



HAL
open science

Encapsulation process of muscle stem cells in alginate matrices to enhance their in vivo post-transplant properties

Mélanie Marquis, Agata Zykwinska, Camille Le Guen, Isabelle Leroux, Cindy Schleder, Stéphane Cuenot, Karl Rouger

► To cite this version:

Mélanie Marquis, Agata Zykwinska, Camille Le Guen, Isabelle Leroux, Cindy Schleder, et al.. Encapsulation process of muscle stem cells in alginate matrices to enhance their in vivo post-transplant properties. journée scientifique de l'Ecole Doctorale Biologie Santé Nantes, Apr 2024, Nantes (France), France. hal-04540739

HAL Id: hal-04540739

<https://hal.science/hal-04540739>

Submitted on 10 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Mélanie Marquis^a, Agata Zykawska^b, Camille Le Guen^a, Isabelle Leroux^a, Cindy Schleder^a, Stéphane Cuenot^c and Karl Rouger^a

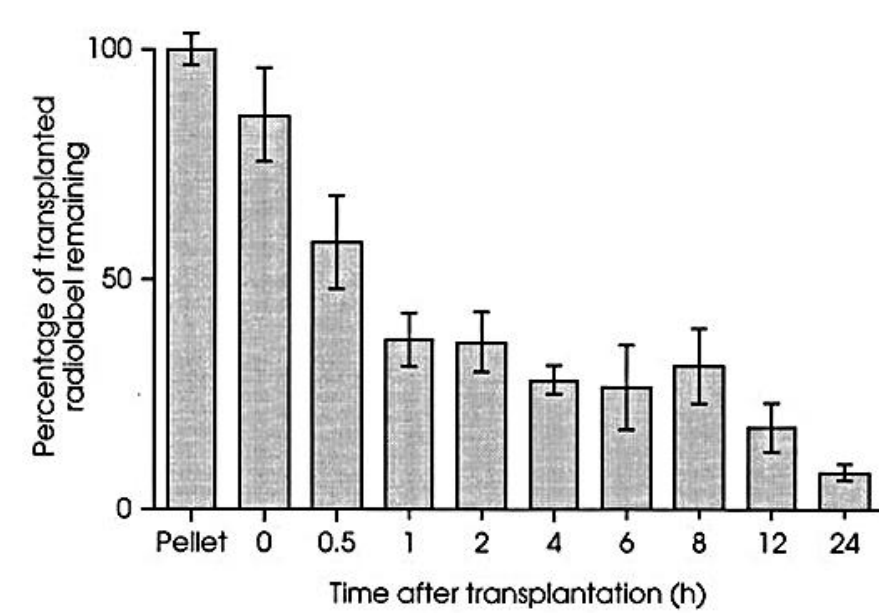
^a Oniris, INRAE, PAnTher, UMR 0703, 44307 Nantes, France

^b IFREMER, MASAE, 44311 Nantes, France

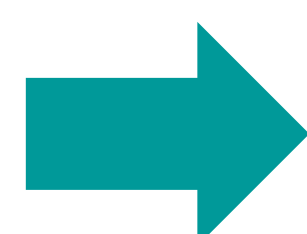
^c Institut des Matériaux de Nantes Jean Rouxel (IMN), Université de Nantes-CNRS, 44322 Nantes, France

Cell therapy and muscle diseases

- Transplantation of myoblasts on dystrophic patients
- **Poor efficiency: low survival and low integration in the host tissue**



Identification of Adult Stem Cells (ASCs)



Context

Human MuStem cells [1-2]

- Skeletal muscle-resident cells isolated based on delayed adhesion properties
- Favorable settings for transplantation: high clonogenicity, strong proliferation capacity
- Contribution to muscle regeneration with formation of new muscle fibers and myogenic progenitors
- Persisting clinical and tissue benefits
- **Good candidate for tissue regeneration in muscle diseases**

but

Clinical potential restricted by moderate ability to survive and engraft after transplantation

Objectives

1-Applied : How to increase the effectiveness of hMuStem cell transplantation?

- Cell engineering to improve *in vitro* conditioning
- Encapsulation inside 3D network to improve cell viability [3]
- **Enhancement of myogenic properties and secretory capacities**
- **Protection against mechanical stress and the immune system**

2-Fundamental : How to better understand the biology of hMuStem cells using 3D systems analogous to targeted tissues?

