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

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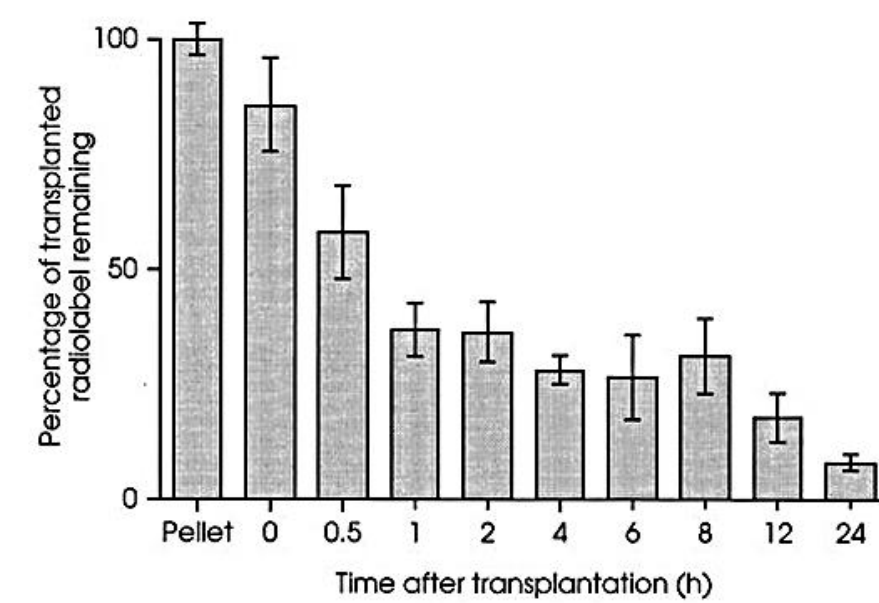
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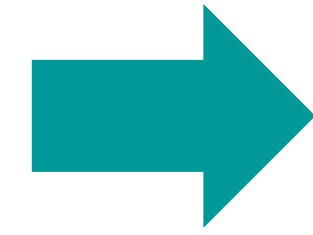
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Cell therapy and muscle diseases

