**Engineering of biohybrid systems by membrane emulsification**

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**Highlights**

* Membrane emulsification is a suitable technology to design biohybrid-engineered systems
* Enzyme-loaded PVA microspheres were produced by membrane emulsification
* Improved operational stability and optimal enzyme-substrate interaction was accessed
* Droplets with controlled structure and glucose-sensitive release properties were developed

**1. Introduction**

Membrane emulsification is a micro-manufacturing process that allow designing micro-nanostructured multi-material components with target particle size and sizing distribution and complex 3D structures []. The method offers many advantages in the micro-manufacturing of particles able to meet modern demand for a new generation of dispersed materials able to assist or replace important physiological functions by cell encapsulation and/or biomolecules delivery. In particular, membrane emulsification is emerging as a more promising formulation method to produce monodispersed particles with target size in mild operative conditions and with low energy consumption.

The present work will demonstrate the suitability of membrane emulsification process for the development of micro-nanostractured biohybrid systems for biomedical applications. The introduction of new strategies in membrane emulsification method to decrease the shear stress conditions maintaining high productivity will be illustrated to promote the use of membrane emulsification when biological systems, living organisms or derivatives thereof are used. Enzyme- loaded particles and glucose-responsive delivery systems will be described as cases study. The general idea is to functionalize droplet interface by using a biomolecule with a specific biological activity to design advanced biohybrid devices.

**2. Methods**

Microporous membranes have been used for liquid droplets production (emulsion). One phase (referred as dispersed phase), containing target biomolecules, is dispersed into the other (referred as continuous phase) by forming droplets at the membrane pore opening, where the two phases meet, as a result of the pressure gradient driving force and the interfacial tension existing between the dispersed phase and the membrane wetted by the continuous phase. The integration of membrane emulsification technology with appropriate secondary reactions (cross-linking) allowed extending to the production of micro-/nano-carriers with different architecture and physical structure. Lipase has been used as a model enzyme to demonstrate the potential of the use of membrane emulsification process in the production of enzyme-loaded PVA particles. A multiple emulsion, containing a bio-receptor (Con A) that specifically recognizes and interacts with an artificial ligand (Glucose), was manufactured by the membrane process and used as a model system. Structural properties of the produced particles (i.e. size, size distribution, surface properties) as well as the functional activity (i.e. catalytic activity for the enzyme loaded particles and glucose-sensitive release for the drug delivery systems) have been evaluated

**3. Results and discussion**

Uniform droplets (span = 0.4) with a size equal of three times the pore size of the membrane has been produced at the optimized fluid-dynamic conditions by membrane emulsification.

The method allow to obtain a specific activity comparable with the one of the free enzyme when has been used for enzyme-loaded particles production. This can be explained considering the structural properties of the enzyme. Lipase is an interfacial enzyme and exhibit the active form in the presence of a water/oil interface. When enzyme immobilization was carried out by entrapment, the carrier is formed in the presence of the enzyme. The lipase is oriented at the interface in the open form that is also the active form. ). On the other end, Con A is able to promote marker substance release as a function of glucose stimulus when membrane emulsification has been employed for biomolecule-responsive particles fabrication. The high affinity between Con A and glucose determined a preferential preferential interaction between them, causing the protein displacement from emulsion interface with phase separation and marker substance release.

**4. Conclusions**

An effective approach for cell and/or biomolecules encapsulatuion and delivery as well as the construction of carriers with tailored properties are crucial for biohybrid sistems engineering. Results demonstrated that the mild operative conditions applied extend the application of membrane emulsification toward the encapsulation of shear-sensitive compounds such as proteins (antibody or enzyme) and cells. It is expected that membrane emulsification can improve the use of particulate materials for many applications in bioscience and bioengineering by tuning the structural and functional properties of micro/nanostructured biohybrid particles.

**References**

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