- Synthesis of artificial biomimetic matrix to model skeletal muscles [4]
- **Impact of the extracellular matrix (ECM) on myogenic activities of hMuStem cells**

Elaboration of a 3D network with tissue-like mechanical properties

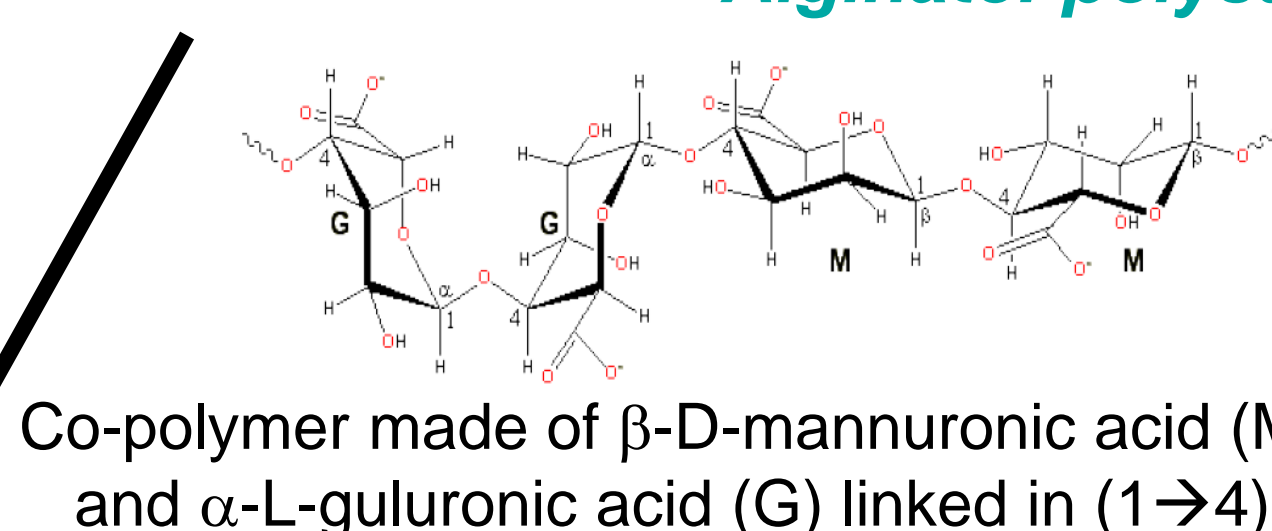
Hydrogels

- **Networks of hydrophilic polymer chains**
- **Strong affinity with water**
- **Matrix with mechanical properties similar to natural tissues**

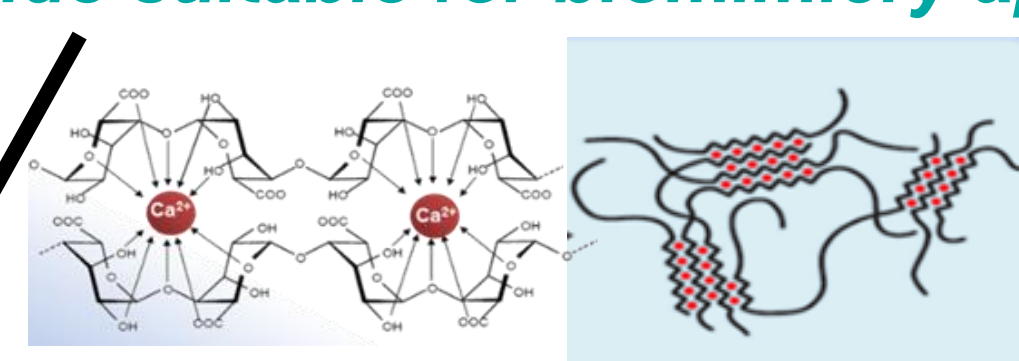
Selection of a biocompatible model polymer



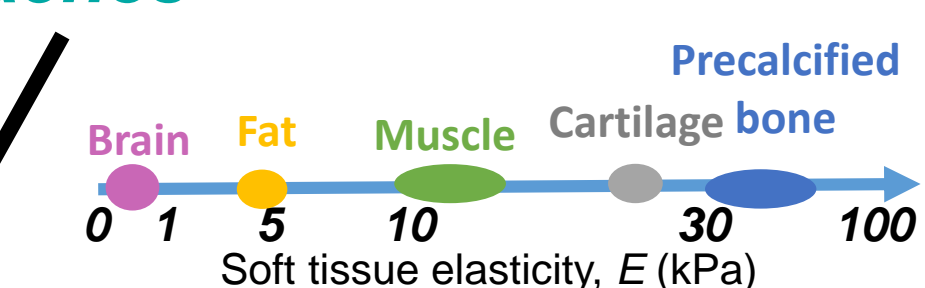
Natural origin: from brown algae



Alginate: polysaccharide suitable for biomimicry approaches



Formation of **physical hydrogel** in presence of **calcium ions (Ca²⁺)** [5]



