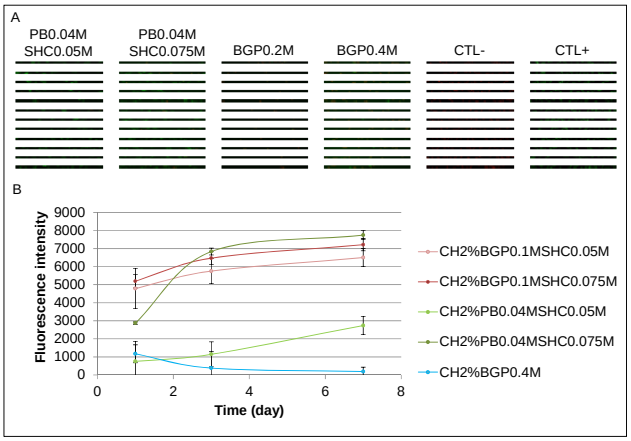
Strong injectable thermosensitive scaffolds for cell therapy

Caroline Ceccaldi, Elias Assaad, Lorene Bonneton, Sophie Lerouge
Laboratory of Endovascular Biomaterials (LBeV), École de technologie supérieure (ETS) / CHUM Research Center (CRCHUM), Montréal, QC, Canada.

Introduction: Cell therapy is an emerging sector which promises to deeply change the medical practices in next years. Unfortunately, benefits of such therapy are limited by poor cell retention and early cell death at the injury site after implantation. Finding new strategies to overcome this problematic is becoming a priority in cell therapy field [1]. In the last two decades, lots of efforts have been focused on the design of new biomaterials able to deliver and sustain cells viability after the graft. Chitosan thermogels are particularly interesting because of their biocompatibility, their ability to degrade *in vivo* and the possibility to be implanted by minimal invasive injections. These gels, created by mixing chitosan with β-glycerophosphate (BGP), are liquid at room temperature, and gelify at body temperature [2]. Unfortunately, the use of chitosan/BGP gel as a cell vehicle is far from ideal due to BGP cytotoxicity when used in concentration required to reach rapid gelation [3]. Moreover their mechanical properties are poor, which limits the resistance of those injectable scaffolds to *in vivo* stresses and their retention ability. The aim of our work was to develop new chitosan thermogels adapted for cell therapy with improved biocompatibility, gelation rate and mechanical resistance.

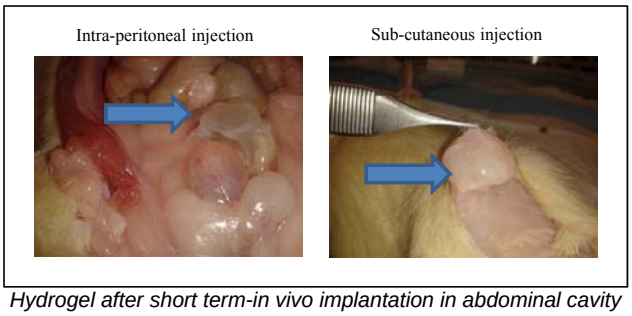
Methods: Chitosan (Mw 250kDa, DDA 94%) thermogels were prepared with novel combinations of gelling agents (BGP, phosphate buffer PB and sodium hydrogen carbonate SHC) with the goal to reduce total salts concentration. Their rheological properties and mechanical strength were evaluated by rheometry and unconfined compression tests (50% of stain) respectively. The pH and osmolility of the hydrogels were measured, and their morphology was observed by scanning electron microscopy. Finally, their ability to homogeneously encapsulate cells and sustain cell viability and proliferation was evaluated by entrapping L929 fibroblasts and measuring their metabolic activity with alamar blue staining during 14 days. All experiments used chitosan/BGP gels as control. For *in vivo* implantation, 2 ml of novel hydrogels were injected through a 1'13G needle in the intra-peritoneal cavity and sub-cutaneously. Rats were autopsied 15 minutes later.

Results: New gelling agents permitted to decrease osmolality of chitosan hydrogels and reach values adapted for cell culture. After mixing with chitosan, resulting hydrogels presented thermosensitive properties. They were liquid and stable at room temperature and able to quickly gelify by heating. Gelling temperature was in physiological range for each formulation. After gelation, mechanical properties of new hydrogels were drastically improved compared to chitosan/BGP. All new hydrogels had a secant Young moduli superior to 88 kPa at 50% of strain, compared with a maximum of only 6.3±1.3 kPa for chitosan/BGP which broke at 30% of strain. Hydrogels morphology presented a macroporosity with a pore size adapted for three-dimensional cell culture. After entrapment in new formulations, fibroblasts presented an excellent viability and were able to proliferate in 3 out of the 4 tested formulations, in contrast to those entrapped in chitosan/BGP formulations.



Cytocompatibility of chitosan based hydrogels. Panel A represents viability of L929 cells after 24 h in hydrogels (Live/Dead staining, live cells in green and dead cells in red). Panel B represents metabolic activity (alamar blue) of entrapped cells in each formulation during 7 days.

Assays on *in vivo* intra-peritoneal and subcutaneous administration by injection showed that new hydrogels are able to gelify in less than 15 minutes leading to a cohesive shave that can serve as a device for cell delivery.



Hydrogel after short term-in vivo implantation in abdominal cavity and under the skin.

Conclusion: This work presents new thermogels with high potential for cell therapy. New formulations presented here permitted to significantly improve their biocompatibility and largely enhanced their mechanical properties while keeping injectable characteristics required for minimal invasion administration way. This work reports the potential of smart natural hydrogels as a new platform for *in vivo* cell delivery.

References: 1. Roche, et al., Biomaterials, 2014. 35(25): p. 6850-8. **2.** Chenite, A., et al., Biomaterials, 2000. 21(21): p. 2155-61. **3.** Wang, L. and J.P. Stegemann, Biomaterials, 2010. 31(14): p. 3976-85.

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