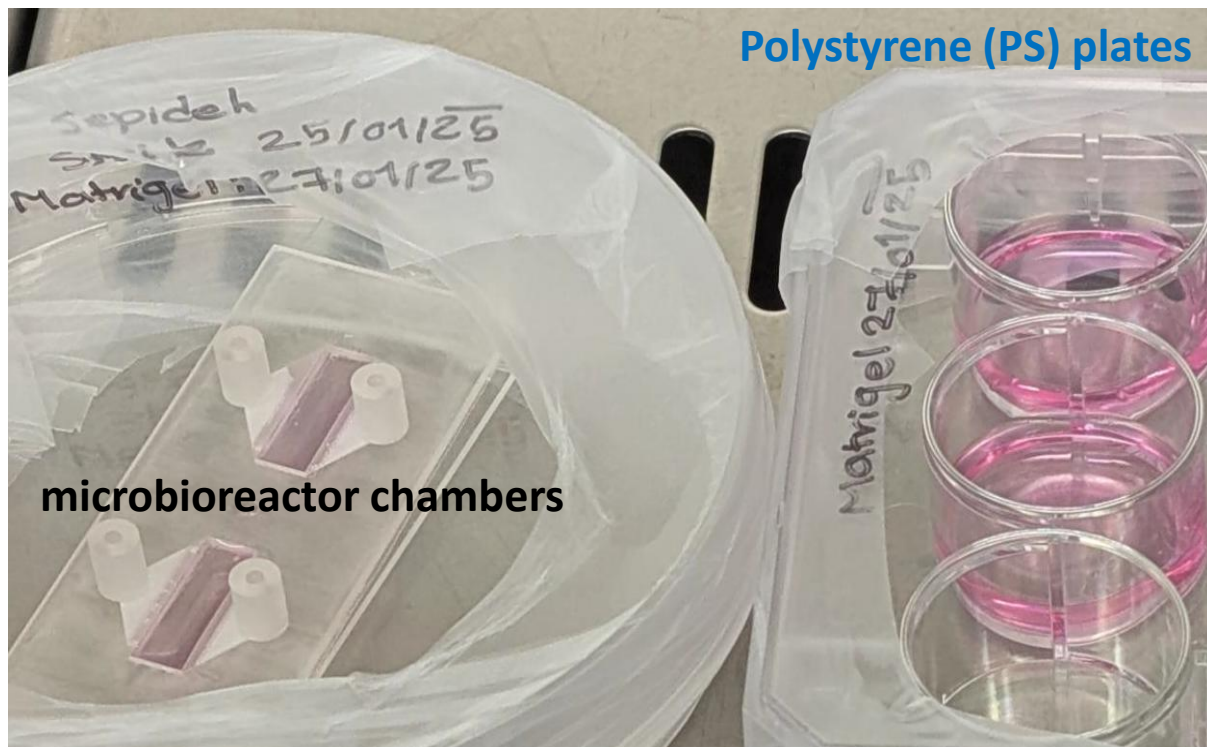
Coloured water flow  
Low Reynolds number (Re)



T3.1

LU → microreactor design & construction  
→ RPC store, thawing & expansion

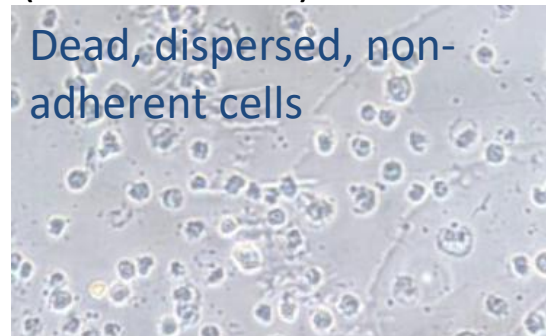
i+12 → UL team training in Madrid  
→ RPC creation & delivery



