

# Chitosan grafted with fibronectin as a building unit for bioactive thermosensitive hydrogels

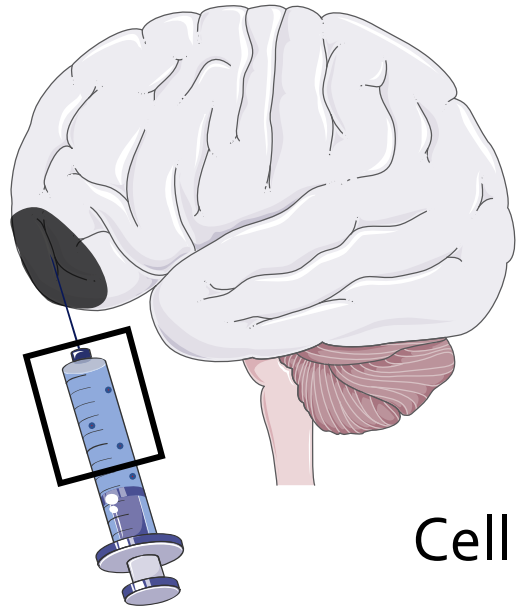
Pierre MARQUAILLE<sup>1,2</sup>, Emmanuel PAUTHE<sup>2</sup>, Laurent CORTE<sup>1,3</sup> and Sophie NORVEZ<sup>1</sup>

<sup>1</sup>C<sub>3</sub>M Laboratory, ESPCI Paris-PSL

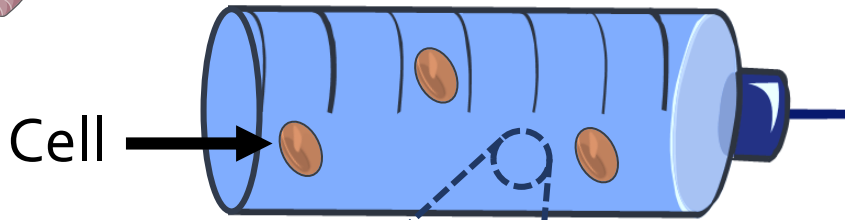
<sup>2</sup>ERRMECe Laboratory, CY Cergy Paris University

