In vivo biocompatibility assessment of 3D printed hydrogels and PCL, MRI follow-up.

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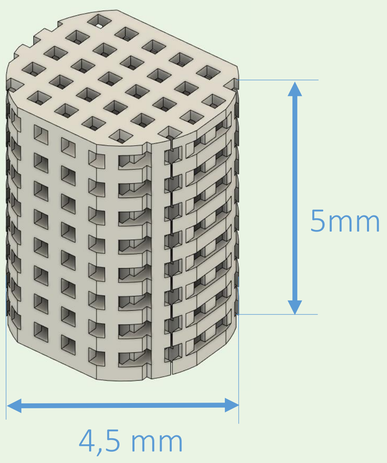
Introduction

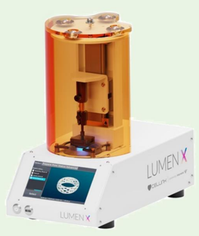
- After a severe traumatic brain injury, there is very little cellular regeneration of the lost part.
- To counteract this, we propose to build a microenvironment that will provide a mechanical support inside the lesion and that could be seeded with cells : a scaffold.
- The first step is to find a suitable material. We tested in vivo the biocompatibility of two hydrogels (**PEGDA** and **GelMA**) and PCL.
- Body response was assessed by behavioral evaluation, MRI follow-up, and histology.

Design



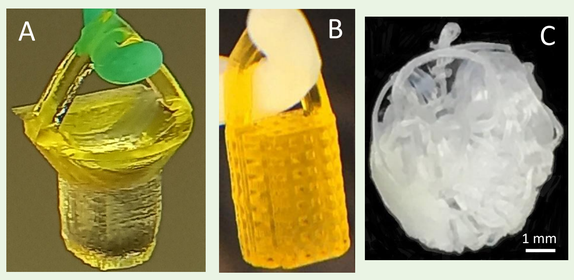
Porous scaffold

Python scripting to repeat patterns

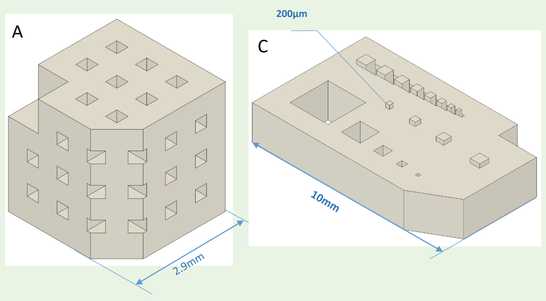


Designed for pillars of 150-230µm, photoprinting with LumenX

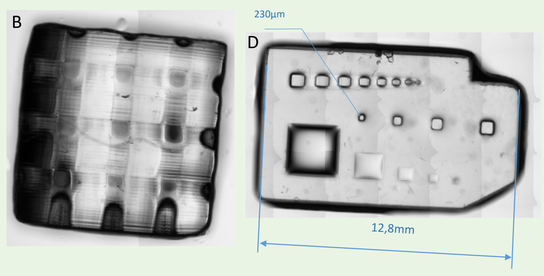
3D printing



(A) Complex design printed in PEGDA (*Poly(ethylen glycol) diacrylate*)
(B) Simpler design with PEGDA-GelMA (*Gelatin Methacrylate*)
(C) PCL thread coiled into a ball (*Polycaprolactone*)



(A) Test design used to adjust exposure time and photo-absorber weight
(B) After printing, we can check the depth of photo-crosslinking with microscopy



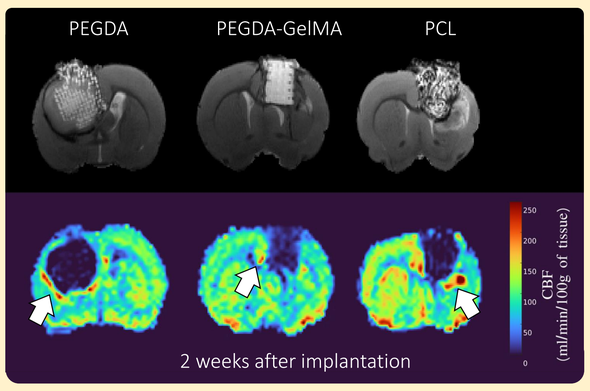
(C) Test design used to measure the difference between what we want to print …
(D) … and what is actually printed

Conclusion

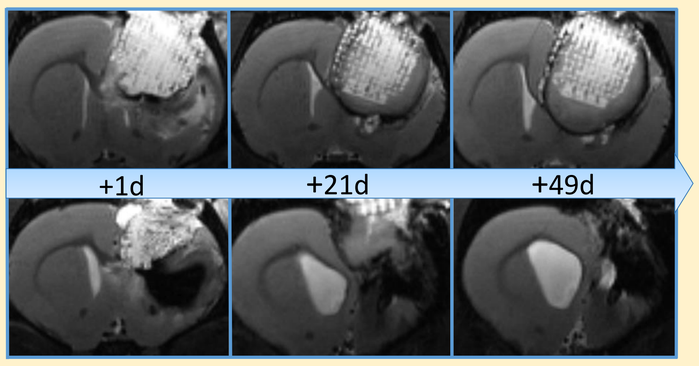
- Complex shape printed
- Specific tools to handle soft materials
- In vivo:
  - Strong inflammatory response for PEGDA only ✗
  - Moderate inflammation for PCL
  - Best results for PEGDA-GelMA ✓
  - Non-invasive follow-up with MRI
- 3D photoprinting of biopolymers is increasingly accessible
- But finding the right material remains challenging

