MINI-REVIEW

F2 Gel Matrix - a Novel Delivery System for Immune and Gene Vaccinations

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Abstract

Exploiting the immune system to abolish cancer growth via vaccination is a promising strategy but that is limited by many clinical issues. For DNA vaccines, viral vectors as a delivery system mediate a strong immune response due to their protein structure, which could afflect the cellular uptake of the genetic vector or even induce cytotoxic immune responses against transfected cells. Recently, synthetic DNA delivery systems have been developed and recommended as much easier and simple approaches for DNA delivery compared with viral vectors. These are based on the attraction of the positively charged cationic transfection reagents to negatively charged DNA molecules, which augments the cellular DNA uptake. In fact, there are three major cellular barriers which hinder successful DNA delivery systems: low uptake across the plasma membrane; inadequate release of DNA molecules with limited stability; and lack of nuclear targeting. Recently, a polysaccharide polymer produced by microalgae has been synthesized in a form of polymeric fiber material poly-N-acetyl glucosamine (p-GlcNAc). Due its unique properties, the F2 gel matrix was suggested as an effective delivery system for immune and gene vaccinations.

Keywords: F2 gel matrix - isolation and purification of poly-N-acetyl glucosamine (p-GlcNAc) - anti-tumor DNA

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Major DNA Delivery Systems and Their Problems

A. Viral Delivery Systems

Nonpathogenic attenuated viruses were widely used as delivery systems for DNA molecules for animal studies, especially plasmids (for reviews, see ref 1. In which, the transgene of interest is inserted in the viral genome, and after injection, the virus uses its innate mechanism of infection to enter the cell and release the expression cassette. The gene then enters the nucleus and integrated with the host gene pool, and is eventually expressed. One significant advantage of viral DNA vectors is their extremely high transfection efficiency in a variety of human tissues 2. However, there are several concerns over the use of viruses to deliver DNA therapeutics in humans. The chief concern is the toxicity of the viruses and the potential for generating a strong immune response owing to their proteinaceous capsid 1.

B. Mechanical and electrical approaches

Mechanical and electrical strategies were initially proposed for the direct injection of naked DNA into cells through many ways.

1): Microinjection: although it is highly efficient, it is highly time consuming-technique, since; it can be achieved only one cell at a time 3.

2): Biolistic particle delivery: can introduce DNA into many cells simultaneously. However, since direct exposure of target tissues is required, particle bombardment is restricted to local expression in the dermis, muscle, or mucosal tissue, where limited local expression of delivered DNA (in cells of the epidermis or muscle) is adequate to achieve immune responses 4.

3): Electroporation: in which a high-voltage electrical current is applied on the cells or tissue to facilitate DNA transfer 5. The drawback of this technique is that it cause high cell mortality and therefore is not suitable for clinical use. Though significant transfection efficiencies have been achieved using mechanical and electrical techniques, they are extremely difficult to standardize in a clinical setting and are considered laborious, impractical, and invasive 6.

C. Chemical delivery systems

The general principle for the chemical delivery systems is the formation of a chemical complex (for reviews, see ref 7). For instance, forming a complex by the attraction of positively charged chemicals e.g. polymers and negatively charged DNA molecules. Or via compaction of the negatively charged polycationic nanomeric particles by the negatively charged nucleic acids. These complexes enter cells usually by endocytosis, and actually are stable enough to retain their bound nucleic acids protected from degradation. The advantages of this approaches, is long-term expression but still low efficiency for the nuclear targeting and toxicity are the

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major restrictions of such methods.

Isolation and Purification of Fully Acetylated p-GlcNAc from Microalgae

The initial source for the production of the p-GlcNAc is the cell wall of the diatoms. There are several ways have been detailed for separating p-GlcNAc fibers from diatom cell bodies 8; among them, the chemical and/or biological method may the best one. Briefly, diatoms could be treated with N-acetylglucosaminyl-P-transferase enzyme inhibitors in order to inhibit p-GlcNAc fiber synthesis, thus releasing the fibers already present. Alternately, diatom cell bodies could be treated with a chemical, which leads to a release of the p-GlcNAc fibers without affecting their structure, e.g. hydrofluoric acid (at a final concentration 0.07 M). For purification and to remove proteins-and other unwanted matters, the suspension of fibers and cell remnants could be washed with 20% sodium dodecyl sulfate (SDS), the final concentration of the liquid 0.5% SDS by volume. Then the solution could neutralize by adding Tris solution (2.9M).

Unique Characteristics of Poly-N-acetyl Glucosamine Fibers

The polymer poly-N-acetylglucosamine (pGlcNAc) fibers is initially utilizing in clinical applications as a topical agent for hemostasis at wound sites. Since, the pGlcNAc polymeric fibers provide hemostasis through redundant mechanisms including platelet activation for fibrin network formation and induction of the red blood cell agglutination and vasoconstriction 9. The polymer also has been tested following United States Food and Drug Administration biocompatibility guidance 10. The primary sugar present in p-GlcNAc is N-acetyl glucosamine, which is fully acetylated into pGlcNAc fibers with a molecular weight of 2.0×10^6 Da, and are fully safe biocompatible molecules 11. pGlcNAc fibers are able per se to elicit a local transitory inflammation in response to the release of cytokine in vivo 12. Some prospective studies indicate the ability of the F2 gel matrix to provide sustained in vitro and in vivo release of cytokines even for those highly toxic ones like IL-12 13. Furthermore, F2 gel/peptide tumor vaccination can prime the immune system in an antigen specific manner 14. Therefore, unlike other delivery methods, F2 gel could add the possibility of formulating each of antigen and adjuvant in a single preparation, which after injection will result in the concomitant release of both components. As shown above, the successful DNA delivery system should have distinct characters including low toxicity, highly uptake across the plasma membrane, offers protection for and provide adequate release of DNA molecules with long-term expression, and lastly but not the least, offers high efficacy for nuclear targeting 7.

F2 gel Matrix Preparation

Unfortunately, in all published studies, none of authors provide detailed information about the method(s) of F2 gel matrix preparation used in their studies. However, Vournakis and Demcheva have invesigated and provided several ways for its prepration 15. The best methods I recommend for polymer/DNA composition is that p-GlcNAc deacetylated by sodium acetate-acetic acid buffer, 0.1 mg/ml polymer in 25 mM sodium acetate-acetic acid buffer, pH=5.7. In order to create a gel called F2 gel matrix, the diluted poly-N-acetyl glucosamine fibers in sodium acetate-acetic acid buffer should be heated in a hot water bath 55°C for 10 min. The final p-GlcNAc concentration after dilution is 0.02% gel. The ration of polymer and DNA should optimize using several sets for the best results. Briefly, 100 micro liters of diluted p-GlcNAc gel to DNA in 100 micro liters of 50 mM sodium sulfate solution, while sample is vortex for 20 seconds. The formulated polymer/DNA composition could keep at room temperature until use for injection into a subject, but not longer than 2 hours.

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