- 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

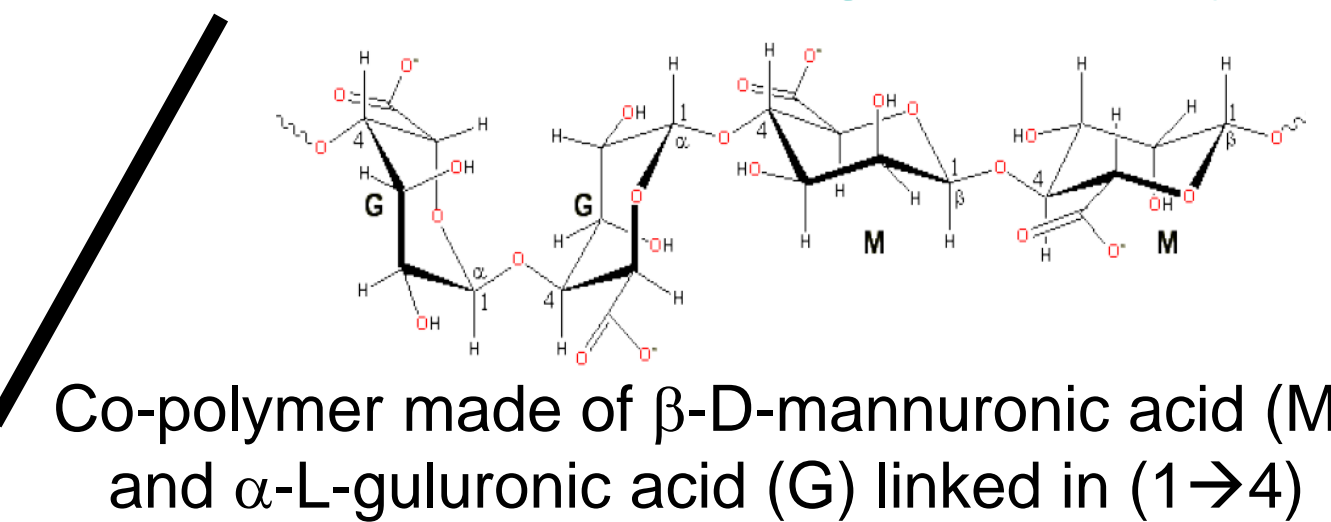
Hydrogels

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

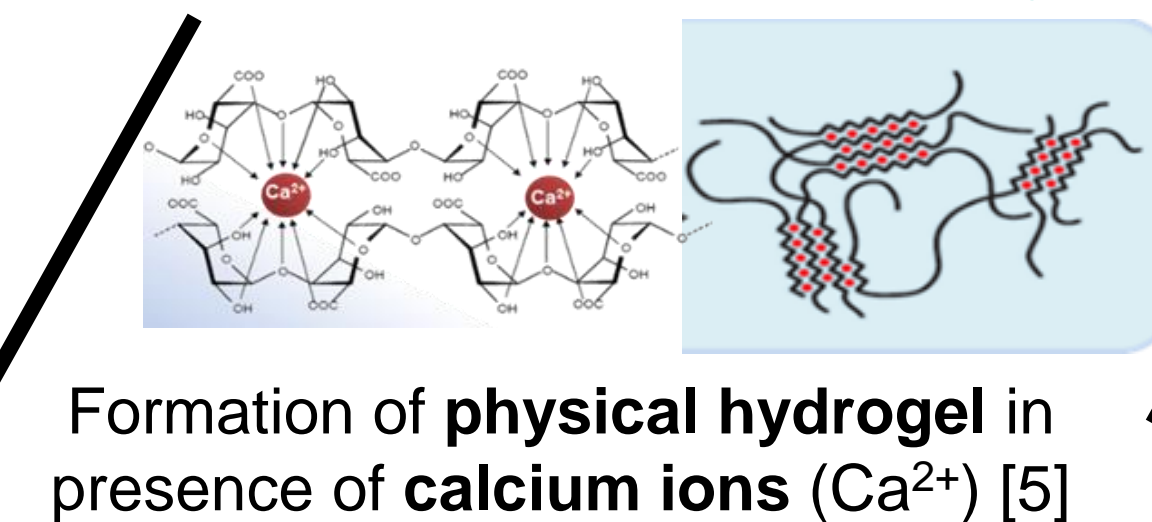
Selection of a biocompatible model polymer



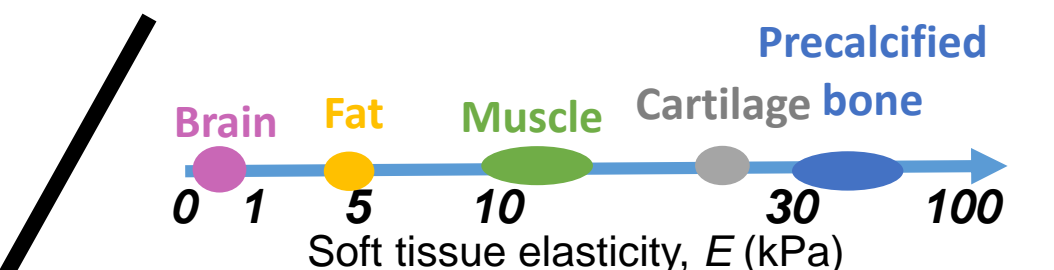
Natural origin: from brown algae



Alginate: polysaccharide suitable for biomimicry approaches



Formation of physical hydrogel in presence of calcium ions (Ca^{2+}) [5]