Polystyrene (PS) plates

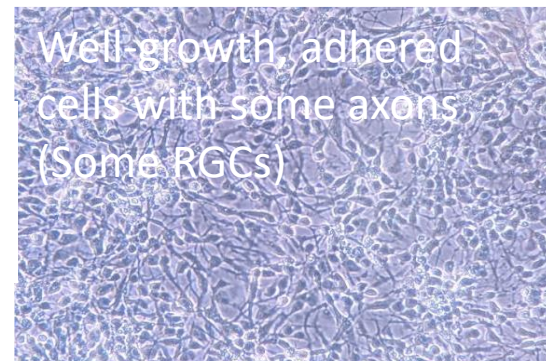
microreactor chambers

On microreactor chamber /Matrigel/ Day 10 (N2B27+BDNF)



Dead, dispersed, non-adherent cells

On PS-plate/Matrigel Day 10 (N2B27+BDNF)



Well-growth, adhered cells with some axons (Some RGCs)

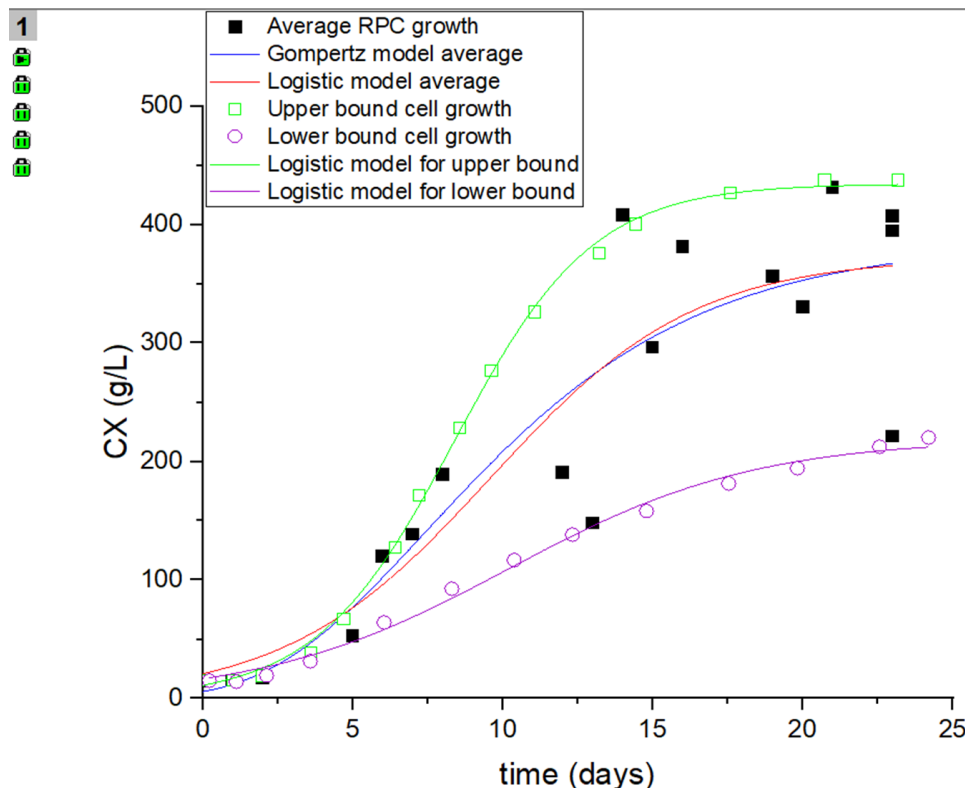
- Results suggest PMMA is not suitable for cell culture, growth, or differentiation.
- It is likely that, the hydrophobicity of PMMA, even when coated with Matrigel or Poly-D-Lysine (chemical treatment), likely interferes with effective cell attachment and growth.

