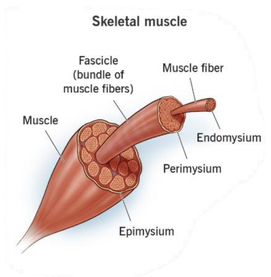
Dual 3D Printing of Dense Collagen and Laponite® to Increase the Cell-to-Matrix Fraction in Muscle ECM Models

Blaise. Hebert[1], Lena.Villerabel[1,2], Maxime. Mauviel[2], Carole Aimé[2], Christophe. Hélary[1]

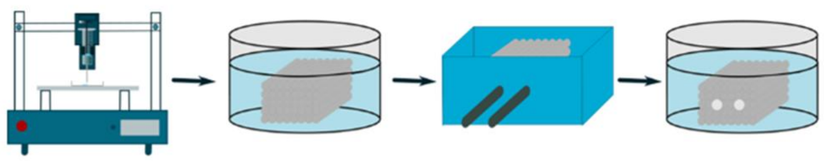
[1] Laboratoire de Chimie de la Matière Condensée de Paris, Sorbonne Université, CNRS, Paris, France
[2] Chimie Physique et Chimie du Vivant, Département de Chimie, École Normale Supérieure, Université PSL, Sorbonne Université, CNRS, Paris, France

INTRODUCTION

Skeletal muscle consists of hierarchically organized fiber bundles embedded in a connective tissue network that ensures mechanical integrity and coordinated force transmission. This extracellular matrix (ECM), representing 10–20% of total muscle volume, combines anisotropic collagen organization, a stiffness of ~15 kPa, and dense vascularization. Reproducing such structural and physico-chemical complexity in vitro remains a major challenge.



3D printing of dense type I collagen provides a promising strategy to recreate the anisotropic and fibrillar architecture of native muscle ECM. Forming internal channels dedicated to cell colonization and nutrient transport without damaging the matrix remains challenging. Needle insertion has been used to generate channels but offers limited channel-to-matrix ratios and often disrupts scaffold integrity. By using this method the number and size of the channels are very limited.



Scaffold printing In liquid 5X PBS | Fibrillogenesis in 5X PBS and NH3 vapor | Needle insertion to generate chanels | Washing in 1X PBS

OBJECTIVES

The objective of this project was to increase the channel-to-matrix ratio inside the collagen matrix in order to reach a more physiological cell density in the 3D muscle model. To do this, we have to increase the number and the size of the channels throughout the collagen hydrogel without disturbing its structure.

STRATEGY

- A printable sacrificial material could be used a strategy to design collagen matrices with a large cell fraction
- A sacrificial ink is a printable material that remains stable during printing and gelation. In addition, it can be easily removed by changing the physico-chemical conditions. Sacrificial inks are currently used in fused filament 3D printing to generate removable support structures, or in bioprinting to create channels.

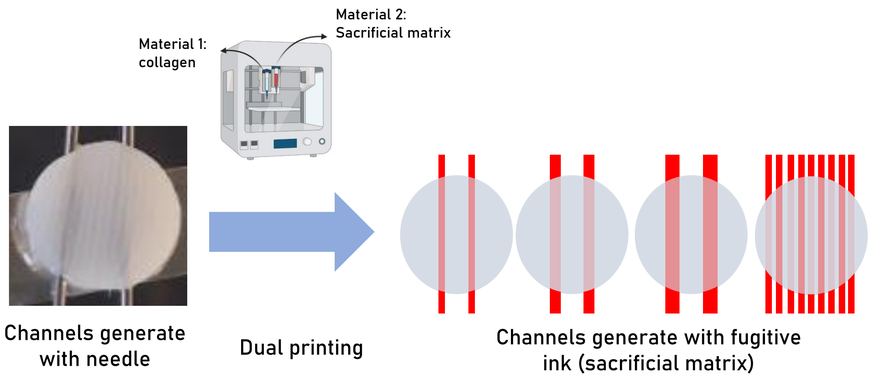


Support structure



Channels

- Our strategy was to print the macroporous matrix dual printing. Collagen filaments were printed beside the sacrificial matrix during the printing.



Channels generate with needle | Dual printing | Channels generate with fugitive ink (sacrificial matrix)

- We need to find a material that meets all the requirements for 3D printing using dual extrusion with collagen. The material should:

- Be easily printable
- Have good adhesion with collagen
- Be stable in 5× PBS and NH_3 vapors
- Be removable without altering the collagen
- Have sufficient mechanical properties to provide structural support