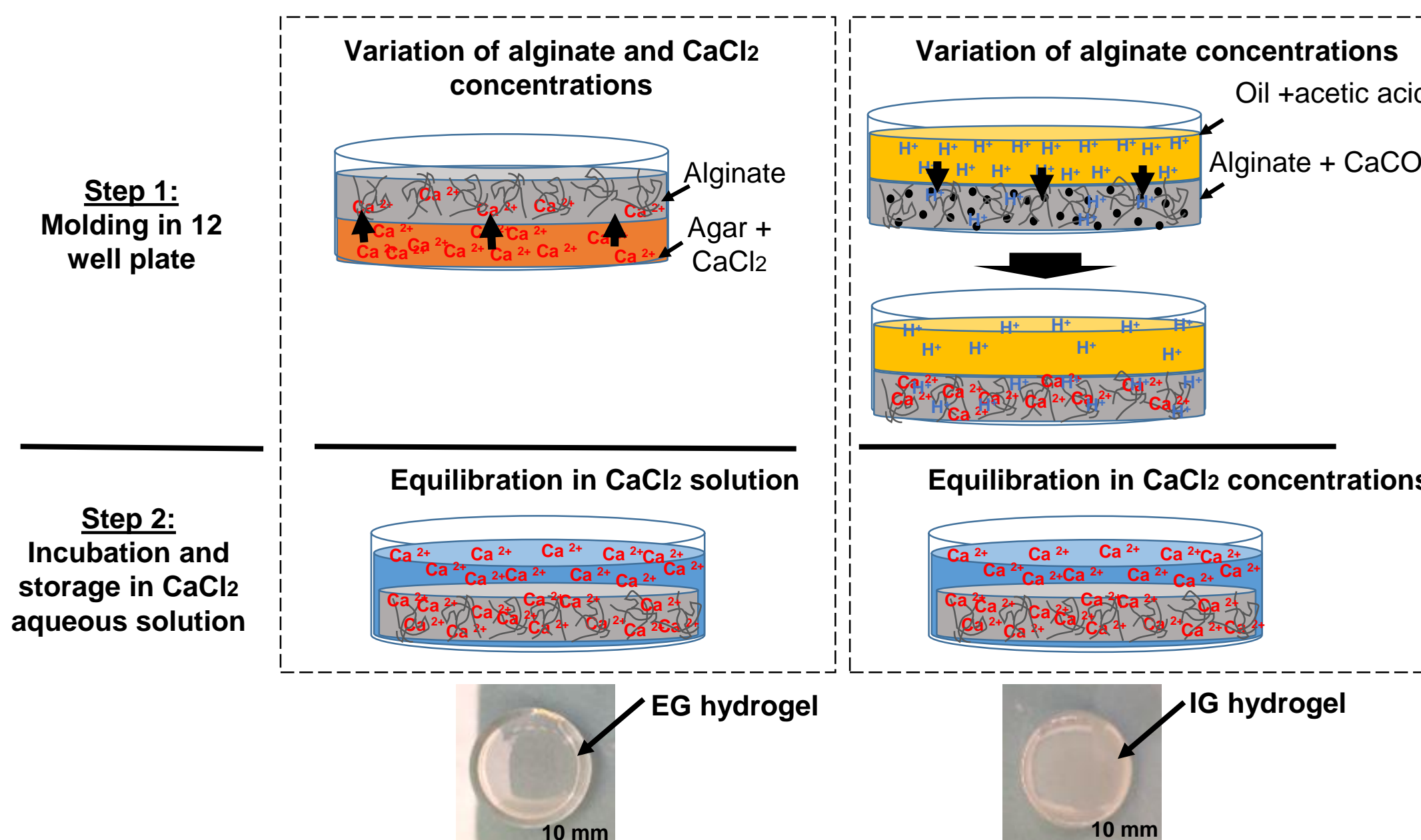


Mechanical properties of alginate hydrogels resembling those of biological tissues [6]

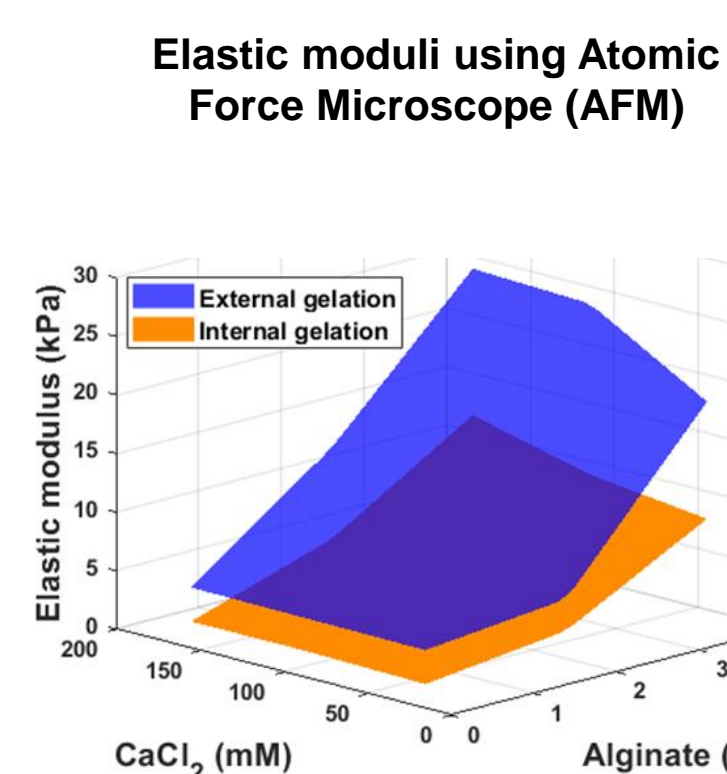
Result 1: Ca-alginate macrogels for hMuStem cell encapsulation – comparative study [7]