Adhesive? Supporting PMMA? → PS or glass substrates

T3.3

i+12 → RPC expansion (control & hydrogels)  
 → RPC differentiation (control & hydrogels)

UCM → liquid composition analysis  
 → kinetic modelling → LU



|                 |                                      |
|-----------------|--------------------------------------|
| Model           | SGompertz                            |
| Equation        | $y = a * \exp(-\exp(-k * (x - xc)))$ |
| Plot            | CX                                   |
| a               | 386.38294 ± 54.46817                 |
| xc              | 7.48369 ± 1.42739                    |
| k               | 0.19197 ± 0.08324                    |
| Reduced Chi-Sqr | 4620.56057                           |
| R-Square (COD)  | 0.81927                              |
| Adj. R-Square   | 0.79518                              |

|                 |                                  |
|-----------------|----------------------------------|
| Model           | Slogistic3                       |
| Equation        | $y = a / (1 + b * \exp(-k * x))$ |
| Plot            | CX                               |
| a               | 372.36074 ± 38.47102             |
| b               | 16.78296 ± 14.59769              |
| k               | 0.29406 ± 0.10196                |
| Reduced Chi-Sqr | 4608.11068                       |
| R-Square (COD)  | 0.81976                          |
| Adj. R-Square   | 0.79573                          |

|                 |                                  |
|-----------------|----------------------------------|
| Model           | Slogistic3                       |
| Equation        | $y = a / (1 + b * \exp(-k * x))$ |
| Plot            | B                                |
| a               | 434.29331 ± 3.56886              |
| b               | 37.74713 ± 3.71584               |
| k               | 0.43301 ± 0.01277                |
| Reduced Chi-Sqr | 38.26524                         |
| R-Square (COD)  | 0.99885                          |
| Adj. R-Square   | 0.99866                          |

|                 |                                  |
|-----------------|----------------------------------|
| Model           | Slogistic3                       |
| Equation        | $y = a / (1 + b * \exp(-k * x))$ |
| Plot            | B                                |
| a               | 219.14122 ± 6.05468              |
| b               | 12.12153 ± 1.70207               |
| k               | 0.24449 ± 0.01748                |
| Reduced Chi-Sqr | 45.9577                          |
| R-Square (COD)  | 0.9937                           |
| Adj. R-Square   | 0.99244                          |

Optical microscopy (Neubauer chamber) → RPC counting during expansion

Glucose → ion exclusion HPLC / glucose enzyme kit

Proteins → Bradford test



Non-structured model for liquid  
 Non/Segregated model for cells

WP4  
hiPSCs → RGCs  
DIFFERENTIATION

i+12, ULEI,  
RGM3D, UL,  
RISE PFI, UCM,  
KUL

T4.1. Differentiation of hiPSCs to RGCs in 2D, 2D+ and 3D systems

➤ **Lee-based protocol, immunofluorescence (off-line) ...**

T4.2. Development of opto-bioelectronic-based monitoring for RGC differentiation and maturation