<sup>3</sup>Centre des Matériaux, MINES Paris-PSL

# Injectable hydrogels for cell encapsulation and delivery



Cell suspension  
in a polymer solution



Cell



Polymer in good solvent

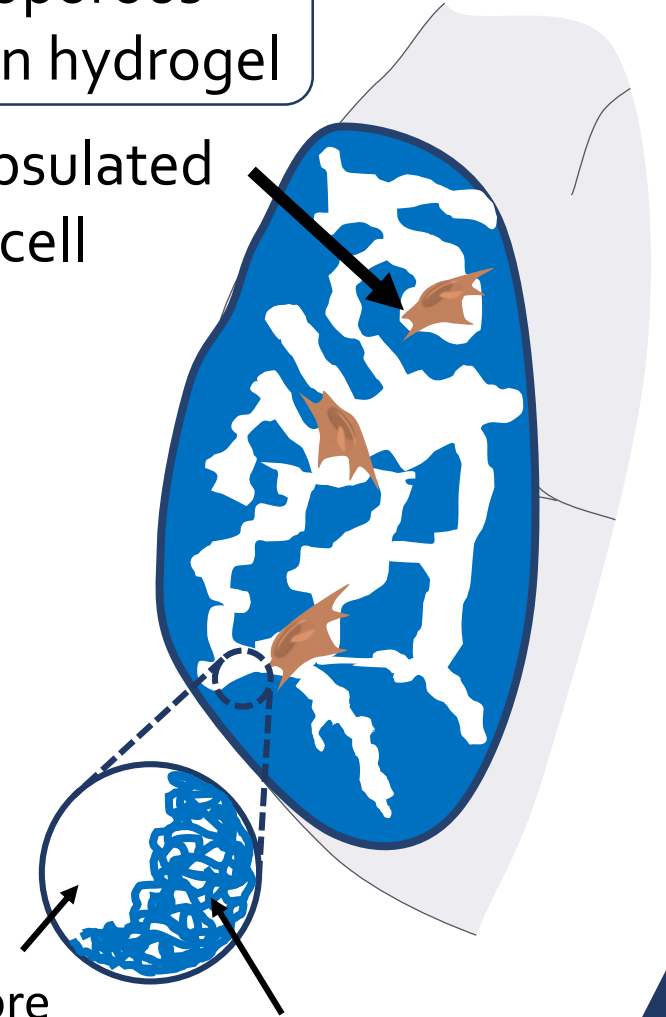
*Heating at 37°C*

Polymer phase  
separation



Macroporous  
cell-laden hydrogel

Encapsulated  
cell

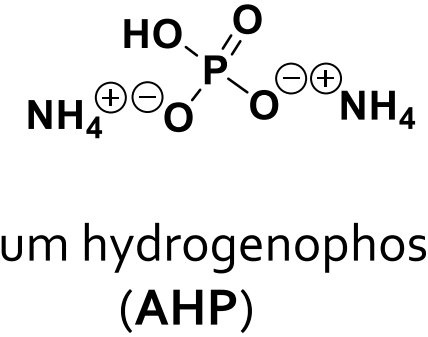
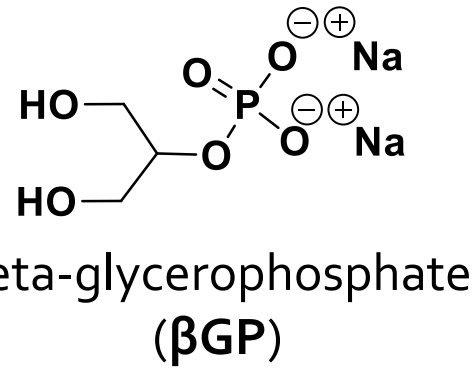
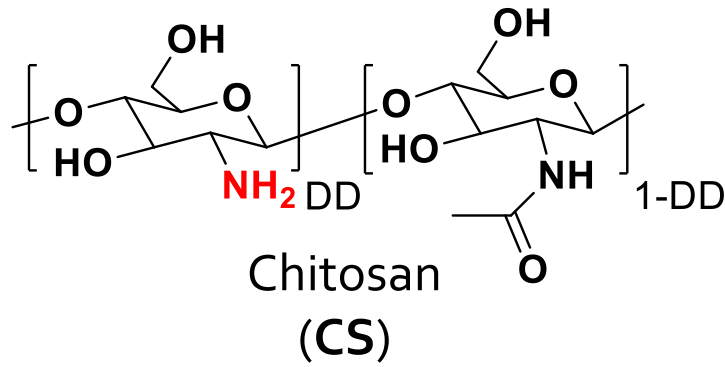


Water-rich macropore

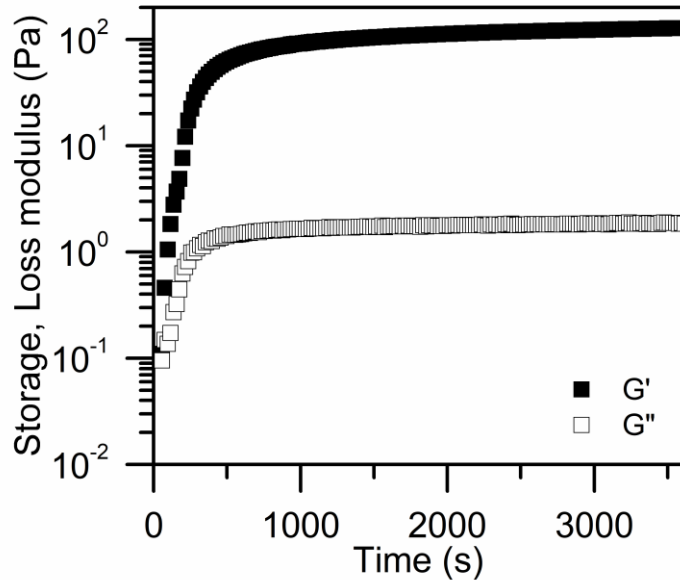
Polymer-rich scaffold

Physiological environment  
Macroporous structure  
Fast gelation properties

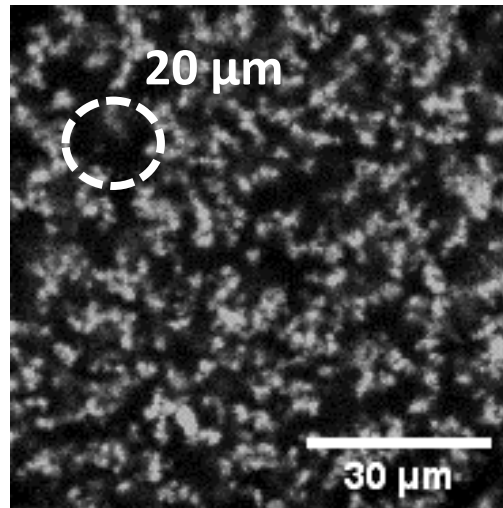
# Chitosan/Phosphate salts thermosensitive solutions



