DNA DELIVERY TO TUMOUR CELLS BY NANOPARTICULATE PLGA SYSTEMS

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Nanotechnology, although not a new concept, has gained significant momentum in recent years. This stems partly from the fact that nanosystems have significantly different biological properties compared to large-size systems. In gene therapy, we face the problems of inefficacy or nonspecific effects; hence, nanosystems are being developed for targeted gene therapy [1]. On the other hand, naked DNA is degraded by deoxyribonuclease I (DNAse I) present in serum. This limitation has led to the investigation of strategies, such as nanoencapsulation, to increase the efficacy of DNA delivery. PLGA is a biodegradable and biocompatible polymer and has been used to develop several drug and gene delivery nanosystems [2]. The choice of DOTAP is based on its previous use also for gene therapy.

In order to improve the efficiency of transfection mediated by the particles, in this study one approach has been to introduce DNA into cells by attaching it to a ligand and exploiting the natural specificity of receptor-mediated endocytosis. One useful cell-binding ligand is asialofetuin (AF), which is of particular interest for liver-directed internalization of therapeutic constructs because his asialoglycoprotein receptor (AsGPr) is expressed selectively on hepatocytes [3]. In this way, the aim of this study was the nanoencapsulation, characterization and “in vitro” evaluation of different cationic PLGA-DOTAP nanoparticles.

The particles were prepared with PLGA RG502H by using a modified solvent evaporation process. We prepared three different formulations. In the Particles 1 the DNA was encapsulated in the cationic PLGA-DOTAP nanoparticles (Strategy 1) and in the Particles 2 and 3 the DNA was adsorbed on the surface of the cationic PLGA-DOTAP particles (Strategies 2 and 3) . The size of the particles ranged between 200-1200 nm depending on the strategy and had a positive zeta potential in all the cases. The plasmid DNA extracted from PLGA-DOTAP particles was analyzed for structural integrity and protection from DNAses by agarose gel electrophoresis. The results suggest protective effects of PLGA on shearing of plasmid DNA during the preparation method and that the PLGA particles protected plasmid DNA against the endonucleases activity, both encapsulated and adsorbed. In the electrophoresis gel, we observed that the naked DNA is completely digested within 5 minutes of incubation with the equal mass of DNAse I, while the DNA encapsulated and adsorbed remained intact in the presence of DNAse I for up to 30 minutes of incubation.

The transfection studies were performed in HepG2 cells (hepatocellular carcinoma) and gene expression efficiency of different nanoparticles was studied in the absence and presence of serum. As we are interested in using these vectors for in vivo applications, we are focusing our study in the particles with the best performance in the presence of serum. Our results indicate that with the polymer 502H the higher gene expression was obtained with the strategy 2 in the presence of 60% serum (Figure 1). Asialofetuin was added as ligand to the particles, which led to an increase in the level of transfection. Cell viability was quantified by a modified Alamar blue assay, and the results showed a viability higher than 80% in all transfected wells.

References:

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Figures:

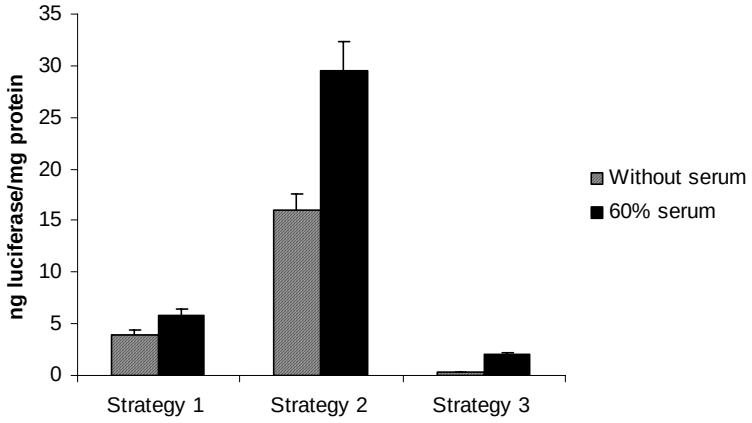

Figure 1. Transfection activity in HepG2 cells