DEVELOPMENT OF CELL-MEMBRANE CAMOUFLAGED NANO-EMULSIONS FOR AN INNOVATIVE LYSOSOME TARGETING APPROACH

Lagreca Elena – Advisor: Prof. Paolo Antonio Netti

Curriculum: Ingegneria dei Materiali e delle Strutture/IIT

Nanoscale drug delivery systems (NDDS) are well-known strategies used to overcome the problems associated with the direct administration of naked drug and to obtain a controlled release of drug content into a selective target site, for this reason represent a challenging area in pharmaceutical research, for the supply of therapeutics to the site of action, without affecting healthy tissue or organs. Among NDDS, the bioinspired nano-carrier, based on biomimetic coating of cell membrane, show interesting features, particularly the ability to avoid the immune response and to improve drug bioavailability.

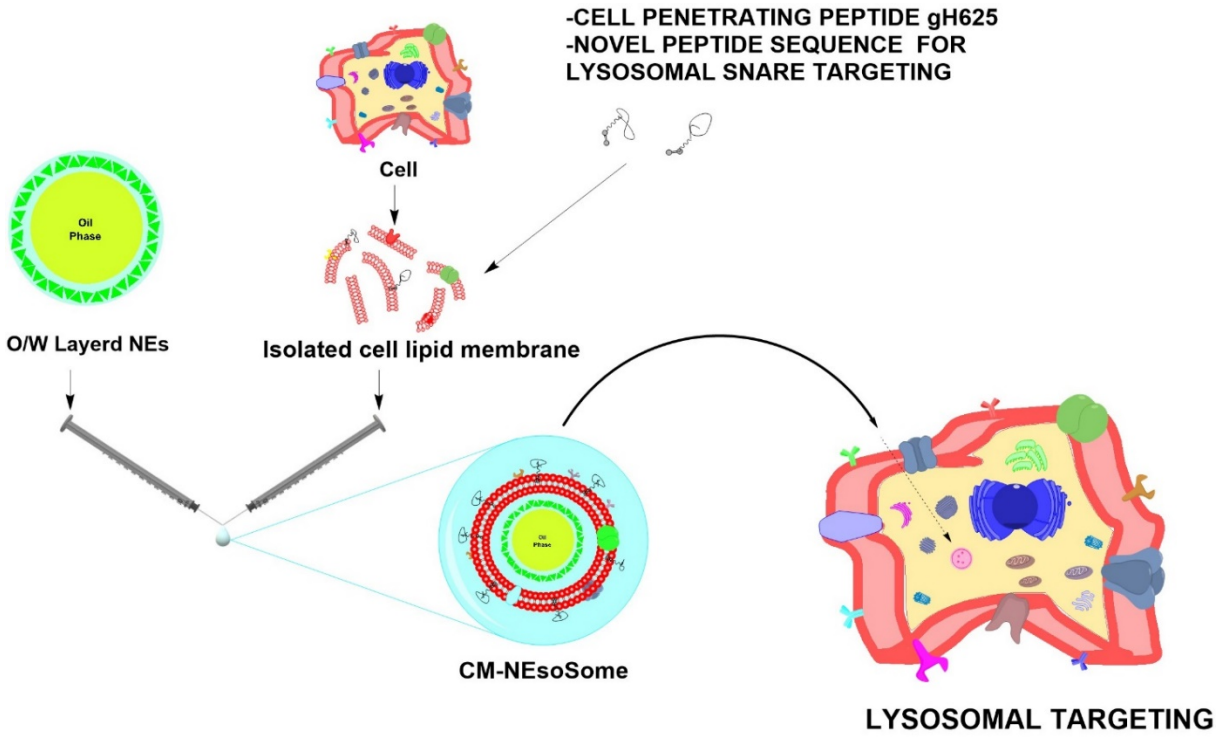
Different types of nano-carriers have been developed and were further functionalized with stealth polymers, targeting moieties to reach the lysosomal compartment. The growing attention to lysosome delivery is related to the central role of these organelles in different pathologies including cancer[1,2]. In particular, lysosomes are essential components of almost all types of cells involved in the degradation of cellular materials, which are responsible of many cellular processes such as autophagy, pathogen degradation, nutrient sensing and plasma membrane repair[3]. The actual treatment of these diseases includes gene therapies, organ/ cell transplantation, substrate reduction therapy, lysosome exocytosis[4,5] and small molecule therapies [2,6–9]. These therapies are related to high cost and invasive approach, which are associated with a low patient compliance. Moreover, the amount of active drug able to reach lysosomes are low because of degradative process related to lysosomal degradative pathway. For that reason, a valid route to overcome this limitation could be a direct (external) interaction with lysosomal membrane. To this aim, nano-carrier surface needs to be opportunely functionalized.

Among active targeting, ligand mimic peptide could also be used to reach specific targets. Peptides represent a valid targeting moiety because they provide several advantageous characteristics such as tissue accumulation capability, lack of immunogenicity, easy production with cost rather low, and relative flexibility in chemical conjugation processes10,11. Recently, cell-penetrating peptides (CPPs), short cationic and/or amphipathic peptides, have been used to facilitate drug delivery into the cell and are promising in the treatment and in the diagnosis of several types of pathologies12.

Herein, we report a novel strategy to engineer oil in water nano-emulsions (O/W NEs) in order to obtain a biomimetic nano-carrier based on peptide-functionalized cell membrane coating. The aim is to design a new biomimetic nano-carrier which is based on cell membrane - O/W NEs able to carry several internal and external cargos (Some) CM-NEsoSome. CM-NEsoSome consist of an O/W NEs coated with peptides-incorporated cell membrane coating. Peptides will be integrated into the surface of CM-NesoSome by lipid -Polyethylene glycol (PEG) derivate and/ or by plasmid technology. This activity will be developed in collaboration with Prof. Marcelle Machluf of Technion (Israel Institute of Technology, Haifa, Israel).

Preliminary cell membrane coatings were obtained through a liquid–liquid interface method with the aim to obtain a fine deposition on O/W NEs droplets. This nano-carrier was based on electrostatic attraction between charged cell membrane and O/W NEs. Overall, the main objective is to deliver therapeutics to lysosomes through an active targeting mediated by a selection of peptides including gH625. gH625 is a membranotropic peptide, well known for its ability to penetrate cell membranes and transport a large variety of cargo molecules/materials inside the cells. The main limitation of gH625 is due to their failure to deliver functionalized NPs to a specific cellular site and recognize it, being able only to cross their membrane13. To obtain an active targeting of CM-NEsoSOme it will also be incorporated a different peptide able to realize a specific targeting to lysosomes. With this purpose, a new peptide sequence will be discovered and developed for lysosomal SNARE active targeting by combinatorial peptide library or phage display.

The final nano-carrier will be finally validated by internalization and co-localization study followed by in vitro and in vivo test of CM-NEsoSome loaded with a select lipophilic drug. Lysosomal targeting will be evaluated in collaboration with Carmine Settembre's group of the Telethon Institute of Genetics and Medicine (TIGEM, Pozzuoli, NA, ITALIA).



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