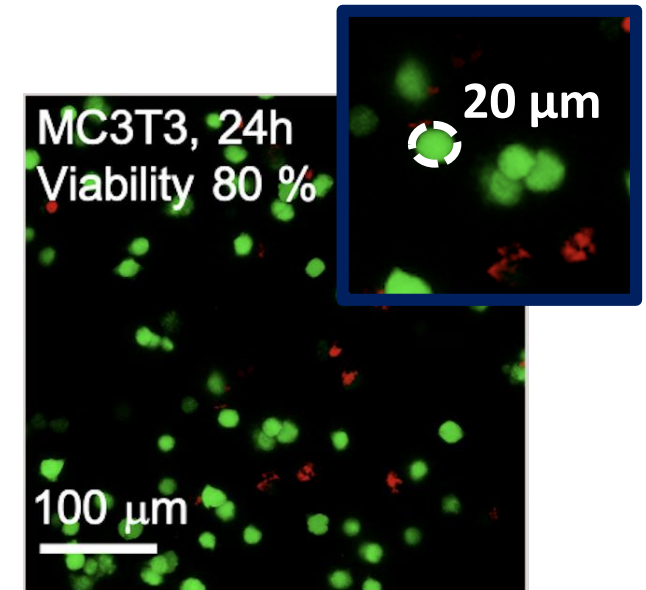
pH = 7.0-7.4 ; 300 mOsm.L<sup>-1</sup>



Quick gelation

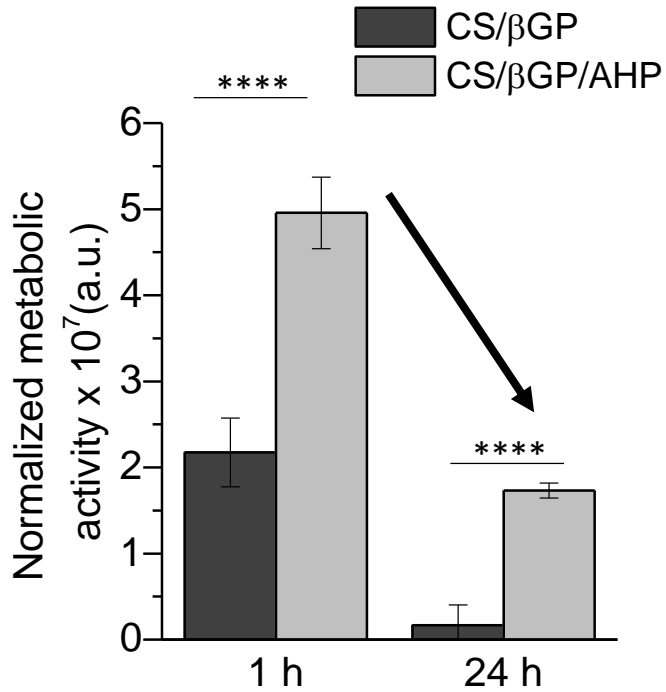


Macroporosity



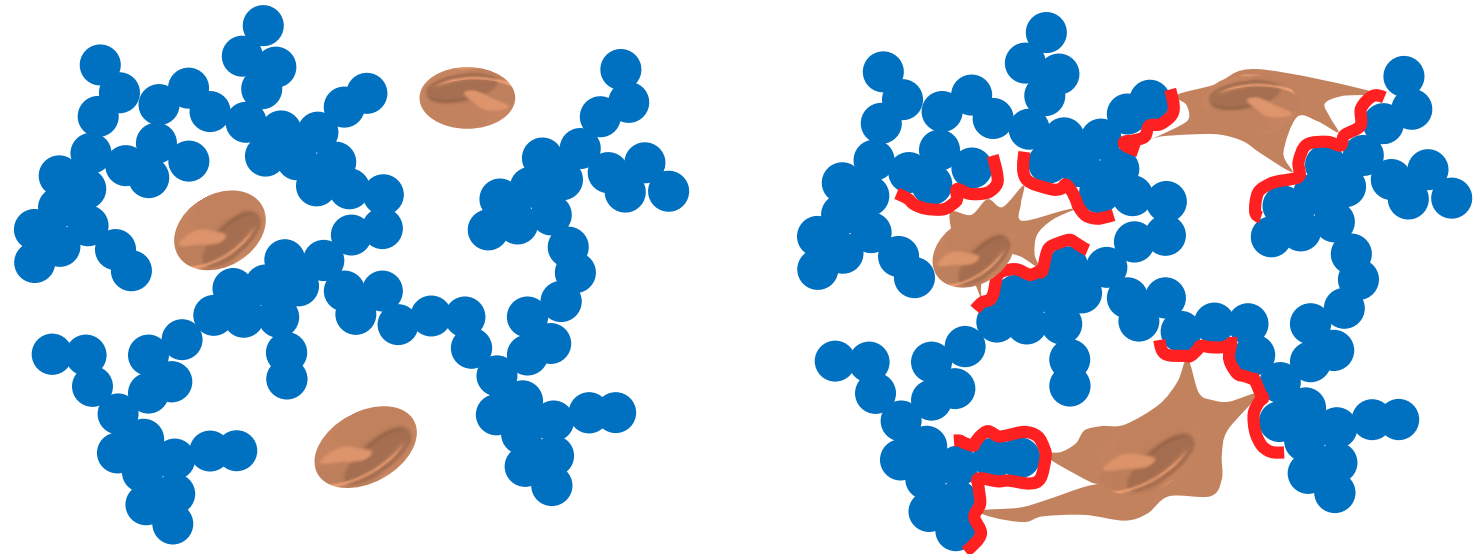
Cytocompatibility

# Improving bioactivity in our chitosan/phosphate salts hydrogel



**Poor bioactivity**  
**Weak cell adhesion**

## Hydrogel network

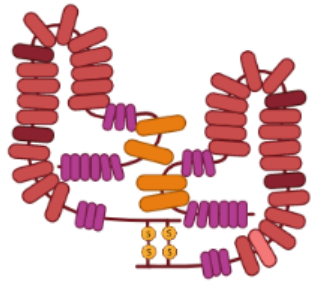