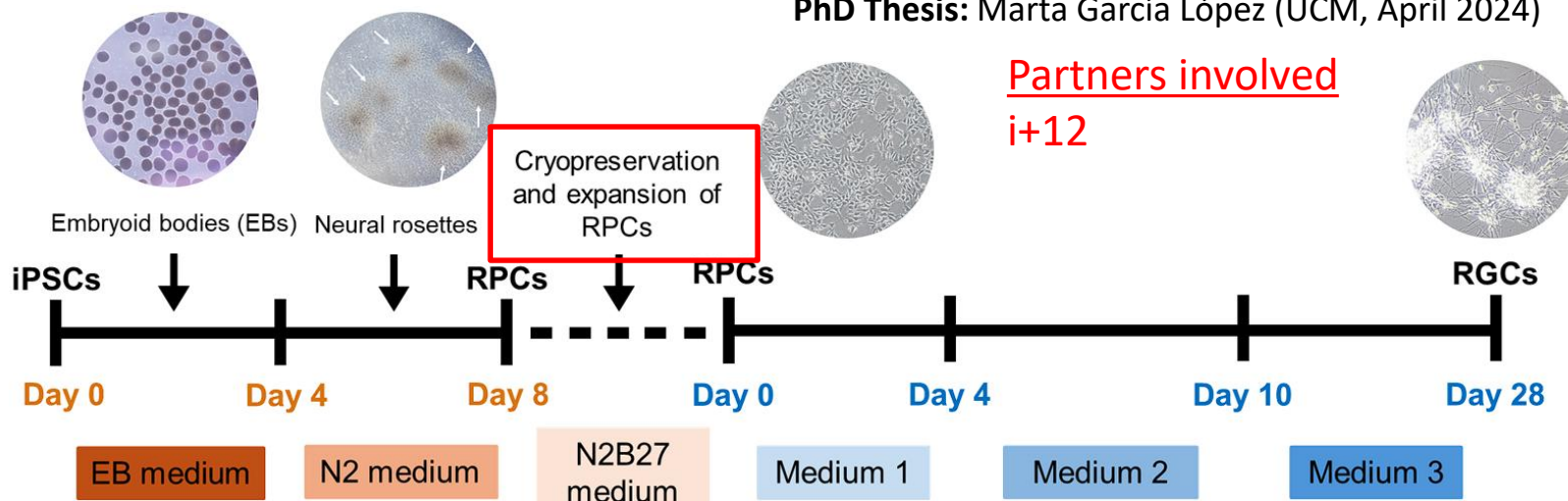
➤ **Microelectrode arrays, FEM, High Content Screening + photonic 7 optic monitoring, integration of sensors in 3D devices...**

T4.3. Differentiation of iPSCs to RGCs in successful sensor-equipped 2D/3D and microfluidic devices

➤ **Patient, OPA1 gene mutation, CRISPR/Cas9 correction...RGCs function monitoring (control in Matrigel).**

T4.1 The Protocol: from hiPSCs to RGCs

Scientific paper: PMID: 39000346. *García-López et al. 2024*  
PhD Thesis: Marta García López (UCM, April 2024)

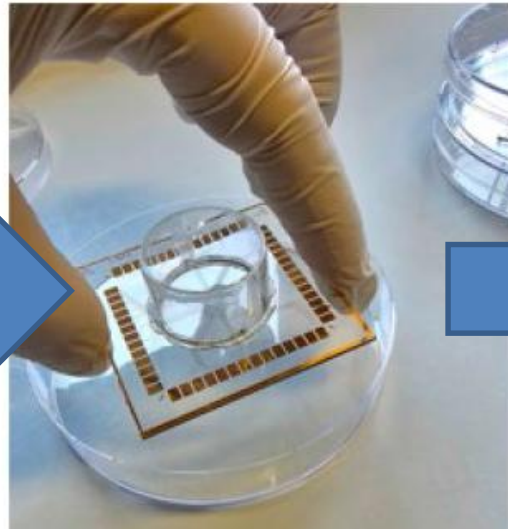


T4.2

→ First multimodal **opto-bioelectronic monitoring system with novel ultrathin HighDense-MEAs** allows highly spatial resolved non-invasive, real-time monitoring of cellular processes and electrophysiological activity

