Liquified Capsules Encapsulating Microparticles to Provide Cell Adhesion Sites Enhance Cellular Functions

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Statement of Purpose: In bioencapsulation, the dense network of hydrogels compared to the liquified environment of capsules jeopardizes the diffusion of essential molecules for cell survival. However, most cells cannot grow in suspension and need to adhere to a solid structure. Encapsulated in the liquified environment of capsules and deprived of a physical support, anchorage-dependent cells are not able to adhere and proliferate. To address this dichotomy we propose the development of a completely new concept of liquified capsules featuring (i) an external shell by layer-by-layer assembly of poly(L-lysine) (PLL), alginate (ALG) and chitosan (CHT), and encapsulating (ii) poly(L-lactic acid) (PLLA) microparticles. We hypothesize that, while the liquified environment enhances the exchange of nutrients, oxygen, metabolites and waste products, microparticles dispersed in the liquified core of capsules provide the physical support required for cellular functions.

Methods: Alginate particles were obtained by ionotropic gelation and used as templates to obtain the LbL membrane or as a 3D cell culture control in biological assays. After sequential adsorption of PLL, ALG and CHT the core was liquified by ethylenediaminetetraacetic acid (EDTA). The assembly was monitored by Q-CMD and thickness measurements by applying the Voigt model were performed. The mechanical resistance of capsules was assessed by a rotational test. PLLA microparticles were obtained by solvent emulsion evaporation and surface functionalized with plasma treatment and collagen I. The biological outcome of capsules with (PLLA capsules) or without (ALG capsules) microparticles and encapsulating L929 cells was assessed up to 28days.

Results: Deformable capsules with an average diameter of 1.8±0.06mm encapsulating PLLA microparticles (figure 1a) were prepared. Microparticles had a sooth surface and diameter of 45±13.5µm (figure 1b). The contribution of PLL in the mechanical strengthening of the LbL membrane was evaluated by a rotational stress test. Capsules coated with the three macromolecular components showed an improved mechanical stability (figure 1c). Moreover, the incorporation of PLL led to an exponential regime growth and to a thicker film compared to the typical linear regime growth of the two-component CHT-ALG LbL system (data not shown).

The contribution of adding PLLA microparticles in cell behavior was assessed. Up to 7 days of culture, cells had a continuous increase in the metabolic activity for all formulations (figure 1d). Specifically, PLLA capsules at day 7 had the highest cell metabolic activity compared to control and ALG capsules. In concordance, PLLA capsules had the highest cell proliferation during culture time, which had a continuous increase up to 28 days of culture (figure 1e). Despite of all formulations presented a good amount of well spread living cells (green spots, figure 1f top row), PLLA capsules had less dead cells (red spots, figure 1f top row) compared to other formulations. DAPI-phalloidin assay (figure 1f bottom row) suggests that cells adhered to microparticles. Interestingly, cells started to form large aggregates recruiting microparticles to construct their own 3D cell culture system inside the capsule.

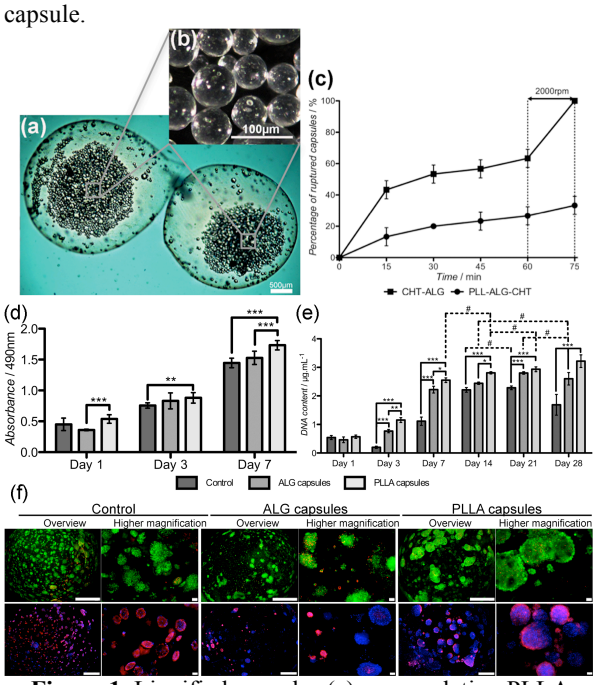

Figure 1. Liquified capsules (a) encapsulating PLLA microparticles (b). Mechanical resistance by rotational stress of capsules with (PLL-ALG-CHT) or without (CHT-ALG) PLL incorporated in the LbL shell (c). MTS viability (d), DNA quantification (e) and live-dead (top row) and DAPI-phalloidin (bottom row) (f) assays of control particles and ALG and PLLA capsules. Scale bar is 500µm (overview) and 40µm (higher magnification).

Conclusions: We were able to validate a new concept of hierarchical robust liquified capsules containing microparticles as solid adhesion sites to support the attachment and proliferation of cells. Capsules containing microparticles were able to offer the great advantage of providing a large surface area for cellular growth compared to traditional 2D cell culture systems or empty capsules. Moreover, the liquified core also allows capsules to adapt to structures with variable shapes. This ability is of huge interest for encapsulation systems since it offers capsules the ability of being implanted by minimal invasive methods in transplantation procedures. We believe the knowledge generated by this research will open new prospects in TE. The most promising feature of our strategy is the ability to tailor different properties in one structure and to create novel multifunctional materials in accordance with the target application field.