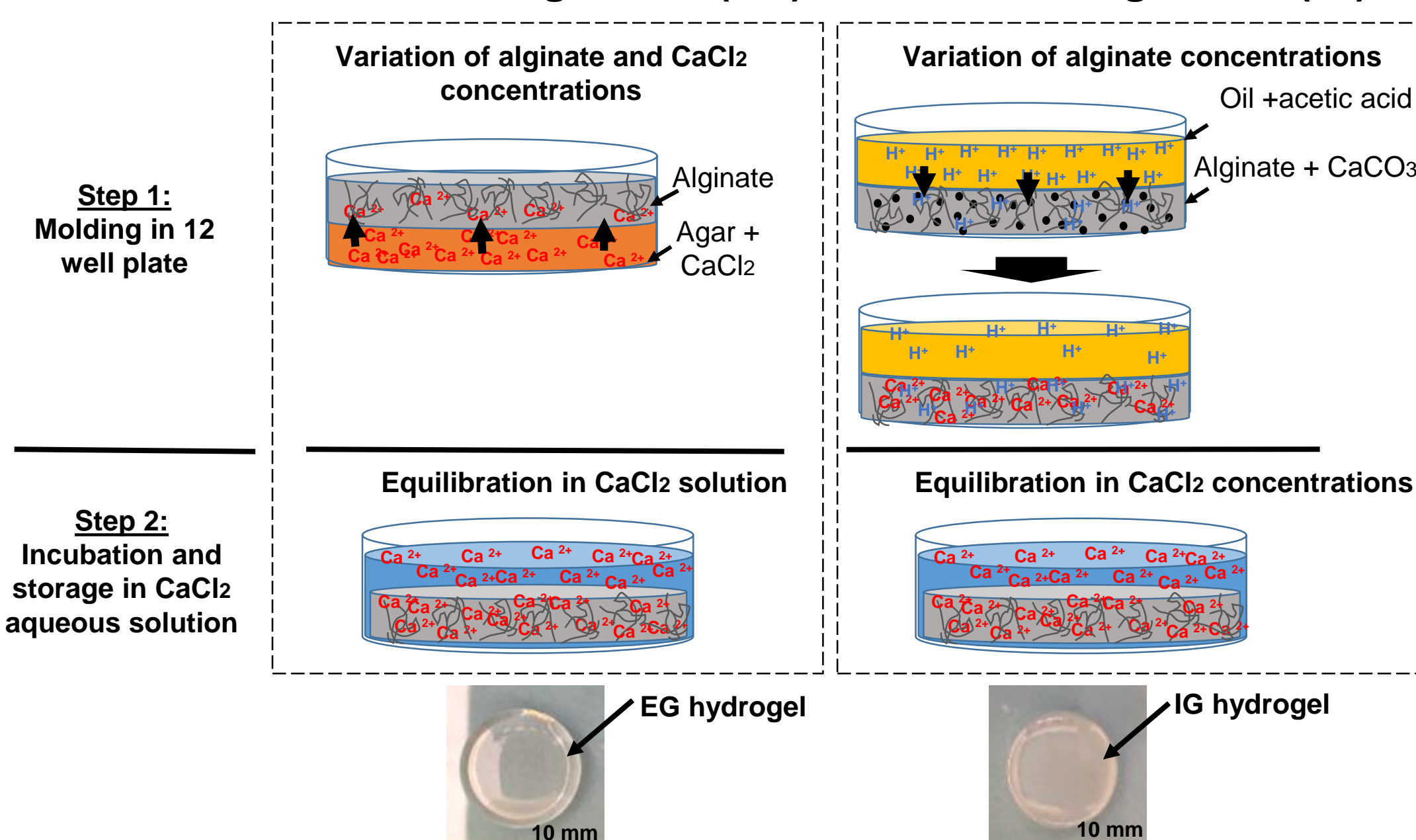
Mechanical properties of alginate hydrogels resembling those of biological tissues [6]

Result 1: Ca-alginate macrogels for hMuStem cell encapsulation – comparative study [7]

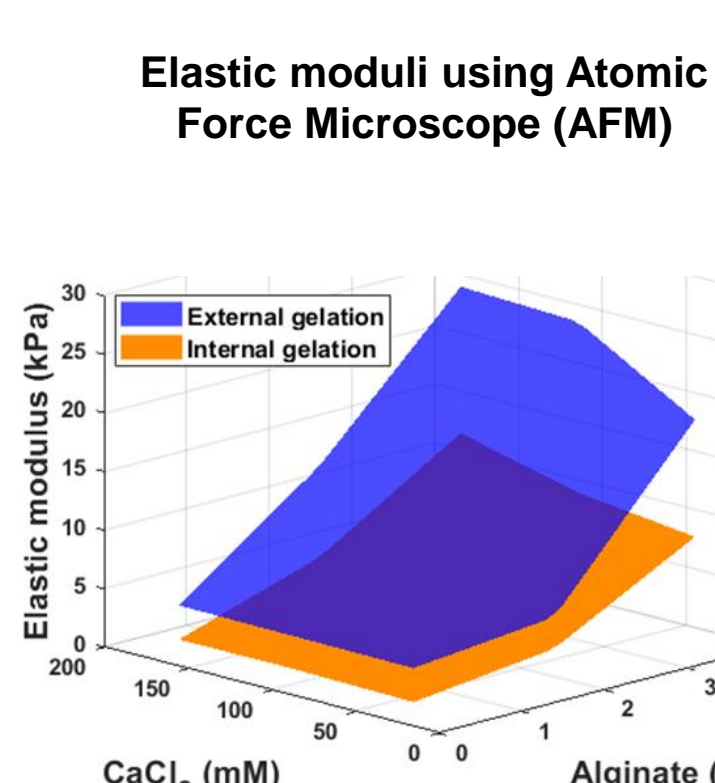
Elaboration of artificial matrix to mimic ECM based on alginate polysaccharide - Two gelation approaches