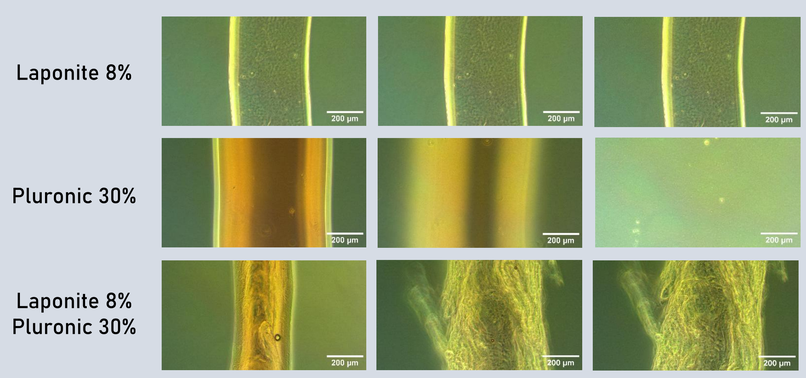
CONCLUSION / PESPECTIVES

- Pluronic-based fugitive inks are unstable in the collagen fibrillation conditions.
- Pure Laponite® is easy to prepare, printable, stable in ionic solutions, and can be flushed out by distilled water.
- No cytotoxicity was observed in C2C12 myoblasts after Laponite removal.
- Laponite was toxic if not removed.
- Excellent printing fidelity and structural reproducibility achieved using Laponite.
- Dense collagen matrices containing up to nine channels were synthesized, thereby mimicking the native muscle ECM architecture.
- The use of Laponite did not affect the ability of cells to colonize, proliferate, and differentiate into myotube when seed within the channels.
- Laponite could be used to generate channels within fibrotic-like collagen matrices to mimic the pathological ECM observed in Duchenne muscular dystrophy.

RESULTS

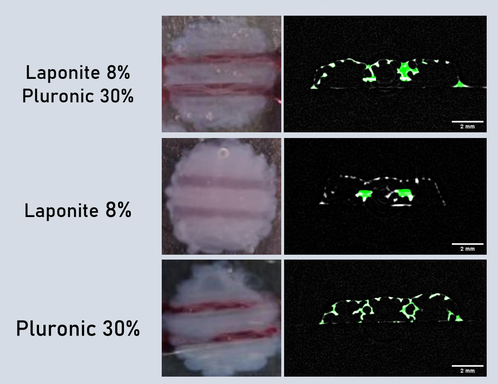
Laponite can be used as a sacrificial matrix

Stability of laponite in 5X PBS



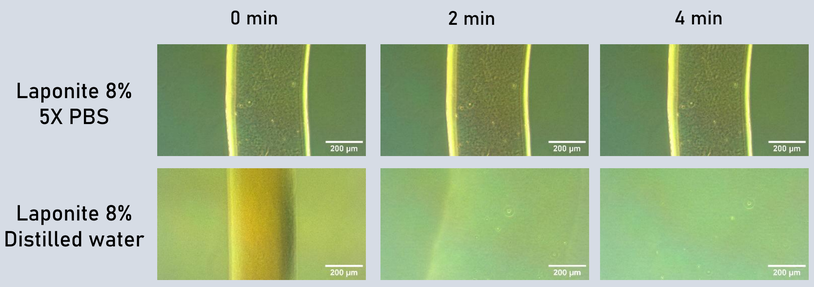
Laponite filaments are the only ones stable in the printing bath that triggers collagen gelation.

Matrices produced by collagen and fugitive ink dual extrusion



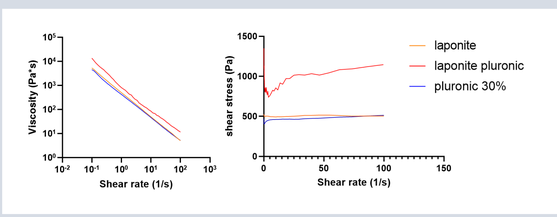
Laponite enable high shape retention of the printed filaments of sacrificial matrix beside collagen filaments.

Laponite can be removed with distilled water



The laponite fugitive ink can easily and quickly be removed using distilled water

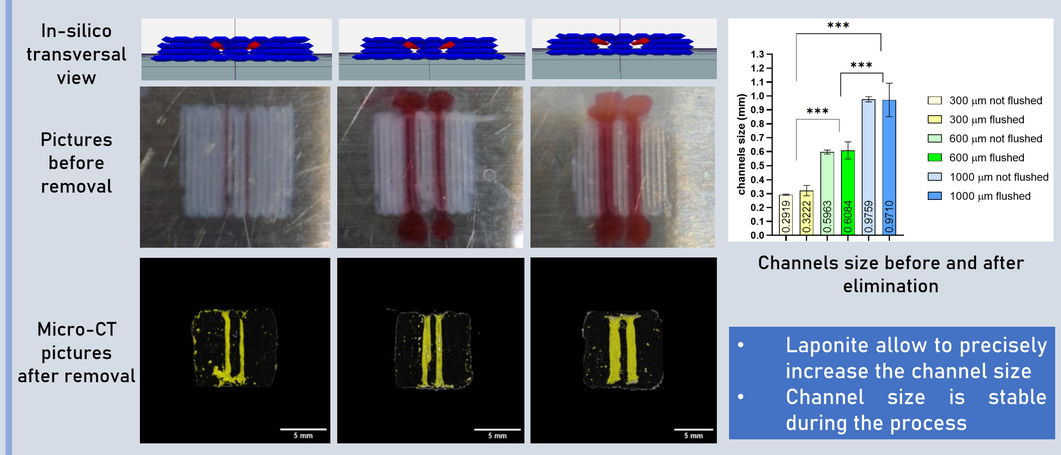
Mechanical properties



All the tested materials share the mechanical properties of a good sacrificial ink:
- Shear-thinning behavior
- Yield stress
- Good viscosity

