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Ice templating as a new strategy for the encapsulation of living cells

Thesis supervisor (HDR) :

Name : Francisco Surname : Fernandes

Title : Maitre de Conferences (Associate Professor)

email : francisco.fernandes@sorbonne-universite.fr

Professional adress :
(site, dresse, bulding, office…) Campus Pierre et Marie Curie, 4 place Jussieu, 75005 Paris

Research Unit

Name : Laboratoire de Chimie de la Matière Condensée de Paris

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Ice templating as a new strategy for the encapsulation of living cells

PhD advisor: Francisco M. Fernandes (francisco.fernandes@sorbonne-universite.fr)
Laboratory: Laboratoire de Chimie de la Matière Condensée de Paris
Address: 4 place Jussieu, 75005 Paris

I - Introduction

Encapsulation of biological entities is one of the most interesting strategies to develop materials that collectively harness the functionality of biological entities.[1] One of the most exciting and challenging cases of biological material encapsulation is that of living cells. Creating an artificial extracellular matrix surrounding cells allows for the maintenance of the cellular metabolic activity while creating a structural envelope that provides protection from external physiological stress. This approach, largely described by previous works in our laboratory[2–5], has been mostly devoted to the fundamental aspects of cell encapsulation and its impact on their viability. The present research project aims at extending the scope of application of encapsulated viable cells within the encapsulation matrix to design tissue like materials.

Recently, we've introduced ice templating as a new materials' processing strategy that enables to control the structure of macroporous cell-containing materials.[5–7] The recruited PhD candidate will continue the development of these macroporous materials with different cell types by developing original biopolymer compositions to effectively protect the encapsulated biological material during the processing step and to promote the subsequent tissue formation. In parallel, the successful candidate will explore the conditions required for the long-term preservation of the metabolic activity of the encapsulated cells, opening a important pathway for cryobiology applications.

The major challenge of this project concerns the capacity to transform mixtures containing cells in suspension into bionanocomposite solids with controlled morphology[5]. The fine control of the construct's texture will permit maintaining the cells' metabolic activity and minimizing cell leaching. The proposed research plan required to attain these objectives is detailed as follows.

II - Research plan

1. Integration period

The first main task of the future PhD student consists on an integration period. This period consists on the development of the necessary skills to accomplish the PhD successfully. The key task consists on a thorough analysis of the bibliography on the subject of cell encapsulation and ice templating. This literature survey will be accompanied by experimental training on ice templating setup development, cell culture and data analysis. The necessary security training will be provided.

2. Ice templating

2.1. Biopolymer solutions and hydrogels

One of the key activities in the research program is to widen the variety of biopolymers processed via ice templating into hydrated, mechanically robust, macroporous gels.[8] Ice templating techniques have been initially devoted to the shaping of inorganic macroporous materials in the presence of a cohesive agent[9]. The technique was later adapted to build biopolymer-based and bionanocomposite materials with a wide range of applications[10–12]. We have recently demonstrated that the same technique could be applied to the encapsulation of viable cells[5,7]. In order to maximize the number of different cells lines that can be successfully encapsulated, a wider variety of biopolymers will be tested. The future PhD student will explore the experimental conditions necessary to shape a range of polysaccharides (alginate, starch, guar gum, arabic gum, carboxylethylcellulose, etc…) and proteins (collagen, gelatin, elastin, etc…) in order to determine the processing conditions that allow for the precise structuration of macroporous materials. Variables such as biopolymer concentration, freezing rate and experimental setup will be considered to fine-tune the frozen materials' morphology. In parallel to the freeze casting experiments, a thorough characterization of the biopolymers in solution will be carried out to establish

the appropriate relationships between the solutions' viscosity, water affinity and freeze point depression and their morphology upon freeze casting.

The final aim of this part us to build a wide base of knowledge for the structuration of biopolymers using ice-templating to maximize the morphological control over the macroporous foams using biopolymers of different nature.

2.2. Bi-component biopolymer foams

The development of multi-component materials for the containment of metabolically active biological species will provide supplementary resources to modulate the water partition between encapsulated cells and the surrounding ice crystals, and thus to maximize cells' viability. In mammals, the water activity in multiple environments is controlled via a judicious balance between polysaccharides and proteins, leading to controlled osmotic and oncotic pressures. Inspired by this approach the candidate will explore biopolymer couples that enable the formation of vitrous domain during freezing, a central requirement for the successful preservation of living cells.

The final aim of this part us to formulate the composition that will maximize cell viability during encapsulation and storage of cellularized materials.

2.3. Biomechanical testing

The generated materials in the previous steps will be fully characterized under tensile and compression testing using a dedicated home-built system. The mechanical characteristics of macroporous biomaterials are a key feature since they determine the tissues that can be ultimately targeted. The impact of the nature of the biopolymers and processing conditions will be established in order to rationalize the interactions between the controllable parameters and the obtained mechanical properties. In addition, the use of fluorescent microparticle encapsulated systems will allow to understand how the mechanical transduction occurs within the macroporous materials. Analyzing the macroporous foam morphology after the compression experiments by SEM and the integrity of fluorescent microparticles will provide invaluable tools to understand the stress transfer inside the macroporous materials.

The final aim of this part us to select the most efficient morphologies and composition to protect cellular material after encapsulation and to determine their target tissues.

3. Encapsulation of living mammalian cells

Following recent successful results (patent pending) the most interesting macroporous morphologies and processing conditions developed in the previous part of the research plan will be selected to develop materials containing living cells. An array of different primary cell types from different tissues (blood and connective) at different stages of their development will be explored. The viability results will be assessed using FACS, as well as functional/metabolic tests and the cellularized materials will be characterized by confocal microscopy, Scanning Electron Microscopy, Transmission Electron Microscopy, as well as other state of the art facilities within the research team environment.

At the end of the doctoral period, we wish to propose a new rational approach to the encapsulation and conservation of different cell lines. Such strategy will be supported by a fundamental understanding of the interdependence between the ice templating processing parameters, the local environment surrounding cells during freezing and cell viability.

III – Candidates

Candidates from disciplines such as physics, biology, chemistry, pharmacy and materials science are equally welcome provided they have prior experience in biomaterials. Successful candidates will be at ease in a multidisciplinary environment (the team is composed by chemists, biologists, physicists and materials scientist in close collaboration with several clinical teams). The candidate is expected to be fluent in English, highly creative, technology driven, and able to work as a part of a team. Previous cell culture experience is highly valued.

IV - References

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For further information direct all queries to Francisco M. Fernandes
francisco.fernandes@sorbonne-universite.fr.