External gelation (EG)

Internal gelation (IG)

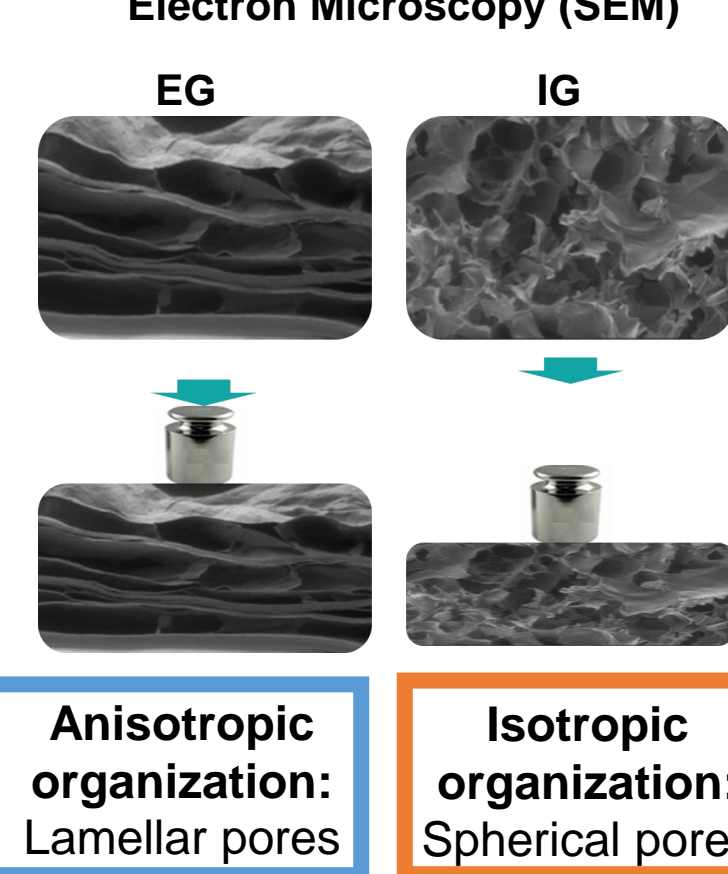


Impact of the gelation mode on the physical properties of hydrogels



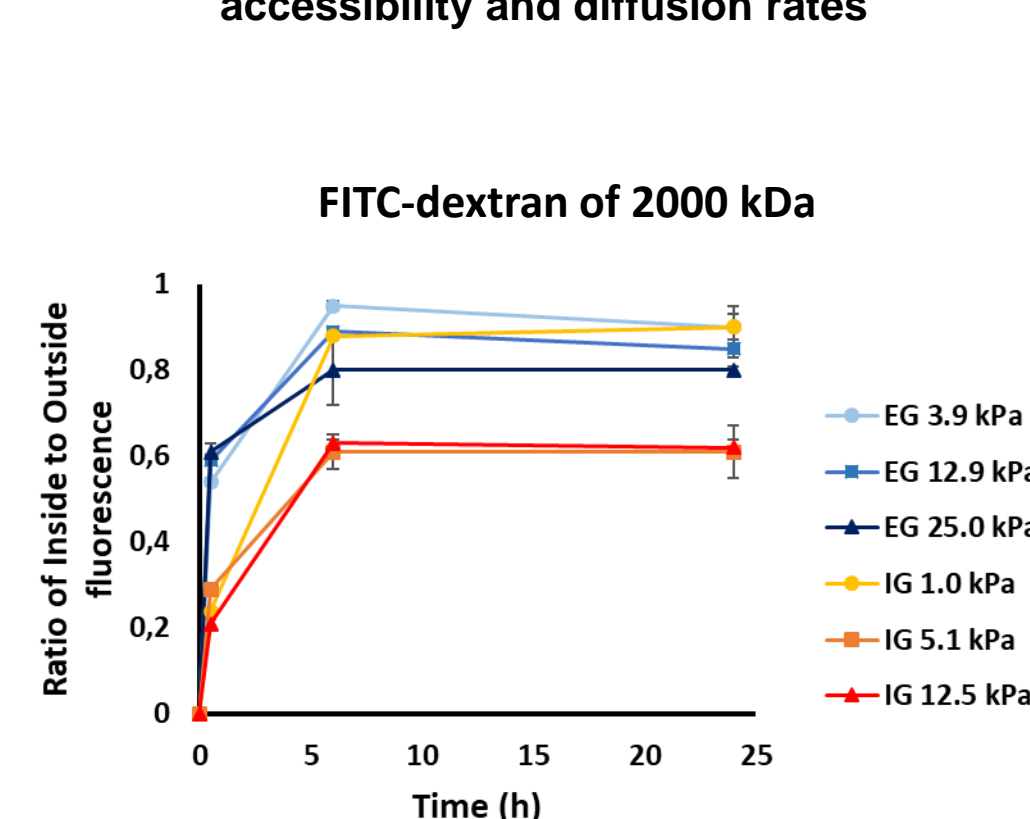
- **Hydrogels with elastic modulus from 1 to 25 kPa**
- **Lower elastic modulus observed for Internal Gelation mode**

