**Optimization of Gene Electro-Transfer protocols of DNA-based drug delivery to improve immunotherapeutic anti-cancer nanomedicine approaches**

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Significant advances have been made in the field of cancer immunotherapy by orchestrating the body’s immune system to eradicate cancer cells. However, the systemic delivery of immunomodulatory compounds present several concerns in terms of safety and efficacy. In this context, nanomedicine may be advantageous for many aspects, such as targeted delivery to cancer cells and reduced adverse events, which are relevant aspects in the delivery of cancer vaccines and immunomodulatory agents.

Over the last 15 years or so, significant efforts have been made to develop nanomedicine-based approaches, integrating nanotechnology, biomolecular engineering, life sciences and medicine. In parallel, many potential applications of nanomedicine have been, or are being, explored, including the development of different delivery strategies and cargos for targeted *in vivo* drug/gene delivery for more efficient therapy. These cargos range from small molecules to proteins and macromolecules, including dyes, impermeable drugs, molecular beacons, proteins, nanoparticles, siRNA and DNA plasmid [1].

Many strategies have been developed to improve immunotherapeutic anti-cancer nanomedicine approaches, such as the use of electroporation to deliver different cargo molecules and materials of interest to the intracellular space.

Electroporation exploits the application of electric pulses on the target organ to allow the transfer of molecules of interest within recipient cells. Application of electric fields leads to transient pore formation on the cell membrane, thus forcing the molecule of interest to enter target cells [2].

On the strength of permeabilization triggered by electric field, electroporation has been used as a crucial component both in nanoparticles delivery and in Gene Electro-Transfer (GET) protocols based on plasmid DNA [3-5].

GET is a method that allows the targeted transfer of plasmid DNA into cells in a tissue and promotes efficient gene expression. It results from the direct application of electric field pulses, avoiding the use of viral vectors. The application of controlled electric pulses induces a transient permeabilisation of the plasma membrane and the uptake of the negatively charged plasmid DNA inside the cell.

We demonstrated that electroporation induces a substantial increase in antigen expression and availability to resident APCs in the muscle cells, which become a long-term antigen reservoir. On the other hand, the tissue damage induced by electroporation, triggers a site-specific inflammatory reaction which enhances the antigen presentation mechanisms through activation of cells and factors belonging to the innate compartment of the immune system [6].

In addition, we showed that hyaluronidase (Hyal) is a good enhancer of intramuscular GET efficiency in anti-cancer preclinical protocols, with increased transfected cells and higher expression of the encoded genes. Pretreatment of muscle before GET with hyaluronidase, which breaks down components of the extracellular matrix of the muscular tissue, permits indeed a wider distribution of inflammatory cells throughout the entire tissue section [7].

Hyaluronidase is quite commonly used into the clinical practice, nevertheless, the use of animal-derived Hyals results limited respect to their potentialities, since such preparations could be affected by low purity, variable potency and uncertain safety with possible side effects.

Here we present an intramuscular GET-based protocol in a murine model, using a new hyaluronidase purified and produced by an innovative methodology [8]. We investigated a new recombinant Hyal, the rHyal-sk, to assess *in vivo* safety and activity of this treatment at cellular and biochemical levels. We evaluated the cellular events and the inflammation chemical mediators involved at different time points after rHyal-sk administration plus GET. We demonstrated the *in vivo* safety and efficacy of rHyal-sk when injected once intramuscularly in association with GET, with no toxicity, good plasmid in-take ability, useful inflammatory response activation, and low immunogenicity [9]. Following these findings, we would recommend the use of the new rHyal-sk for the delivery of DNA-based vaccines and immunotherapy, as well as into clinical practice, and to improve nanotherapeutics approaches.

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