Microfluidic and enzyme replacement therapy: plga nps towards the development of new versatile therapeutic solutions

Sabrina CUOGHI - *University of Modena and Reggio Emilia*

Enzyme replacement therapy (ERT) plays a crucial role in genetic diseases like lysosomal storage disorders (LSD), characterized by lysosomal enzymes deficiencies. Lamzede , which contains the recombinant enzyme Velmanase alfa, is the first ERT approved treatment of the non-central nervous system manifestation of the LSD alpha-mannosidosis[1]. Unfortunately, Velmanase alfa is unable to cross the BBB and manage CNS symptoms, and the administration of free-form enzymes may result in strong immunogenic reactions and faces challenges related to enzyme sensitivity to biological milieu. In this landscape, the development of a tunable nanoplatform for brain delivery of therapeutic proteins stands as a milestone for overcoming ERT drawbacks and achieving new therapeutic solutions, not only targeting LSDs.

In this regard, a formulative protocol for the encapsulation of the enzyme Velmanase alfa in FDA-approved polymer poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) has been optimized. Given the high cost of the freeze-ried marketed product, the enzyme β-glucosidase has been used as a model during the optimization steps, for which an efficient encapsulation protocol using the double emulsion and solvent evaporation method (DE) was previously optimized and improved with the use of stabilizers such as Tween and Bovine Serum Albumin (BSA)[2,3]. However, the DE technique is a bench top method displaying shortcomings in terms of scalability and reproducibility, and involves the use of harsh process conditions, which are not suitable for sensitive molecules. All these drawbacks can be overcome with the microfluidic (MF) technology allowing a versatile and reproducible NPs formulation while minimizing solvent and reagent waste, which is fundamental when using high-cost products. Therefore, a MF formulative protocol has been optimized, even with the aid of biocompatible stabilizers, firstly for the encapsulation of β-glucosidase and then for the one of Velmanase alfa. In this view, several parameters have been evaluated, including polymer concentration, enzyme to polymer ratio, type and concentration of surfactants and stabilizers. Due to the similarity of the results observed (∽200 nm, PDI <0.3, ∽ -20 mV), it can be established that a tunable and scalable nanoplatform has been obtained and it is noteworthy that preliminary enzymatic activity assays asserted the maintenance of the enzymatic activity. Moreover, sophisticated *in vitro* systems were developed to evaluate the biological characteristics of the nanomedicines.

All these results taken together set the stage for the transition, in the landscape of enzyme encapsulation, from a bench top method to a reproducible and scalable technique, aiming at overcoming drawbacks of approved ERT, achieving new effective therapies for several LSDs, and closing the gap between academy and industry. Moreover, the tunability of the developed nanoplatform hold promises to target a wide range of diseases, paving the way to new versatile therapeutic solutions.