Liposomes as novel immunostimulant delivery systems in aquaculture

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In the last three decades, world food fish production from aquaculture has expanded by almost 12 times. Nowadays, aquaculture contributes nearly half (47.3%) of the world's food fish consumption. In this sector, most economic losses related to fish diseases are mainly caused by mortality and reduce growth. It is estimated that approximately 50% of all fish are lost due to disease before they reach the market [1]. Therefore, the development of sustainable aquaculture, a strategic sector to feed the ever-increasing human population, relies on disease prevention through implementation of preventive immunostimulation and effective vaccination strategies. With the advent of liposomal vaccines, one can begin to conceive new non-invasive, non-stressful and easy-to-manage methods for administering immunostimulants and vaccines to a large number of cultured fish at any time of its life cycle [2]. Liposomes are hollow spherical, safe and well-tolerated assemblies formed by lipid bilayers that can be tailored (via composition, size and charge) to efficiently entrap a wide variety of immunostimulants and vaccines. This encapsulation provides the obvious potential advantages of increasing their stability and protection, thus enhancing their immune response and disease protection, and opening up the possibility to design more efficient immunostimulant-vaccine cocktails.

Herein we present a unique delivery system based on the encapsulation of a cocktail of immunostimulants in nanoliposomes with the ability to protect them against a pathogenic challenge and to stimulate, for the first time, two potent innate immunity pathways virtually present in all fish species, which represents a breakthrough in fish health. The immunostimulant cocktail selected for this study are the bacterial lipopolysaccharide (LPS) and the synthetic analogue of dsRNA virus, poly(I:C), both virtually present in all fish species.

Liposomes encapsulating both immunostimulants are prepared by thin film hydratation method using the DLPC:Cholesterol:Cholesteryl:PEG lipid mixture [3]. Through this methodology, highly homogeneous small unilamellar vesicles with a mean particle size of 125.8 nm can be prepared (see Figure 1). Liposomes that show a positive surface charge (+1.37 mV) seems to be ideal for encapsulating both LPS and poly (I:C). Indeed, the attractive interaction between the negative charge of both immunostimulants and the positive surface charge of liposomes results in the near-perfect conditions to achieve the highest encapsulation efficiencies of 22.3 % for LPS and 99.6% for poly (I:C). Confocal microscope images of fluorescent-labeled liposomes demonstrate that both LPS and poly (I:C) are incorporated into their cationic lipid bilayer.

We show that this liposomal carrier presents a low toxicity *in vitro* using three different cellular models and *in vivo* using zebrafish embryos and larvae at the therapeutic and immunomodulatory doses. In addition, liposomal uptake is confirmed by incubating fluorescent-labelled liposomes with zebrafish hepatocytes and trout macrophage plasma membrane, observing high liposome internalization mainly through caveolae-mediated endocytosis. Importantly, we anticipate that this liposomal cocktail elicits a specific pro-inflammatory and anti-viral response in both zebrafish hepatocyte cells and trout macrophages, after studying the changes in the expression of different immune related genes, which represents a completely new approach in fish health [4].

In conclusion, the induction of specific immune responses with liposomal immunostimulant formulations is found to be a very promising strategy to improve disease control in fish farms. Further work is ongoing to evaluate the *in vivo* biodistribution of this liposomal cocktail in aquacultured fishes and their affinity to specific fish organs. Different administration routes will be evaluated - injection, oral and immersion- to finally establish a rational immunisation protocols. Liposome stability and therapeutic efficacy studies with bacterial and viral challenge are also in progress.

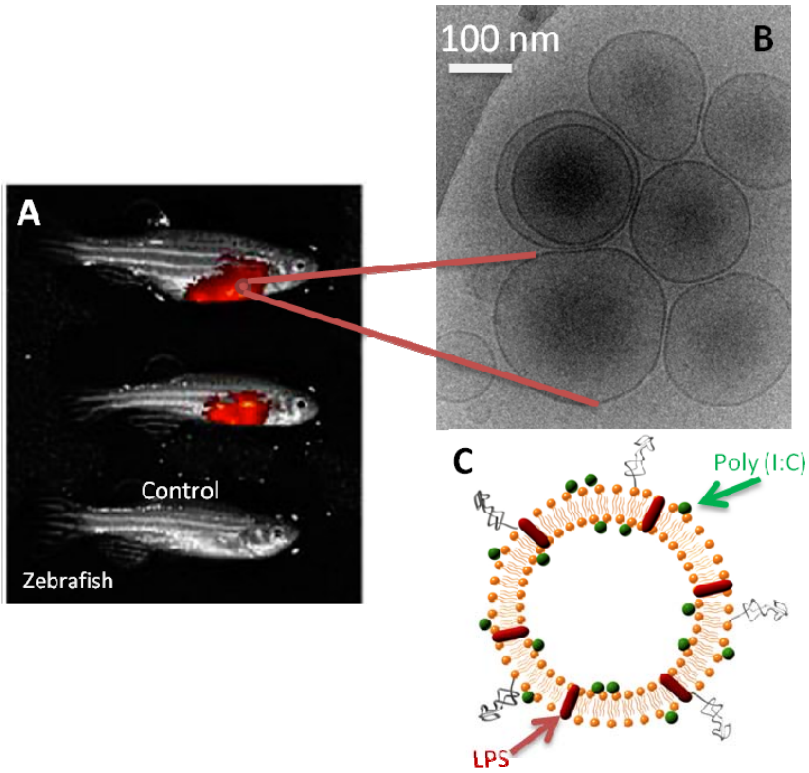

Figure 1. a) Biodistribution of fluorescent liposomes after their intravenous injection in zebrafish. b) Cryo-TEM image of DLPC:Cholesterol:Cholesteryl:PEG liposomes containing both immunostimulants. C) Schematic representation of a liposome encapsulating both LPS (red) and poly (I:C) (green).

REFERENCES

[1] O. Evensen, **Options Mediterraneennes** (2009) 1.
[2] P. Nordly, H.B. Madsen, H.M. Nielsen, C. Foged, **Expert Opin. Drug Deliv.** 6 (2009) 657.
[3] A.D. Bangham, M.M. Standish, J.C. Watkins, **J. Mol. Biol.** 13 (1965) 238.
[4] L.-Y. Zhu, L. Nie, G. Zhu, L.-X. Xiang, J.-Z. Shao, **Dev Comp Immunol.** (2012) 1.