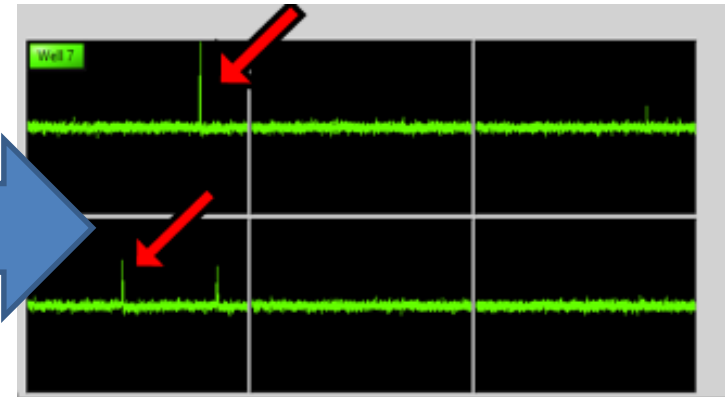


Clean Room



MEAs

Partners involved  
i+12, ULEI

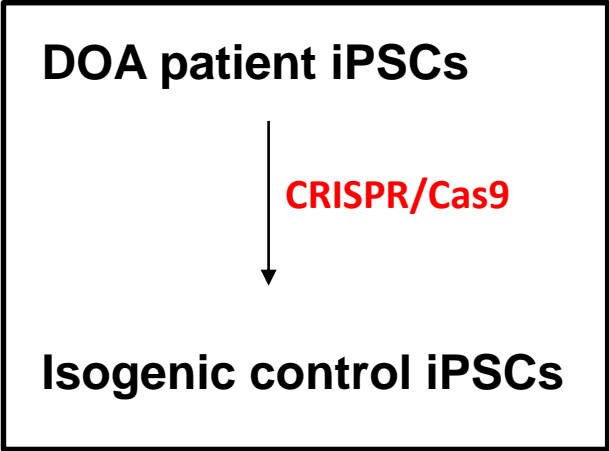


Action potentials detected in RGCs

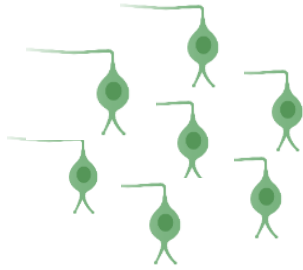
- first proof of electrophysiological activity of hiPSCs-derived RGCs
- it is now possible to quantitative monitor differentiation and functionality of RGCs
- developed Matrigel alternatives can now be tested in RGC differentiation experiments



T4.3 Differentiation of iPSCs to RGCs in sensor-equipped 2D/3D and microfluidic devices

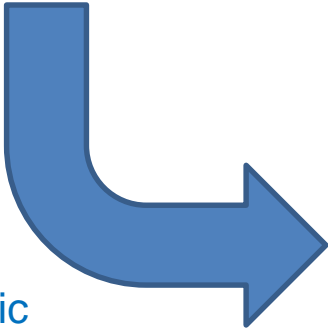


Partners involved  
i+12, ULEI

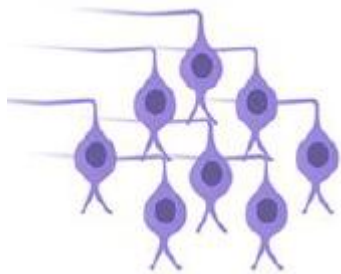


Patient RGCs

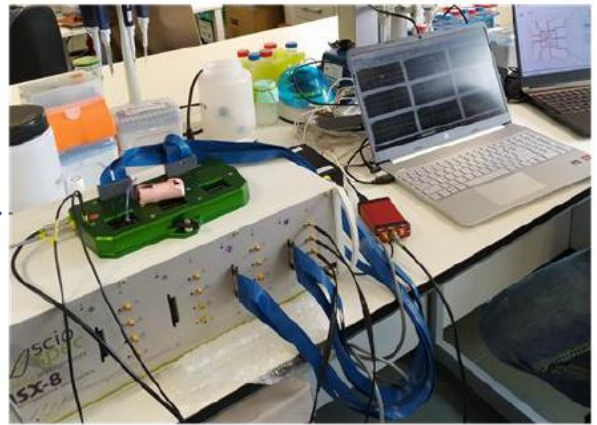
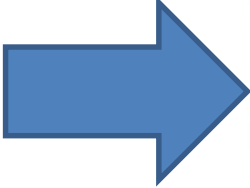
?



