ABSTRACT SUBMISSION

To be submitted before Feb. 22nd, 2019 online at https://biomatsante.sciencesconf.org

Biomat – Materials for Health Congress – June 3-7 2019 – La Grande Motte, France

Design of an injectable porous hydrogel as support for muscle regeneration

Louise Griveau[1], Emilie Christin[2], Romain Debret[3], Vincent Gache[2], Jérôme Sohier[1]

[1]MATEIS, CNRS UMR5510, Lyon, France; [2]INMG, CNRS UMRS5310, INSERM U1217, Lyon, France; [3]LBTI CNRS UMR5305, Lyon, France

Volumetric muscle loss (VML) resulting from traumatic incidents drastically decreases muscle regeneration capacity and lacks treatments [1]. Hydrogels are promising therapeutic candidates by providing muscle cells support through tailored mechanical properties and potential injection in the wound for a perfect fill. However, as the regeneration potential requires the implanted hydrogels to be porous to allow cell infiltration [2], it is crucial to obtain a porosity during or after injection. Recently, we have developed a biocompatible and biodegradable hydrogel composed of poly-lysine dendrimers (DGL) and an elastic derived peptide (EDP), cross-linked by polyethylene glycol (PEG-NHS), which requires a preformed porosity to be colonized by cells and is thus non injectable *per se* [3]. Therefore, the aims of this study were to investigate the potential of this hydrogel as a candidate for skeletal muscle repair, together with the creation of a spontaneous porosity through a novel effervescent approach, compatible with injection.

To determine the most suitable environment for myoblasts, dense hydrogels were prepared by mixing different ratio of DGL and PEG-NHS in PBS with or without EDP, and their mechanical properties measured by DMA. Mouse myoblasts (C2C12) were cultured on the hydrogels in proliferative conditions during 24h. Time-lapsed cell spreading and mobility were quantified by image analysis.

To obtain a porosity compatible with injection, DLG and PEG-NHS were mixed with a carboxylic acid and a carbonated base at different ratios in the presence of a non-ionic surfactant at various concentrations. Cytotoxity was studied by immersing the porous hydrogels in culture medium for 24h and applying the supernatant onto normal human dermal fibroblasts, followed by a live/dead viability cell assay after 24 and 48 hours.

As the hydrogels stiffness could be modulated from 10 to 150kPa (Fig. 1B), the C2C12 behavior in terms of cell spreading and mobility on the support was strongly influenced with highest values observed for 150kPa (Fig. 1A). Interestingly, the presence of the EDP induced similar behavior but for lower rigidities (70 kPa). These two hydrogels of interest were therefore used to develop an effervescent porous formulation.

Herein, a selection of acid/base ratios and specific mixing order to the hydrogel components allowed a strong effervescence, concomitant to the hydrogel cross-linking. As a striking result, an interconnected porosity was created, remnant of the effervescently-generated CO_2 bubbles (Fig. 2). Interestingly, the ratio of acid/base and the addition of different types of surfactants allow to modulate the resulting porosity. However, cell viability was negatively correlated with increasing concentrations of surfactants and acid/bases couples.

Through a versatile hydrogel, environments of interest for muscle cells in regard of stiffness and composition were highlighted, while a spontaneous, interconnected and tailorable porosity could be induced through an effervescent approach. Cells viability further comforts their potential for *in situ* injection. On-going experiments focus on the optimal porosity for muscle cells infiltration along with potential to enhance functional tissue repair.

The French ANR is acknowledged for financial support.

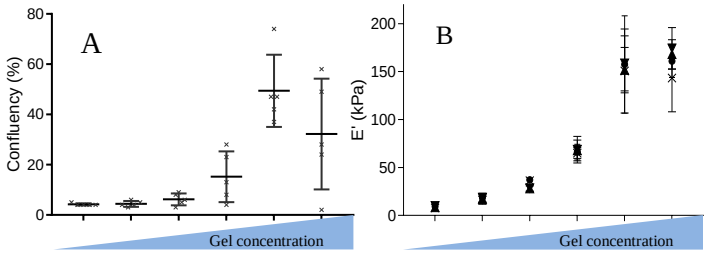

Figure 1: Cell confluence after 24hours (A) on hydrogels of increasing rigidity (B).

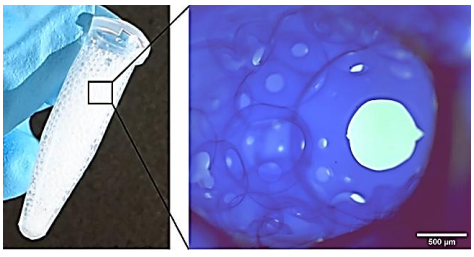

Figure 2: Effervescent hydrogel with interconnected porosity

[1] Corona *et al.* Cells Tiss Org 2016;202:180-188, doi : 10.1159/000443925

[2] Annabi *et al.* tissue eng: Part B 2010; 16 (4), doi: 10.1089/ten.TEB.2009.0639

[3] Debret *et al.* patent WO2017EP6151