In vivo

- 2 rats PEGDA + 1 PEGDA-GelMA + 1 PCL + 1 control
- Malonate injection to simulate an acute brain injury
- Scaffold implanted in the brain 8 days after injury



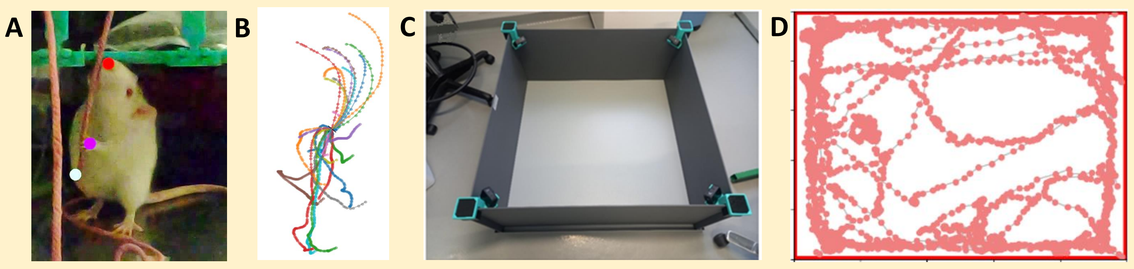
- High water content of hydrogels: hyper intensity on T2 images
- Low water content of PCL: hypo intensity on T2 images
- Measurement of cerebral blood flow (CBF) by ASL: hyperperfusion around the lesion
- CBF measured inside the implant suggests some functional vascularisation with PEGDA-GelMA.



(Top) Gradual encapsulation of a PEGDA implant by fibrosis

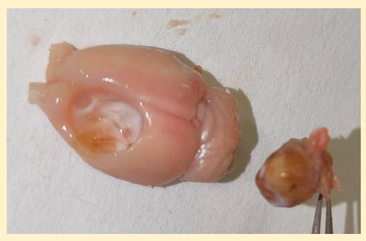
(Bottom) Another PEGDA implant, ejected by intracranial pressure

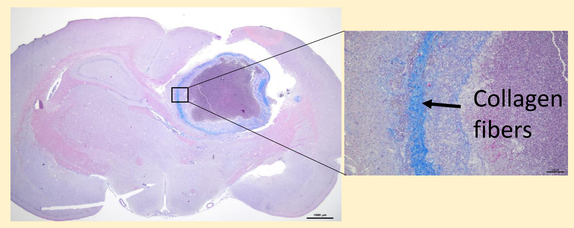
Longitudinal MRI study of PEGDA, allows non-invasive in vivo follow-up



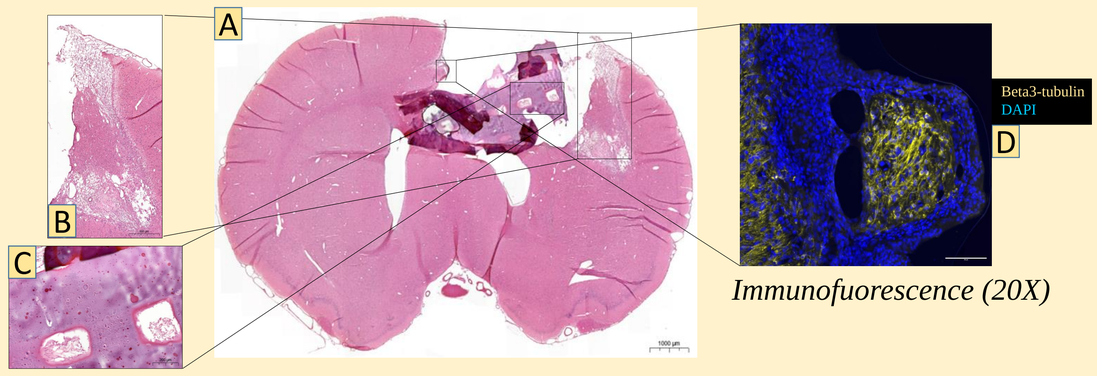
(A) String pulling experiment to measure dexterity, pose estimation with deep learning **(B)** All trajectories of one arm during an experiment **(C)** Open field to measure behavior and anxiety, 4 cameras **(D)** Exemple of a rat trajectory during 10 minutes in the open field

Ex vivo & Histology



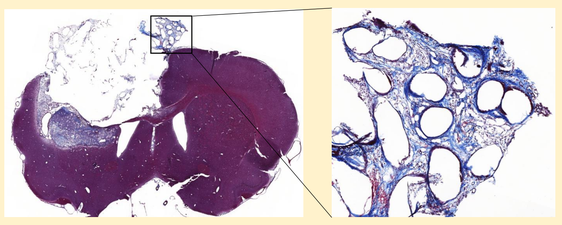


- **PEGDA**: 2 months after implantation **(left)** Scaffold encapsulated in an abscess **(right)** Masson's trichrome shows the abscess surrounded by fibrosis



Immunofuorescence (20X)

- **PEGDA + GelMA** : 1 month after implantation **(A)** Hematoxylin and eosin staining **(B)** Perilesional reconstructed tissue with vascularization and low inflammatory response **(C)** Migration and colonisation inside the scaffold. **(D)** B3-tubulin immunofluorescent labeling of neuron progenitors



- **PCL :** Masson's trichrome shows a coolagenous matrix and fibrovascular tissues in the vicinity of PCL thread.

Cirillo C. *et al*., J. Cereb Blood Flow Metab. 40(1)3-22, 2020
Le Friec A. *et al*., Transl Stroke Res, 12(1) :98-11, 2021