Internal structure using Scanning Electron Microscopy (SEM)



- **Anisotropic organization: Lamellar pores**
- **Isotropic organization: Spherical pores**
- **Lamellar structure more resistant to mechanical pressure**

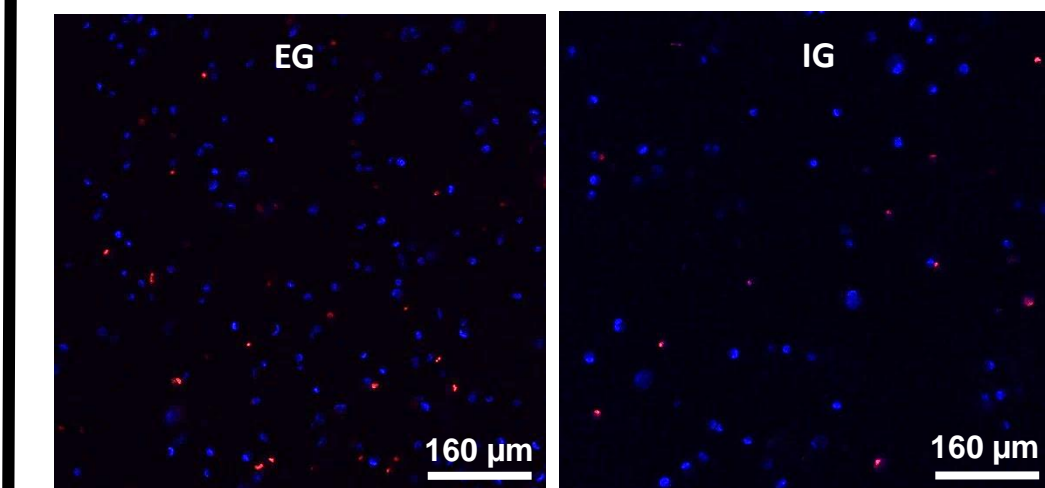
Effect of pore shape and size on solute accessibility and diffusion rates



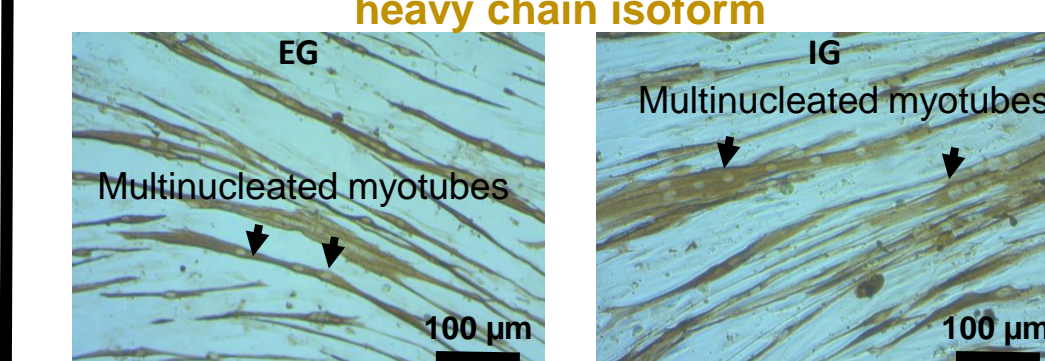
- **Diffusion of small molecules inside hydrogels**

Impact of the mechanical properties of the mimetic matrix on hMuStem cells

Good cell viability (>70%) inside hydrogels mimicking muscle elastic modulus (12 kPa) – Live/Dead labeling (Hoescht 33342 / Ethidium D1)



Good myogenic properties after contact with Ca-alginate matrices – labeling of sarcomeric myosin heavy chain isoform



