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Cationic star homo- and co-polymers for gene delivery

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Several groups of star polymers were synthesized and evaluated as gene delivery vehicles. All polymers were synthesized by group transfer polymerisation and were based on 2-(dimethylamino)ethyl methacrylate (DMAEMA). In particular, one group of DMAEMA star homo-polymers of different molecular weights and three groups of star copolymers of different architectures were prepared. The three groups of copolymers were based on the DMAEMA monomer and a second hydrophilic monomer comprising either poly(ethylene glycol) methacrylate, methacrylic acid or glycerol methacrylate. All series of star polymers were characterized by gel permeation chromatography and nuclear magnetic resonance spectroscopy. Aqueous solutions of the star polymers were studied by turbidimetry, hydrogen ion titration, and dynamic light scattering. All but the most recent star polymers were evaluated for their ability to transfect cells. The transfection efficiency was affected by the molecular weight of the star polymer, the star architecture and the nature of the second co-monomer.

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Gene electrotransfer: comparison between 2D cultured cells and multicellular tumor spheroid model

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Electroporation is a physical method to deliver molecules into cells and tissues. Clinical applications have been successfully developed for antitumoral drug delivery and clinical trials for gene electrotransfer are underway [1]. However, little is known about the mechanisms involved in these processes. The main difficulties stem from the lack of cell models which reliably replicate the complex *in vivo* environment. To increase our understanding of the DNA electrotransfer mechanisms, we recently exploited multicellular tumor spheroids (MCTS) as an *ex vivo* model of tumor [2]. This 3-dimensional model can replicate the *in vivo* in complex environment and therefore enables us to develop new strategies for studying mechanisms of molecules delivery by electric field pulses. In the present study, we observed cells response to electric field pulses for propidium iodide and plasmid DNA delivery. HCT116 cells were pulsed either in suspension (2D culture) or in MCTS (3D culture) and 10 pulses lasting 5 ms were applied at different voltages. Confocal and biphotonic microscopy allowed us to visualize the repartition of permeabilized and transfected cells in MCTS subjected to electric pulses. Flow cytometery analysis was used to obtain quantitative analysis both on cells pulsed in suspension or on cells pulsed in MCTS (in that case, cells were dissociated by an enzymatic treatment). Results show differences in electric field sensitivity between cell in suspension and MCTS. Permeabilization process (revealed by propidium iodide uptake) is affected only the first cell layers of MCTS. A maximum of 30% of cells being permeabilized was obtained at 400 V cm^{-1}. Increasing the field strength above that value did not further increase the number of permeabilized cell. On the contrary, in the case of cells pulsed in suspension, up to 90% of cells were shown to be permeabilized at 700 V cm^{-1}. DNA delivery process (revealed by GFP expression) showed that less than 5% cells were transfected when present in the spheroid model while, under the same conditions, about 25% of them were transfected when pulsed in suspension. These results point out the difficulty DNA has to cross the multicellular barrier and give an explanation for the different of responses of cells *in vitro* and *in vivo* [3]. Taken together, these results are in agreement with the ones obtained in tumors and indicate that the spheroid model is more relevant to an *in vivo* situation than cells cultured as monolayers. They validate the spheroid model as a way to study electromediated gene delivery processes.

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Combination of a triblock copolymer L64 with electrotransfer increases gene delivery *in vitro*

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Gene transfer into muscle cells is a key issue in biomedical research. Indeed, it is important for the development of new therapy for many genetic disorders affecting this tissue and for the use of muscle tissue as a secretion platform of therapeutic proteins. Electrotransfer is a promising method to achieve gene expression in muscles. However, this method can lead to some tissue damage especially on pathologic muscles. Therefore there is a need for the development of new and less deleterious methods. Triblock copolymers as pluronic L64 are starting to be used to improve gene transfer mediated by several agents into muscle tissue.

