Company/laboratory/public institution: Institut Galien Paris Saclay (Team 6) and Microfluidic laboratory of SOLEIL.

Address: Faculty of Pharmacy, Paris-Saclay University. 17 avenue de Sciences, Orsay 91400. L'Orme des Merisiers Départementale 128, 91190 Saint-Aubin

Supervision of trainee:

Name of tutor: Eloisa Berbel Manaia

Position: associate professor

E-mail: eloisa.berbel-manaia@universite-paris-saclay.fr

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Title of the project: The use of microfluidic technology to design alginate beads for mammalian cell encapsulation and cryopreservation.

General context: The rapidly growing demand for readily available living cells in cell-based research and medicine demands highly efficient cell storage. Currently, the only efficient way to achieve long-term storage of cells is cryopreservation. Great efforts have been made to overcome the main limitations of classical cryopreservation protocols, which originate in ice formation, growth, and recrystallisation. Intracellular and extracellular ice formation leads to cellular dehydration, which is the leading cause of mechanical and osmotic stress. The overall cell viability after cryopreservation strongly depends on cell tolerance to these sources of cryoinjuries [1,2].

The encapsulation of cells in a hydrogel matrix is one of the strategies investigated to improve the cryopreservation process. Although encapsulation was not initially intended for this purpose, several studies have reported how it may confer some protection against cryoinjuries. They suggest the protection against mechanical damage occurs by preventing contact between cells or between cells and ice crystals, confining the extracellular ice growth, and delaying the formation of intracellular ice [3]. Among the polymers commonly used for hydrogel-based cell encapsulation, alginate plays a prominent role. It displays easy gelling properties, allows capsule production at physiological conditions (room temperature, physiological pH, isotonic solutions), and has relevant availability, biocompatibility, and biodegradability [4].

Recently, we have set up and characterized a library of alginate-beads for mammalian cell encapsulation and cryoconservation, based on the extrusion of a cell suspension in alginate by various means (manual syringe, syringe pump, air-flow nozzle and vibration nozzle), resulting in a series of capsules spanning a wide range of sizes (0.2-2 mm) and morphologies [5]. These results have unveiled a strong correlation between bead size and post-thawing cell survival after a slow cooling cryopreservation process, with cell viabilities ranging from 7 to 70% depending on the capsule size, with the smallest capsules achieving the highest level of survival (Figure 1). This approach highlights the importance of controlled hydrogel matrix with a reduced deformability, identified as a potential mechanism for cell protection. It also shows the potential of cell encapsulation in even smaller alginate beads, and prompts the exploration of further physicochemical parameters of hydrogels to better understand the mechanisms behind cell protection.

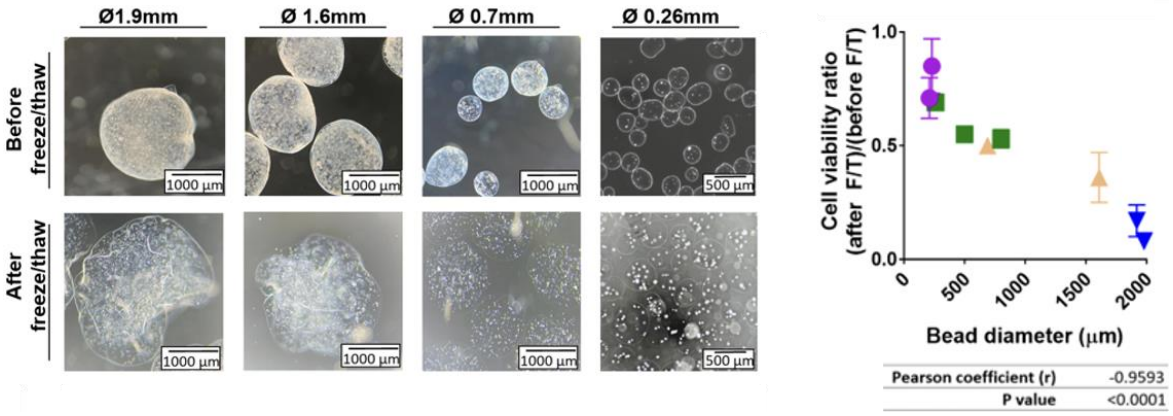

Figure 1. Left: Selected alginate hydrogel beads formulated with various extrusion methods, encapsulating Raw 264.7 cells, observed before cryopreservation and after freeze and thaw. Right: Correlation of cell survival and alginate bead diameter.

In this study, it was also observed the limitation of the techniques used to obtain smaller size of alginate beads. Microfluidic technique remains the most prominent to control the size and morphology of the cell loaded alginate beads. The self-fabrication of polydimethylsiloxane (PDMS) chips is a cost-effective alternative. It allows designing an "intelligent" chip to generate alginate beads able to encapsulate the cells with most appropriate experimental conditions that reduces cell stress.

Objective: Optimization of a microfluidics technology able to optimize the production of 50-80 µm alginate beads able to entrap functional eukaryotic cells. The beads will be characterized in terms of size and morphology. The effect on their freezing will be investigated in terms of cell metabolic activity, membrane integrity and cell functionality.

Scientific technical program:

- Optimization and production of alginate micro-beads by microfluidics;
- Morphology and structure during bead freezing and on frozen state;
- Cell survival and functionality.

The practical work will be conducted at both SOLEIL and IGPS laboratories.

We expect to continue this research in a PhD project.

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5. Ortiz-Silva et al., Int J Pharm 2023, DOI:10.1016/j.ijpharm.2023.123491.