Bioelectronic  
differentiation  
monitoring and  
functional analysis



Isogenic control RGCs



T4.3

Creation of an isogenic control hiPSC line (done & published)

Partners involved  
i+12

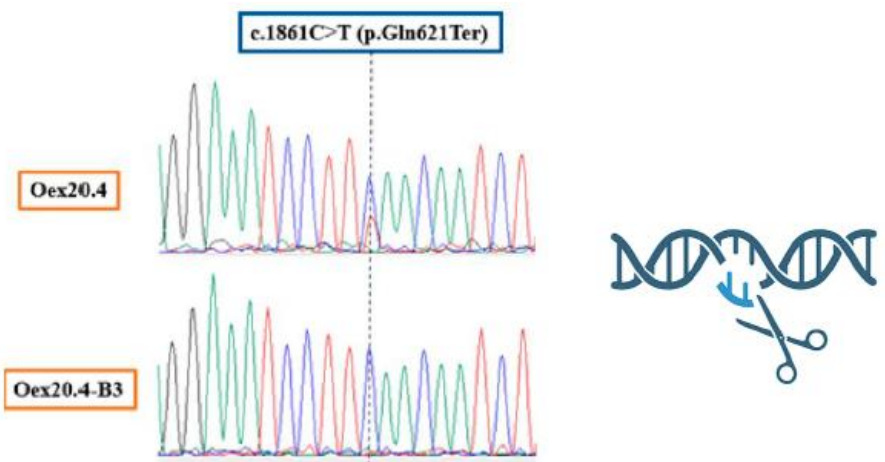
International Journal of Molecular Sciences MDPI

Article  
**Creation of an Isogenic Human iPSC-Based RGC Model of Dominant Optic Atrophy Harboring the Pathogenic Variant c.1861C>T (p.Gln621Ter) in the OPA1 Gene**  
 Marta García-López <sup>1</sup>, Lydia Jiménez-Vicente <sup>1</sup>, Raquel González-Jabardo <sup>1</sup>, Helena Dorado <sup>1</sup>, Irene Gómez-Manjón <sup>2</sup>, Miguel Ángel Martín <sup>2,3,4</sup>, Carmen Ayuso <sup>4,5</sup>, Joaquín Arenas <sup>3,4</sup> and María Esther Gallardo <sup>1,\*</sup>

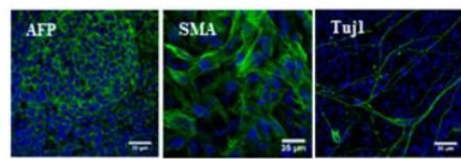
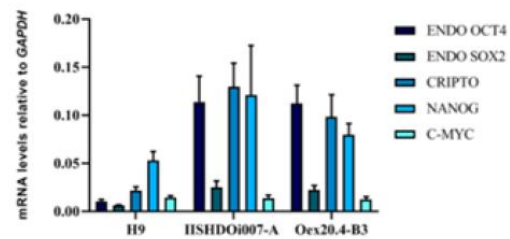
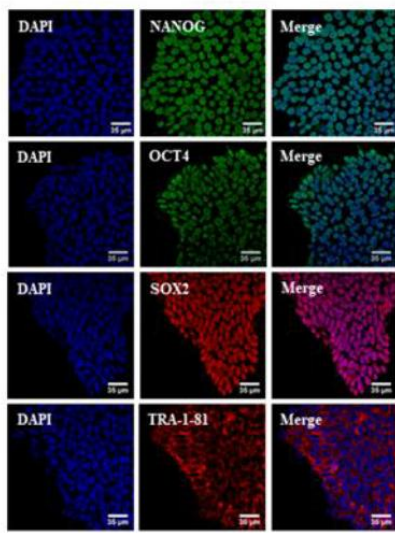
Line created by CRISPR/Cas9  
 Bona-fide hiPSC line  
 (fully pluripotent – high quality line)  
 First i+12 scientific paper (published)  
 partially supported by bioMAT4EYE