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

- External gelation (EG)
- Internal gelation (IG)

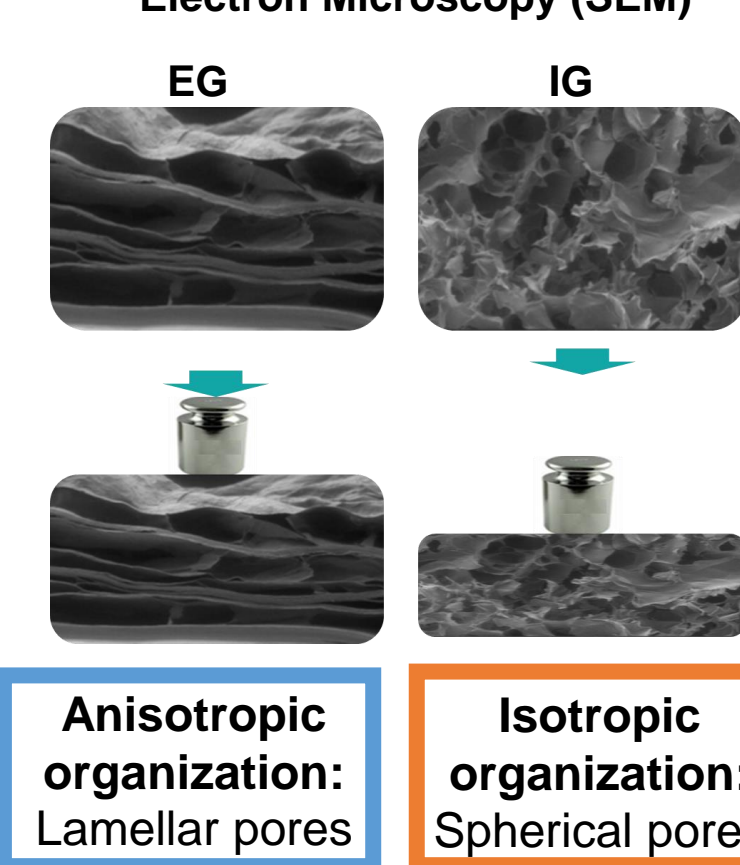


Impact of the gelation mode on the physical properties of hydrogels



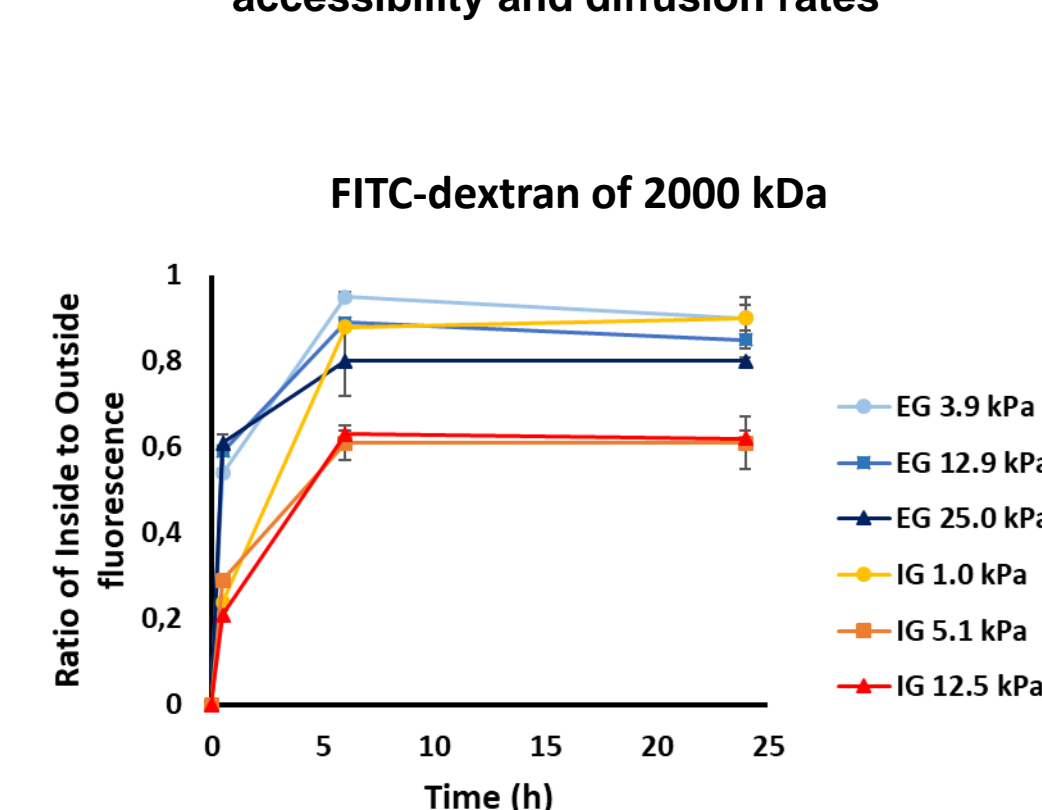
- Hydrogels with elastic modulus from 1 to 25 kPa
- Lower elastic modulus observed for Internal Gelation mode