No adhesion

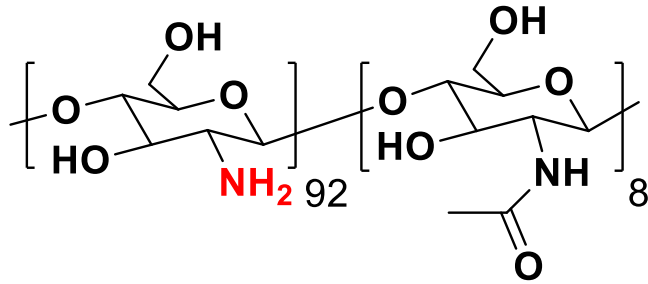
Adhesion

Biofunctionalization of pore surfaces to promote adhesion and confer bioactivity

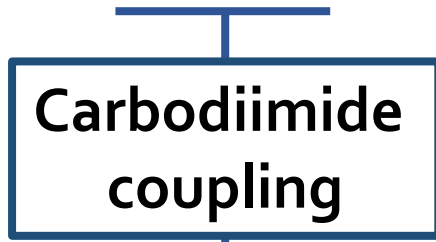
# Incorporation of an adhesion protein: the fibronectin



Fibronectin (Fn)  
550kDa

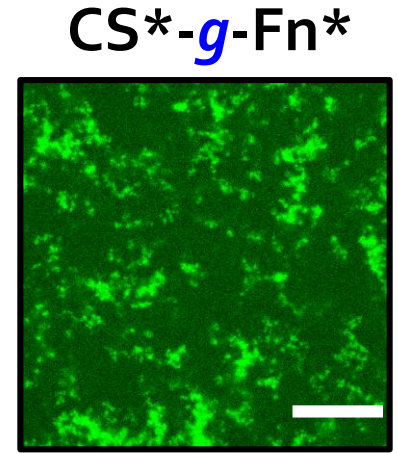
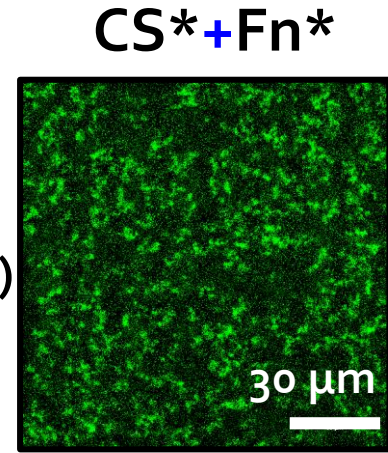