Creation of an isogenic healthy iPSC line with CRISPR/Cas9



| Marker     | Oex20.4 |     | Oex20.4-B3 |     |
|------------|---------|-----|------------|-----|
| D2S1338    | 168     | 169 | 168        | 169 |
| D7S820     | 209     | 213 | 209        | 213 |
| D13S317    | 182     | 187 | 182        | 187 |
| D19S433    | 202     | 206 | 202        | 206 |
| D21S11     | 218     | 222 | 218        | 222 |
| VWA        | 141     | 159 | 141        | 159 |
| Amelogenin | X       | Y   | X          | Y   |



## Publications

1. García-López, M., Jiménez-Vicente, L., González-Jabardo, R., Dorado, H., Gómez-Manjón, I., Martín, M. Á., Gallardo, M. E. (2024). Creation of an Isogenic Human iPSC-Based RGC Model of Dominant Optic Atrophy Harboring the Pathogenic Variant c. 1861C> T (p. Gln621Ter) in the OPA1 Gene. *International Journal of Molecular Sciences*, 25(13), 7240.
2. **Ladero, M., Reche-Sainz, J. A., Gallardo, M. E.** (2024). Hereditary Optic Neuropathies: A Systematic Review on the Interplay between Biomaterials and Induced Pluripotent Stem Cells. *Bioengineering*, 11(1), 52.

## Meetings

&...>2 more from KUL (EPNOE conference Graz 2023...)

1. Raquel González-Jabardo, Marta García-López, Natalia Robles-Anda, Lydia Jiménez-Vicente, Helena Dorado, Pablo Rueda de Arriba, M. Esther Gallardo. Generation of an isogenic control from an induced pluripotent stem cell line of a patient with dominant optic atrophy harbouring the genetic variant c.1024 A>G (p.K342E) in the OPA1 gene. 27th European Association for Vision and Eye Research (EVER) Congress. (November 2024). Type of communication: oral poster.
2. Raquel González-Jabardo, Marta García-López, Natalia Robles-Anda, Lydia Jiménez-Vicente, Helena Dorado, Pablo Rueda de Arriba, M. Esther Gallardo. Generación de un control isogénico a partir de una línea de iPSCs de un paciente con atrofia óptica dominante portador de la variante genética en el gen OPA1, c.1024 A>G (p.K342E). II Jornada Investigadores Junior i+12. (September 2024). Spain. Type of participation: Scientific and organising committee and poster.
3. **Natalia Robles-Anda, Amalie Solberg, Helena Dorado, Eva Pasquier, Raquel González-Jabardo, Lydia Jiménez-Vicente, Pablo Rueda de Arriba, Miguel Ladero, Gary Chinga Carrasco, M. Esther Gallardo.** Empleo de estructuras 3D de biomateriales para el cultivo de células progenitoras de la retina. II Jornada Investigadores Junior i+12. (September 2024). Spain. Type of participation: Scientific and organising committee and poster.
4. **Amalie Solberg, Eva Pasquier, Miguel Ladero, M. Esther Gallardo, Gary Chinga Carrasco.** Chitosan-based inks for 3D-printed scaffolds. 17th Scandinavian Society for Biomaterials Meeting. (2024). Helsingør (Denmark). Type of communication: oral.
5. M. Esther Gallardo. Jornada Investigando Juntos: Jornada de Accesibilidad ONCE-H120. (2023). Madrid, Spain. Type of communication: Invited conference.
6. Ainhoa Porroche; Belén Ponce; **Miguel Ladero; M. Esther Gallardo.** Novel alginate-derived matrices for cultivation and differentiation of iPSCs. BIOTEC 2023. XVIII Congreso de la Sociedad Española de Biotecnología. (2023). Madrid, Spain. Type of communication: oral poster.
7. M. Rosa Zabaleta; Ainhoa Porroche; **M. Esther Gallardo; Miguel Ladero.** Optimization of alginate production by *A. vinelandii* at flask scale. BIOTEC (2023). XVIII Congreso de la Sociedad Española de Biotecnología. (2023). Spain. Type of communication: poster.
8. Marta García-López; Lydia Jiménez; Raquel González-Jabardo; M. Esther Gallardo. Generación de RGCs derivadas de iPSCs para modelizar la atrofia óptica dominante. I Jornada Investigadores Junior i+12. (2023). Spain. Type of participation: Scientific and organising committee and oral communication.
9. Ainhoa Porroche; Belén Ponce; **Miguel Ladero; M. Esther Gallardo.** Nuevas matrices derivadas de alginato para el cultivo y diferenciación de iPSCs. I Jornadas Investigadores Junior i+12. i+12. (2023). Spain. Type of participation: Scientific and organising committee and oral communication.
10. Marta García-López; M. Esther Gallardo. Generation of iPSC-Derived RGCs for Modeling Dominant Optic Atrophy. 2nd International Electronic Conference on Biomedicines. (2023). *Med. Sci. Forum* (2023), 21, 3. DOI: 10.3390/ECB2023-14087. Type of participation: oral communication.