Internal structure using Scanning Electron Microscopy (SEM)



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

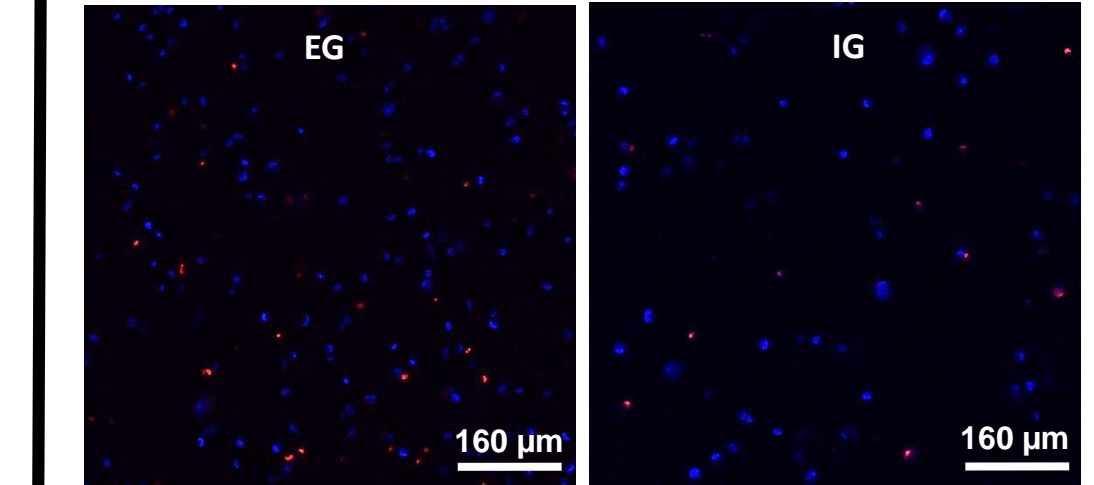
Effect of pore shape and size on solute accessibility and diffusion rates



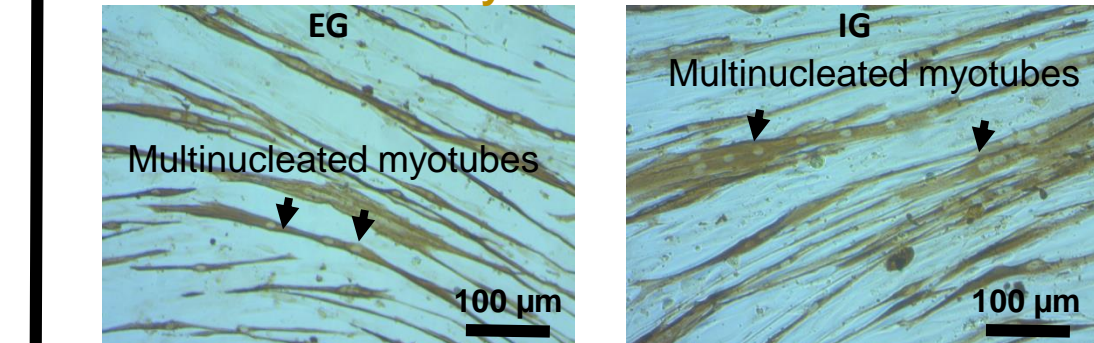
- Diffusion of small molecules inside hydrogels