Chitosan (CS)  
250kDa

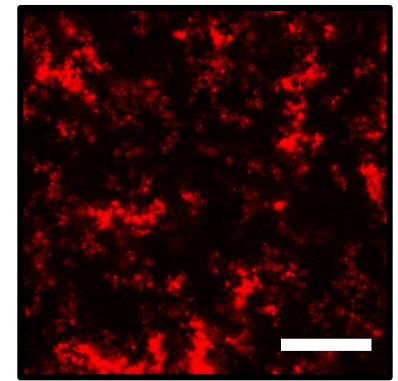
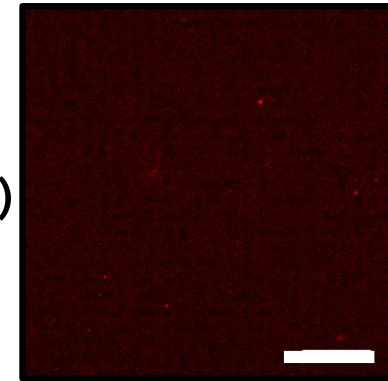


Chitosan-grafted-Fibronectin  
CS-*g*-Fn

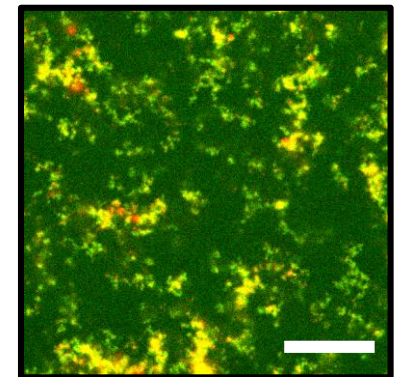
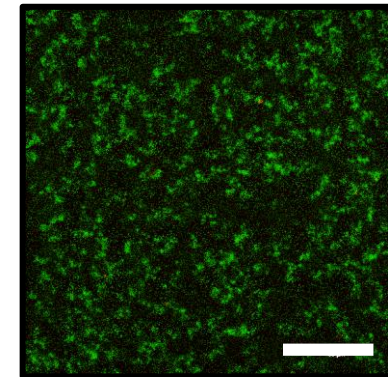
CS\* channel  
(AlexaFluor488)



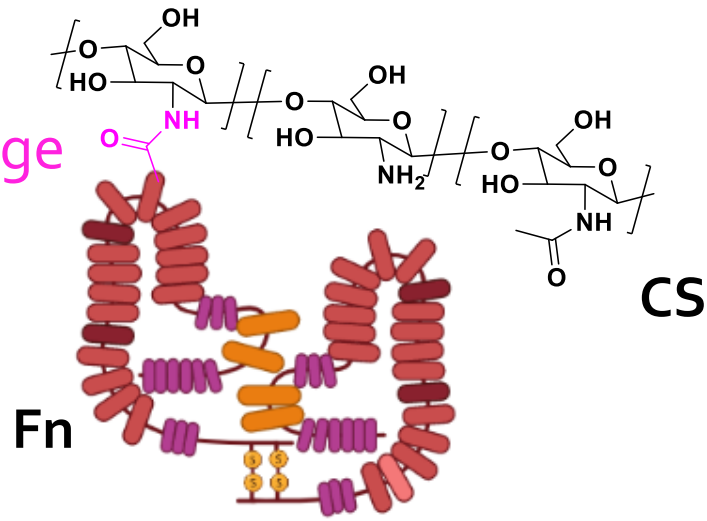
Fn\* channel  
(AlexaFluor568)



Merged  
channels



Amide linkage



Significant difference in  $M_w$

Effect of fibronectin on ...

- Macroporosity?
- Gelation properties?
- Cell metabolism?



Come to poster n°43 !