**PhD thesis**  **i+12** 1 more in progress (Ms. Natalia Robles-Anda i+12+UCM)

**PhD thesis**  **i+12** 1 more in progress (Ms. Femke de Ceulaer KULeuven+UCM)

1. Title: Generación de un modelo humano de RGCs para el estudio y aproximación a terapia de la atrofia óptica dominante. Marta García López. (Defence date: April 2024). Universidad Complutense de Madrid. Qualification: Outstanding cum laude unanimously.

**MSc thesis**  **i+12** i+12/UCM: 3 more / Professional training internships: i+12/UCM=8...)

1. Title: Caracterización de alginato algal y quitosano para la creación de hidrogeles con aplicaciones biomédicas. Mounat El Jarmouni. Master in Biomaterials. (2024). Universidad Complutense de Madrid (UCM).
2. Title: Atrofia óptica dominante: iPSCs como modelo de enfermedad, mejora en el diagnóstico y aproximación a terapia. Pablo Rueda de Arriba. Master's Degree in Biochemistry, Molecular Biology and Biomedicine. (2024). Universidad Complutense de Madrid (UCM).

## Dissemination to society

1. Radio: Programmes: 'Más de uno' (Onda Cero), 'Estamos como queremos' (RNE). 'Tarde o temprano' (Radio Canarias), "Buenos Días" (Onda Madrid), Cope, etc.
2. Television: Soy de Madrid (Televisión Digital de Madrid). Telediario de la 1, la 2 and Antena 3.
3. National press: Diario Médico, El Mundo, La Vanguardia, La Razón, 20 Minutos, NIUS, IM ópticas, El gacetín de Madrid, Europa Press. Consalud, Servimedia, among many others.
4. Regional press: diario de Mallorca, el periódico mediterráneo, el faro de Vigo, la opinión de Zamora, la opinión Coruna, Levante, diario de Ibiza.
5. Local press: actualidad21.net, el noticiero de Madrid.
6. Social networks (e.g. Twitter) and the institutional website.
7. Training programmes (Programa 4º ESO + Empresa, semana de la Ciencia e Innovación y el Día internacional de la mujer y la niña en la Ciencia).

# Acknowledgements to...

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**M-ERA.NET**

**el** AGENCIA ESTATAL DE INVESTIGACIÓN

**CDTI**

**fwo** Research Foundation Flanders  
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**The Research Council of Norway**

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MINISTRY OF EDUCATION,  
SCIENCE AND SPORT**

**STAATSMINISTERIUM  
FÜR WISSENSCHAFT  
UND KUNST** | **Freistaat  
SACHSEN**



*It hasn't been all work and no play*

*...and our teams, institutions, people...supporting us along the way*

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# THANK YOU FOR YOUR ATTENTION