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

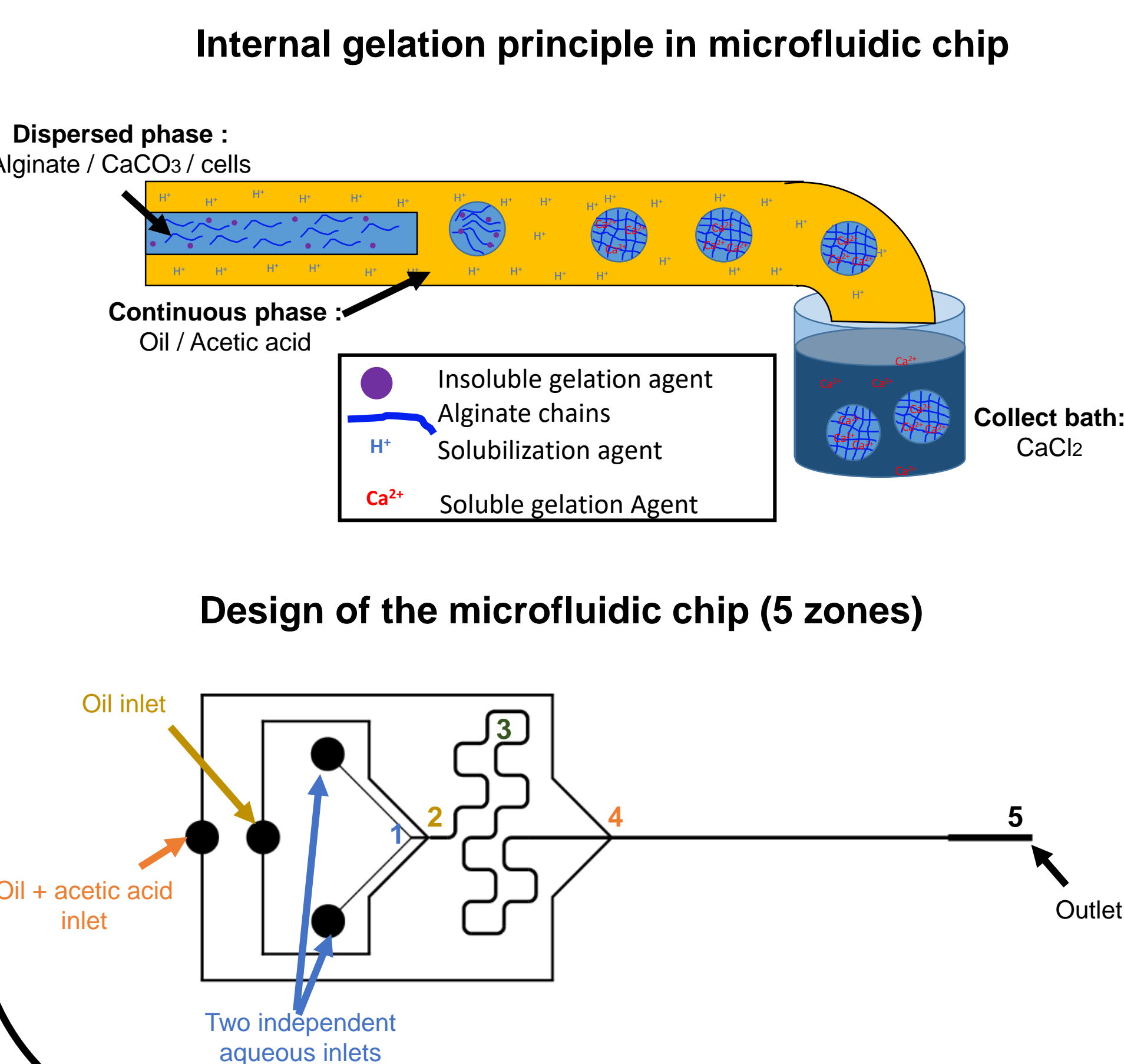


- Structural constraints do not influence biological activities

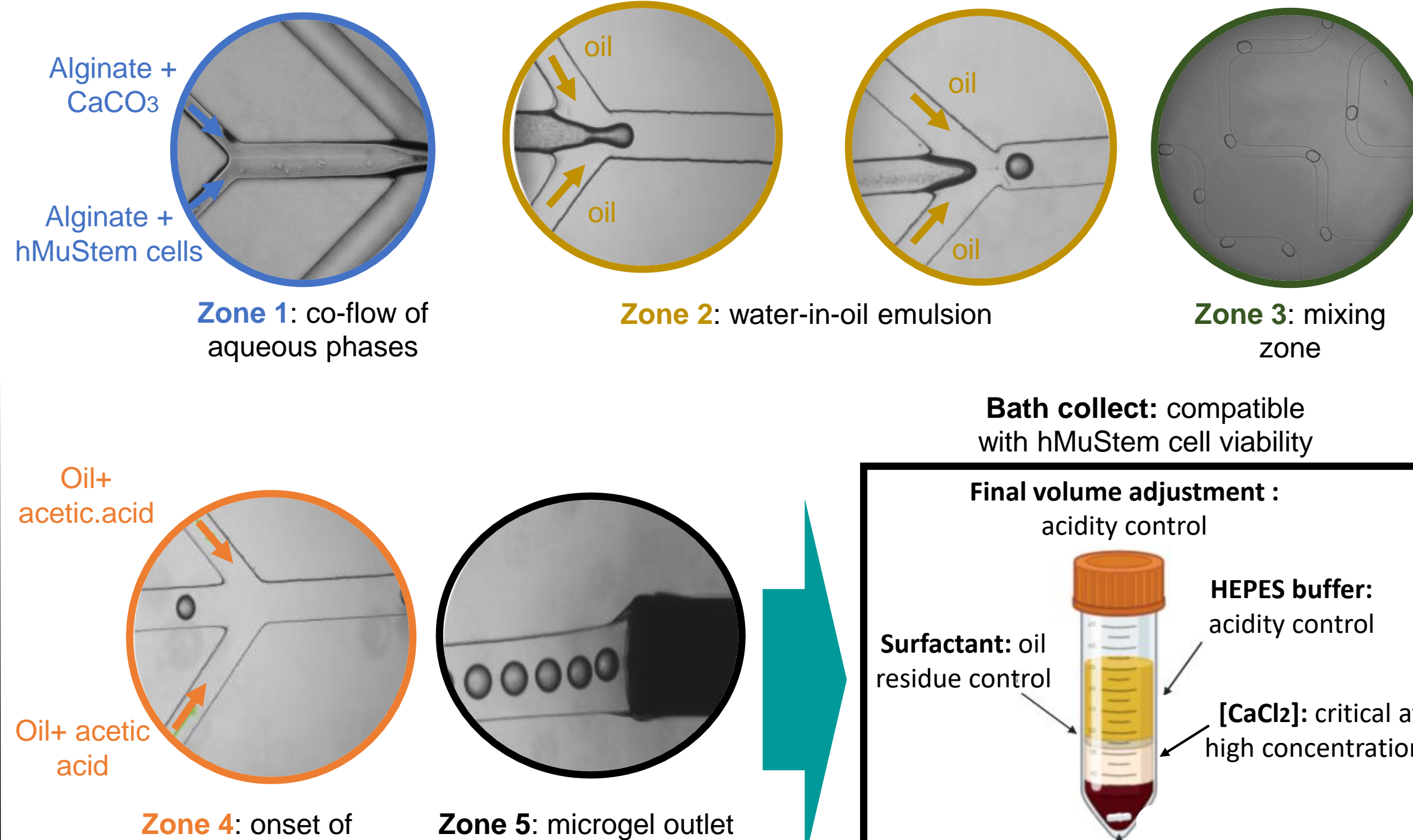
Obtention of macroscopic hydrogels using molding methods whose mechanical, structural and diffusion properties have been characterized as a function of the gelation methods used. However, the large size of these hydrogels limits their therapeutic applications to subcutaneous implant.