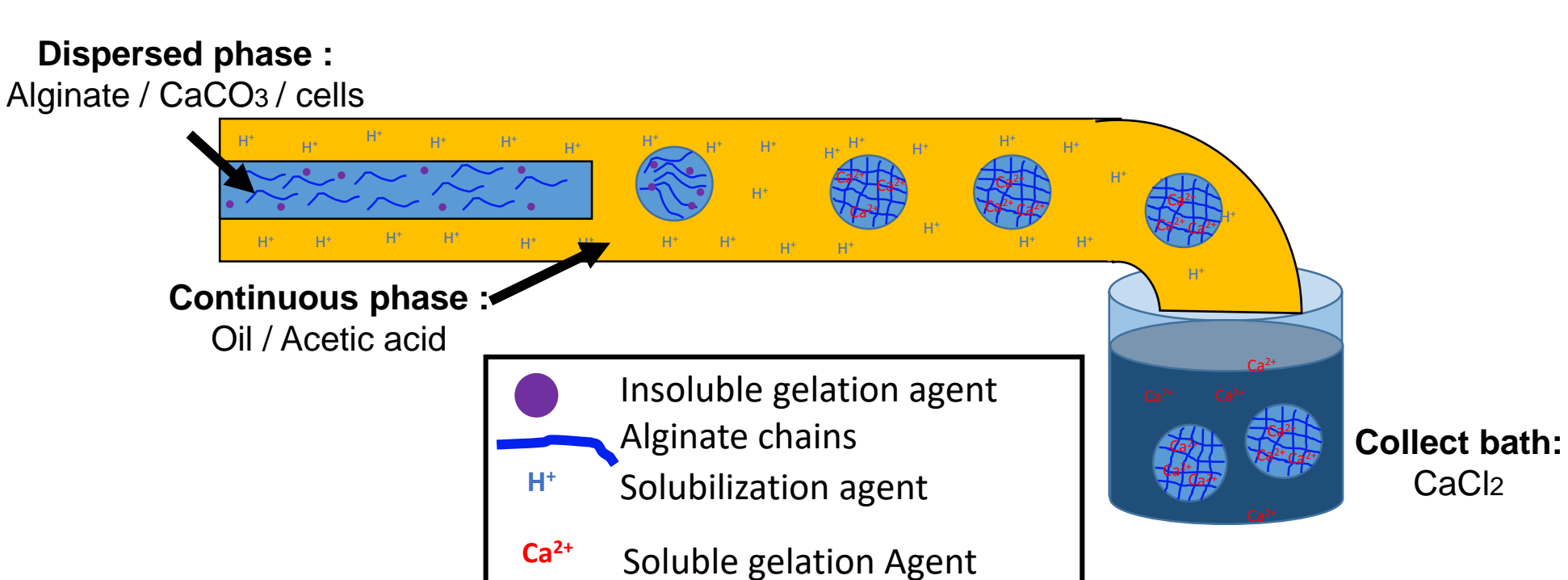
- **Structural constraints do not influence biological activities**

Obtention of macroscopic hydrogels using molding methods whose mechanical, structural and diffusion properties have been characterized as a function of the gelation methods used. However, the large size of these hydrogels limits their therapeutic applications to subcutaneous implant.

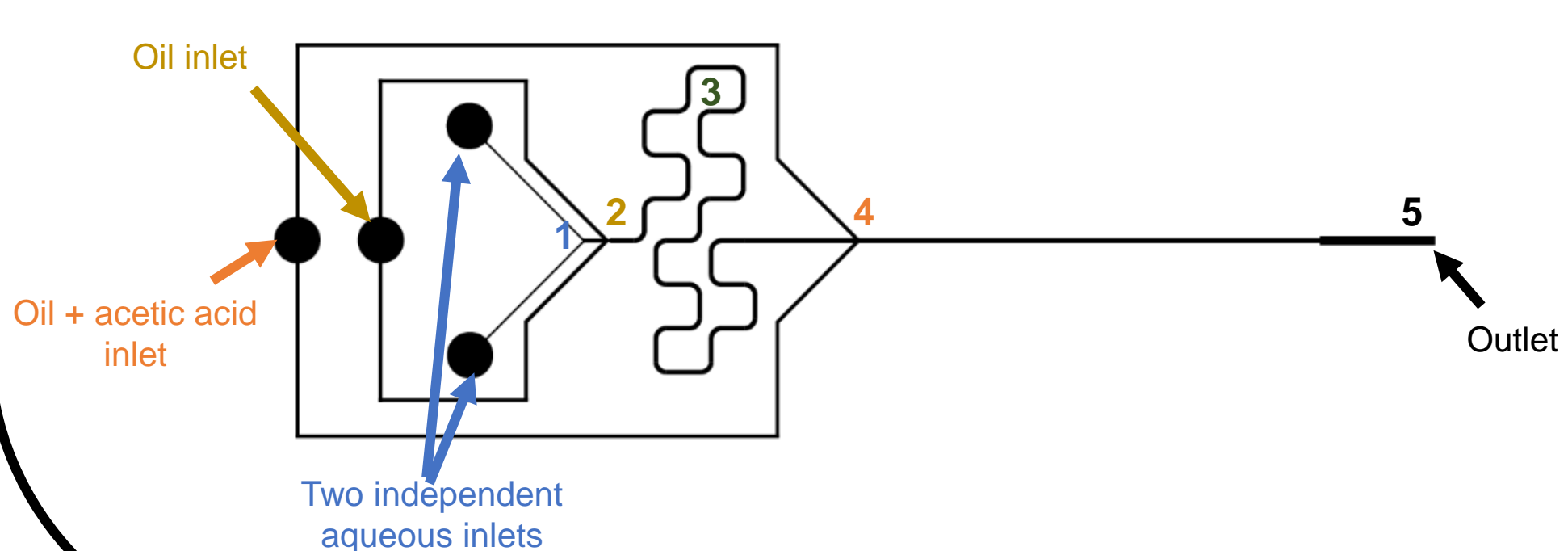
Result 2: Transposition to droplet microfluidic approaches – change of scale

