Alginate-based Microcapsules for Cell Therapy: A Combination of Techniques Designed to Characterize their Stiffness and Surface Properties

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Abstract

Cell encapsulation in hydrogel-based microspheres has demonstrated successes in regenerative cell therapy. We developed a workflow based on Atomic Force Microscopy (AFM) and a microfluidic constriction system to characterize stiffness and surface properties of microcapsules.

Introduction

Cell encapsulation in porous hydrogel microspheres allows the diffusion of nutrients inside, and therapeutic products outside, while avoiding the immune system surveillance [1]. Effects of additives or storage conditions on microcapsule properties must be carefully assessed to ensure a correct *in vivo* performance of microcapsules.

Microcapsule modifications are of particular importance on the microcapsule surface, where interactions with the host cells may occur. Atomic Force Microscopy (AFM) is a powerful set of techniques for the characterization of surface properties of materials under liquid physiological environments [2].

In addition to surface properties, microcapsule global stiffness must be taken into account. Microcapsules are subjected to high shear stresses. The breaking of a microcapsule could lead to the release of its contents and, therefore, the triggering of a foreign body response. Experimental determination of stiffness using large deformation approaches is not trivial, but the pressure/deformation relationship can be used as an approximate measure of stiffness to compare different microcapsule compositions [3].

Methods

We used two complementary methods for the characterization of microcapsule properties. Force Spectroscopy, an AFM technique, was used to acquire simultaneous topography and stiffness maps of microcapsule surface [4]. These maps were then used for a stiffness-topography colocalization analysis to investigate the effects of microcapsule additives in polymer conformation (Fig. 1).

In parallel, we also developed a custom-designed constriction test to perform global stiffness measurements of the microcapsules. This assay was performed inside a custom-made methacrylate microfluidic device, similar to a previously described system for microcapsule aspiration assay [5]. The microdevice consists of a single 400 μm tubular channel, which presents a constriction that reduce its diameter to 200 μm. Via a pressure micropump, microcapsules suspended in a liquid solution were forced to pass through this narrow channel (Fig. 2). The pressure-deformation ratio determined in this assay can be analyzed as a mesure of microcapsule stiffness.

Results

We applied this methodology to study the effects of Graphene Oxide (GO) as an additive in alginate-based microcapsules and the changes in properties of microcapsules subjected to cryopreservation.

This workflow allowed us to determine the effects of GO on the conformation of the alginate network, which becomes more apparent with subsequent surface coatings. The methodology also demonstrated that long periods of cryopreservation did not translate into problematic alterations of microcapsules mechanical properties.

Conclusions

The use of these combined techniques to characterize microcapsule mechanical properties could significantly shorten the time and cost needed to validate each new microcapsule formulation prior to *in vivo* testing. Our workflow represents an interesting tool for rational microcapsule design, which could accelerate microencapsulation research and its clinical translation.

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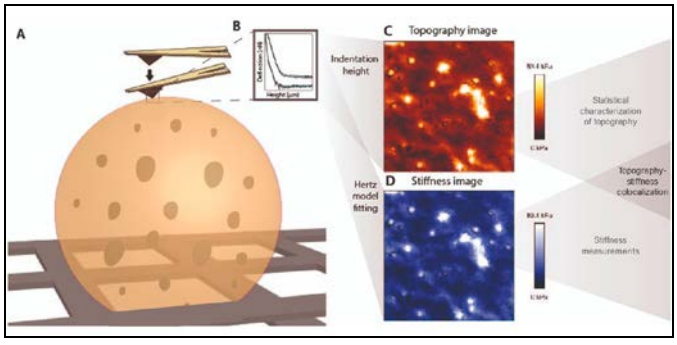

Fig. 1. Schematic representation of the Atomic Force Microscopy methodology. A) Immobilized microcapsules. B) Spectroscopy-based measurements via Atomic Force Microscopy (AFM). C-D) Force spectroscopy-based grids were acquired and processed to obtain both (C) a topography and (D) a stiffness map of the same microcapsule surface area.

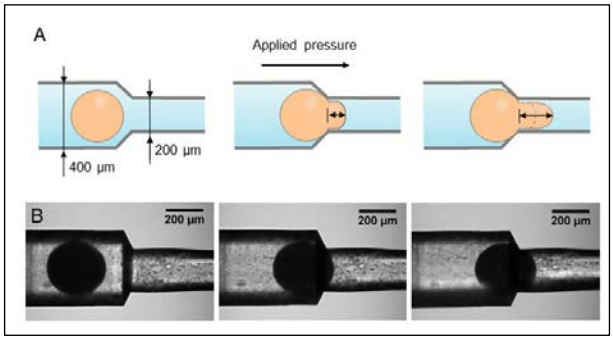

Fig. 2. Microcapsule constriction assay. A) Scheme of the constriction assay process B) Microscopy images (Leica) of a microcapsule constriction assay.