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

Microfluidic chip for Ca-alginate microgels



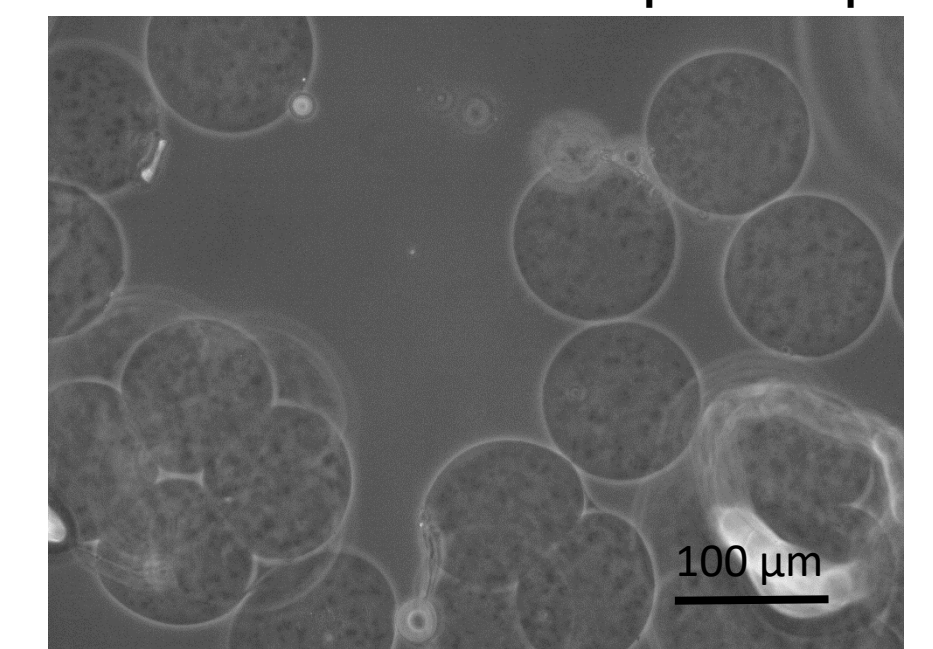
Microencapsulation steps on-chip



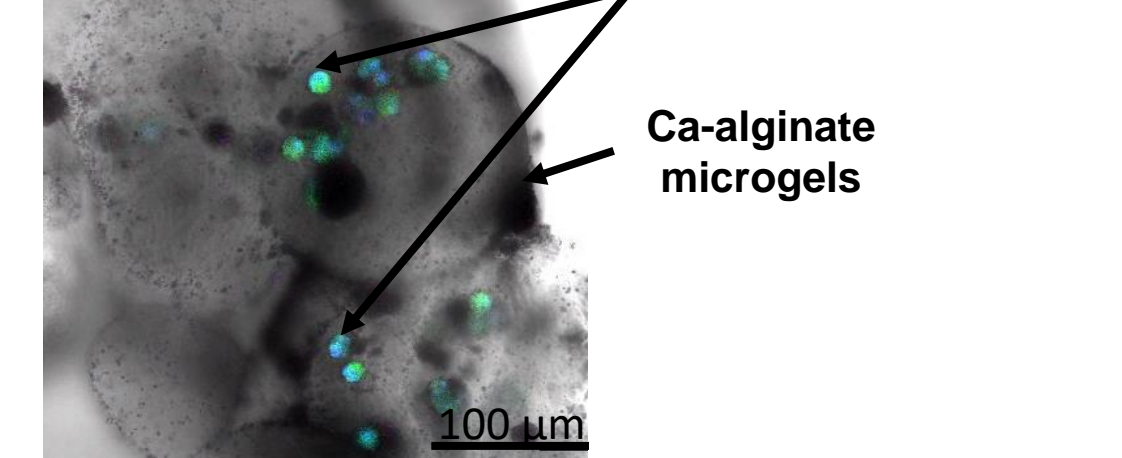
- Limit of impact of CaCO_3 crystals on cells through shear until the droplets formation at the first junction (Zone 2)
- No coalescence of droplets
- Bath collect optimized

Characterization of microgels containing hMuStem cells

Ca-alginate microgels of 100 μm 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

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