Channels size and number can be modulated using Laponite

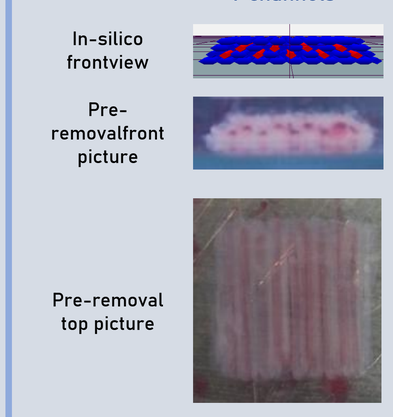
Channels size



Channels size before and after elimination

- Laponite allow to precisely increase the channel size
- Channel size is stable during the process

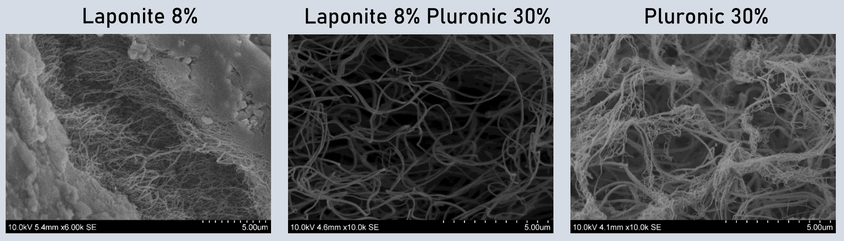
Channel density



This precision allow us to highly increase the channels density

Ultrastructure of channels generated by Laponite removal

SEM Observation

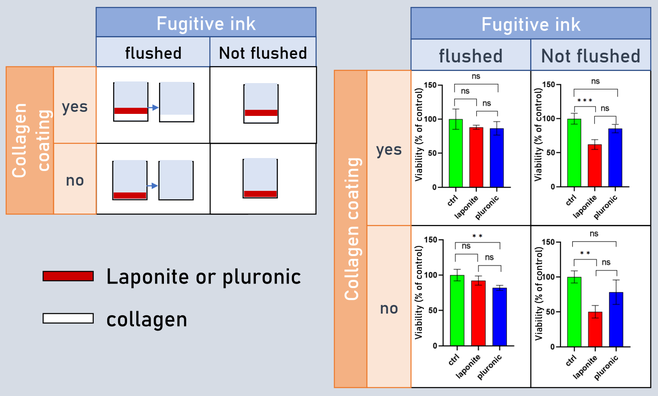


- Damaged collagen network in channels generated by Pluronic
- Fibrillar collagen network in channels generated by Laponite and Laponite–Pluronic
- Residues of Laponite observed inside the channels

Channel colonisation after Laponite removal

Evaluation of cytotoxicity

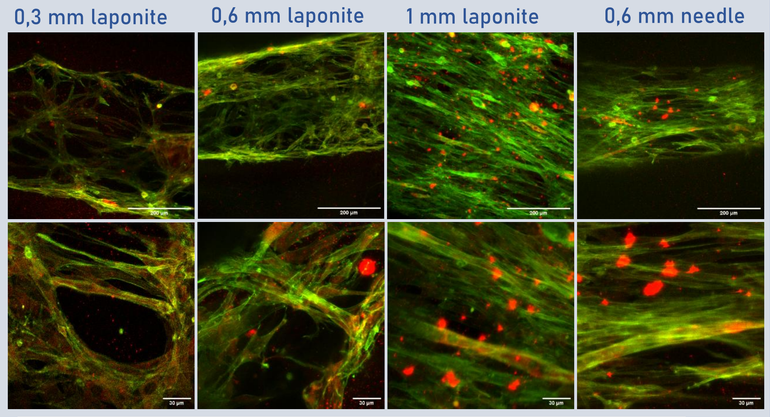
Alamar-Blue



- No toxicity of Laponite after its removal by flushing in distilled water (Alamr blue Test)
- Toxicity of Laponite observed when not removed

Channel colonisation by C2C12 myoblasts after Laponite removal

Confocal images



Cellularization shows that cell density and C2C12 differentiation into myotubes are not impacted by the use of Laponite to generate channels. A slight decrease in density can be observed in 0.3 mm diameter channels, which may be explained by more a challenging cellularization process.

Acknowledgement

We gratefully acknowledge AFM-Téléthon for funding this project.