Microfluidic chip for Ca-alginate microgels

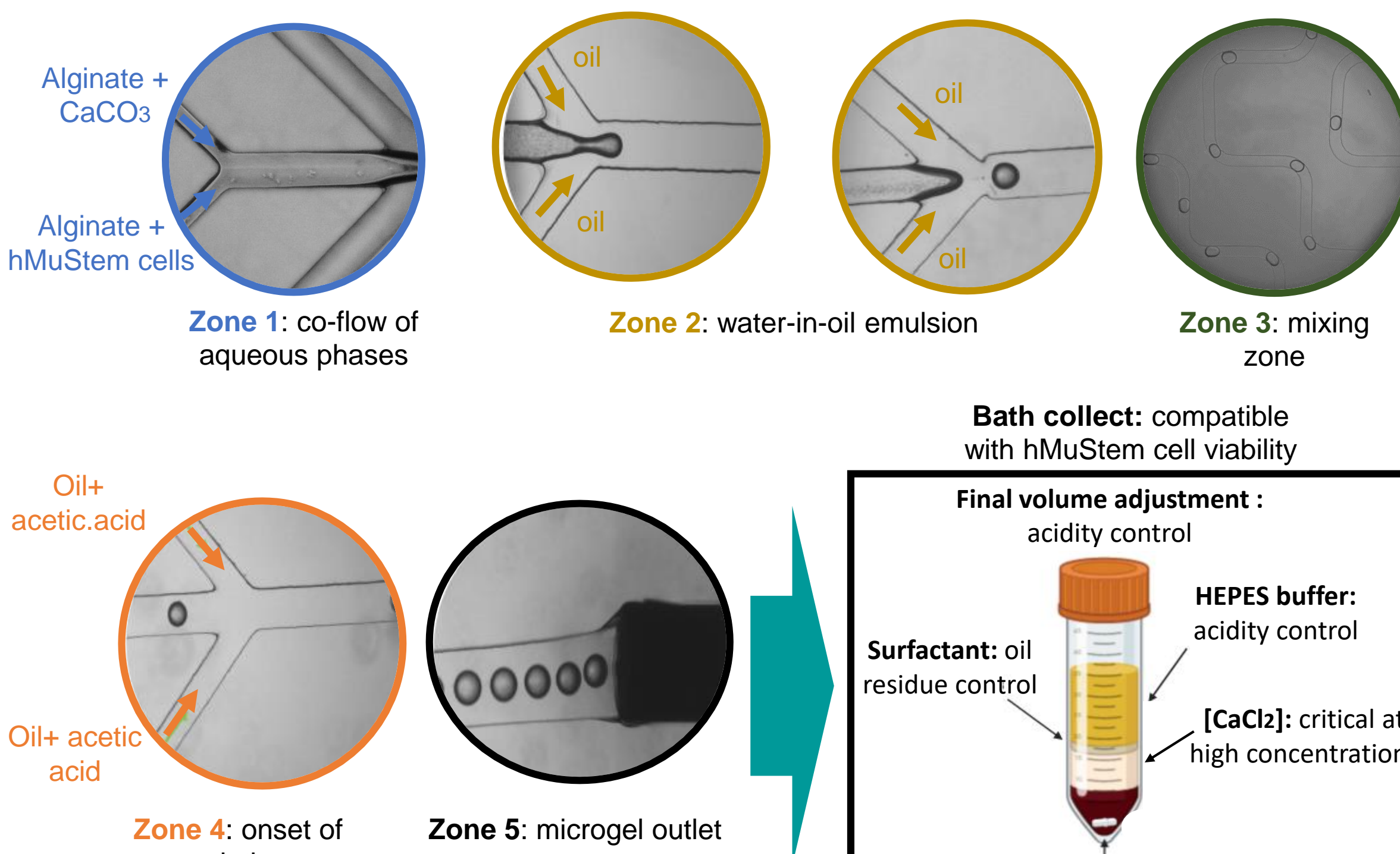
Internal gelation principle in microfluidic chip



Design of the microfluidic chip (5 zones)