## Chitosan grafted with fibronectin as a building unit for bioactive thermosensitive hydrogels

Pierre Marquaille<sup>1,2</sup>, Carla Palomino Durand<sup>2</sup>, Emmanuel Pauthe<sup>2</sup>, Laurent Corté<sup>1,3</sup>, and Sophie Norvez<sup>2</sup>

<sup>1</sup> Molecular, Macromolecular Chemistry and Materials, C3M, ESPCI Paris PSL, CNRS, PSL University, Paris, France

<sup>2</sup> Équipe de Recherche sur les Relations Matrice Extracellulaire-Cellule, ERRMECe, CY Cergy-Paris University, Neuville-sur-Oise, France

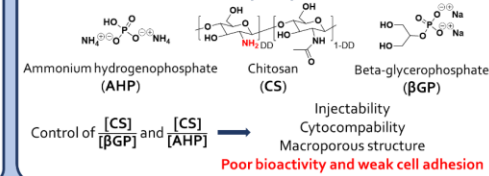
<sup>3</sup> Centre des Matériaux, MINES Paris-PSL, CNRS, PSL University, Evry, France

### Context

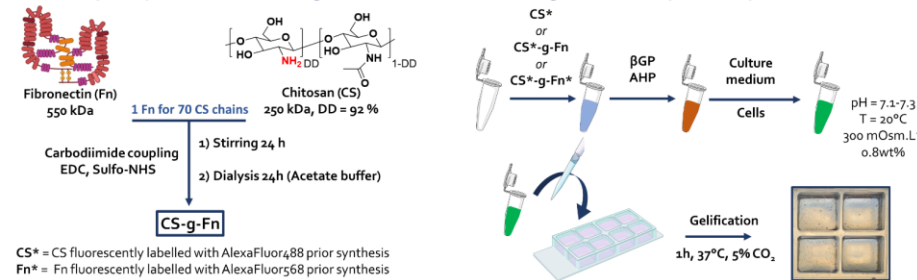
Thermosensitive hydrogels with a sol/gel transition at 37°C are promising tools for cell encapsulation. Their ability to remain liquid at room temperature allows to mix cells homogeneously in the solution. Once heated at 37°C, cells are trapped in the polymer gel, and can survive and proliferate within the structure if the following criteria are respected:

- pH and osmolarity close to physiological values
- Controlled gelation properties matching with cell encapsulation
- Microstructure compatible with nutrients diffusion and cell migration
- Cell-polymer matrix interactions for cell adhesion<sup>1</sup>

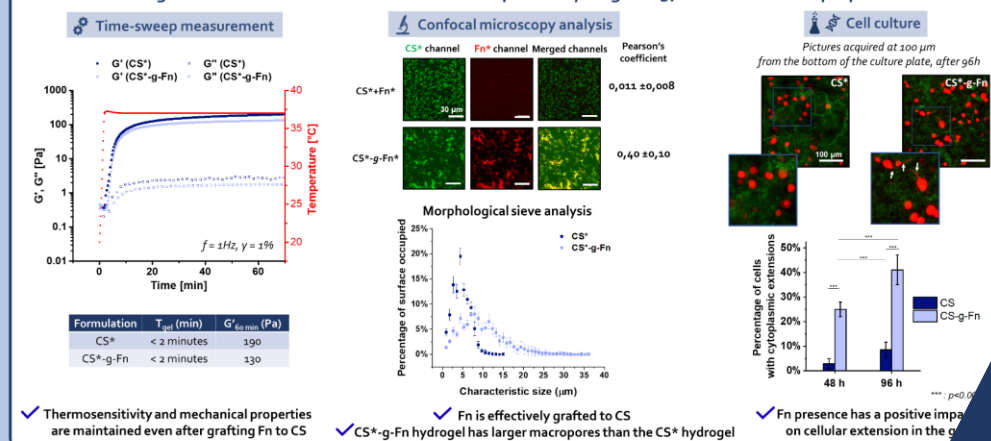
### Chitosan with phosphate salts<sup>2,3</sup>



### "One-pot" synthesis of Chitosan grafted with Fibronectin (CS-g-Fn) and encapsulation procedure



### CS\*-g-Fn is thermosensitive and forms a macroporous hydrogel at 37°C with bioactive properties



### Conclusions

- Grafting of Fn to CS using EDC/NHS chemistry is demonstrated
- Thermogelation and mechanical properties are preserved in CS-g-Fn hydrogels
- Macroporosity is increased, while maintaining good mechanical properties
- Cells sense Fn presence in the hydrogel, and extend their cytoplasm overtime

### Perspectives

- Quantify the amount of grafted protein with A4F technique
- Investigate Fn conformation at the surface of the pores
- Evaluation of cell survival and metabolism over long time culture
- Adapt the grafting protocol to other type of adhesion proteins (

References <sup>1</sup>Palomino Durand C. et al., Appl. Sci. 11, 2021. <sup>2</sup>Chenite A. et al., Biomaterials 21, 2000. <sup>3</sup>Dang P.A. et al. Carbohydr. Polym.