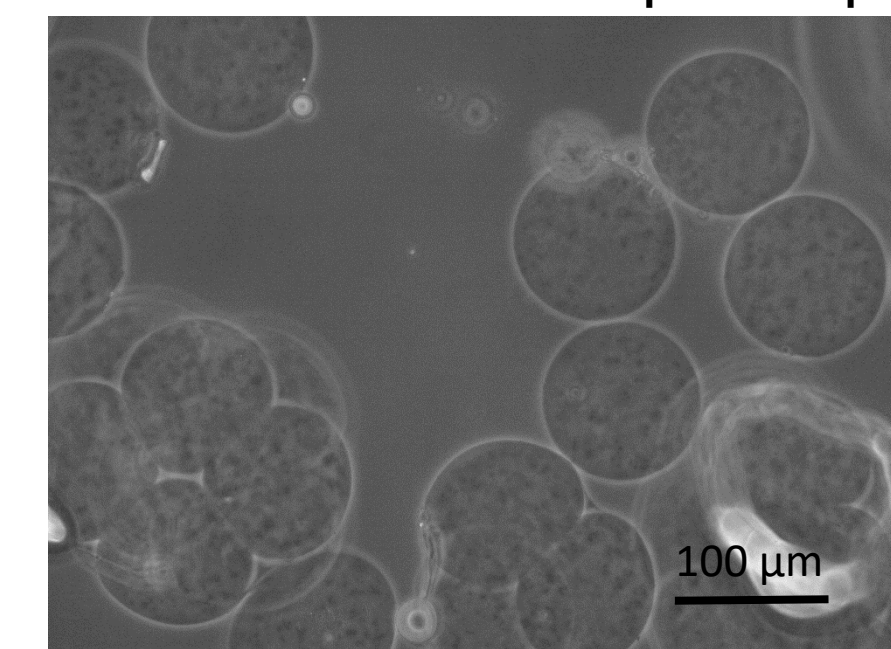
Microencapsulation steps on-chip



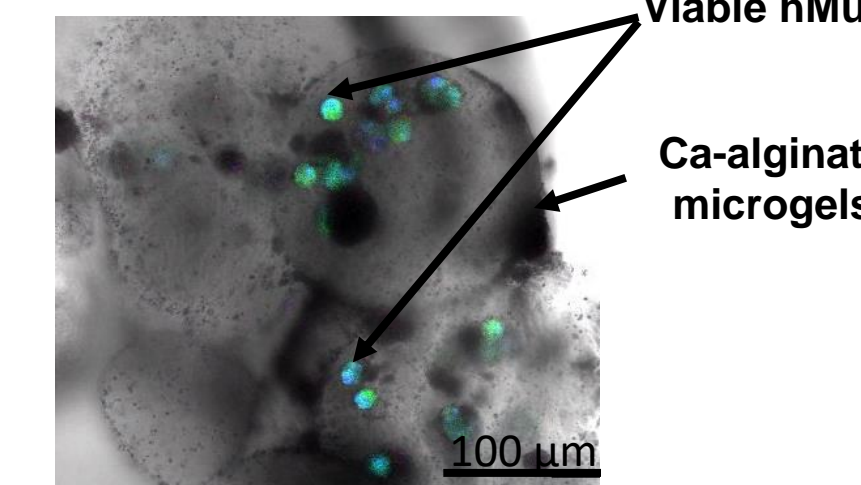
- **Limit of impact of CaCO₃ crystals on cells through shear until the droplets formation at the first junction (Zone 2)**
- **No coalescence of droplets**
- **Bath collect optimized**

Characterization of microgels containing hMuStem cells

Ca-alginate microgels of 100 μ m in diameter containing hMuStem cells at the end of the encapsulation process



High cell viability inside microgels, in culture medium, 8 days after encapsulation – Live/Dead labeling (Calcein AM-Hoescht / Ethidium D1)



- **Process of microencapsulation compatible with hMuStem cells**

Conclusion

We synthesized calcium-alginate macrogels. We showed that the elastic modulus of the hydrogels ranged from 1–25 kPa and was directly dependent on the gelation mode and on Ca-alginate concentrations. Gelation parameters impacted hydrogel structure and diffusion properties, while leaving small molecules free to circulate. Encapsulation did not alter the viability of hMuStem cells that also preserve morphological and myogenic differentiation characteristics after release. To pave the way for new strategies of cell transplantation treatment by injection, following these first results, we demonstrated the effectiveness of hMuStem cell encapsulation on a microscopic scale through the development of a microfluidic process. As this stage of study, all of our results showed that hMuStem cells can be encapsulated in Ca-alginate hydrogels without compromising functionality, opening the way to new transplant protocols.

References

[1] Rouger K., et al., American Journal of Pathology, 2011. DOI:10.1016/j.ajpath.2011.07.022
 [2] Lorant J., et al., Molecular Therapy, 2018. DOI: 10.1016/j.yjth.2017.10.013
 [3] Lejis M., et al., Eur Cell Mater, 2017 DOI:10.22203/eCM.v033a04
 [4] Raab M., et al., Stem Cell Research and Therapy, 2010. DOI:10.1186/s13287-010-0004-0
 [5] Grant et al., FEBS Letters, 1973. DOI:10.1016/0014-5793(73)80770-7
 [6] Jang J., et al., J Mech Behav Biomed Mater, 2014. DOI:10.1016/j.jmbm.2014.05.004
 [7] Marquis M. et al., International Journal of Biological Macromolecules, 2024. DOI: 10.1016/j.jbiomac.2024.130823