Their mechanism of action is still under investigation. The combination of electrotransfer and triblock copolymers, in allowing softening electric field conditions leading to efficient DNA transfection, could potentially represent a milder and more secure transfection method. In the present study, we address the possible synergy that could be obtained by combining the copolymer triblock L64 and electroporation. The synthesis of fluorescent probes L64-rhodamine and DNA-rhodamine is presented here. These probes allowed us to gain some insights into the mechanism of transfection of the combined physical and chemical methods. We have found that a pretreatment of cells with L64 could improve the transfection efficiency. Neither interaction of DNA with the cell membrane, nor L64 membrane interaction seemed to be related to the gain obtained in these transfecting conditions.

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A receptor-mediated gene delivery system using CXCR4 ligand-conjugated cross-linking peptides

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Application of DNA as therapeutics requires efficient cell and tissue-specific targeting which can be achieved by modification of vehicles with a ligand for certain receptor. CXCR4 is a receptor of chemokine SDF-1 and is expressed on some types of cancer and stem cells. Cystein-flanked peptides which are capable of forming small and stable DNA condensates because of cross-linking are considered to be a perspective group of non-viral vehicles. The aim of this project is to characterize a CXCR4 ligand-conjugated cross-linking peptides as a receptor-mediated gene delivery system. We studied four types of DNA/peptide complexes with different ratio between cystein-flanked arginine-rich peptide modified with N-terminal sequence of the chemokine SDF-1 (residues 1–17) and peptide (CHRRRRRRHC) – 100%, 50%, 10% and 0% (ligand-free control). The peptides modification with histidine residues facilitates the escape of DNA from endosomes. Template polymerization of cross-linking peptides was used to form DNA/peptide complexes. EtBr exclusion and DNA retardation assays proved peptides ability to condense DNA. Transfection activity was studied in CXCR4(+),(A172 and HeLa) and CXCR4(−) (CHO) cell lines with lacZ as a reporter gene. Transfection efficacy of ligand-conjugated vehicles in CXCR4(+) HeLa and A172 cells was 10-times higher compared to control peptide. The level of transgene expression with ligand-conjugated peptides in low N/P ratios was comparable with the efficacy of control PEI. Otherwise transfection efficacy of ligand-conjugated peptides on CXCR4(−) CHO cells was lower than in control PEI. Thus these results demonstrate that ligand-conjugated peptide-based vehicles reported can be a perspective approach for effective gene delivery to CXCR4 expressing cells.

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Antibody targeting of lipid nanocapsules for directed drug delivery: physico-chemical characterization and *in vitro* study

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Lipid nanocapsules are recently developed as nanocarriers for lipophilic drugs delivery. The surface characteristics of these colloidal particles are determinant in order to provide a controlled and directed delivery on target tissues with specific markers. We report the development of immuno-nanocapsules, in which antibodies are conjugated to nanocapsules offering the promise of selective drug delivery to specific cells. Several nanocapsule systems were prepared by the solvent displacement technique obtaining an oily core surrounded by a functional shell with surface carboxylic groups. Antibodies were conjugated with nanoparticles by the carbodiimide method that allows it the covalent immobilization of protein molecules through these carboxylic surface groups. A complete physico-chemical characterization of the immuno-nanocapsules was developed confirming the immobilization of protein molecules on the colloidal nanoparticles via electrokinetic and colloidal stability experiments. The immunoreactivity of the protein–nanocapsules complexes was studied following the changes in the turbidity after addition of specific antigens, showing an adequate surface disposition of the covalent bound antibodies in order to a specific immunological recognize. Finally, nanocapsules were conjugated to a specific antibody to HER2 oncoprotein. In this case, in addition to the colloidal characterization, an '*in vitro*' study was developed using this surface modified system with different lipophilic anti-cancer drugs entrapped in their oily core. Flow cytometry experiments were used in order to evaluate the cytotoxicity (IC50) of our modified nanocapsules with wild-type and HER2 over expressing tumoral, cell lines. The obtained results have shown the capacity of the immuno-nanocapsules to increase their uptake in tumoral cells, suggesting their ability to a selective deliver drugs.

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Characterization of polymer-coated nanoparticles based on DNA condensation via spermine

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The combination of the complete human genome sequence and the understanding of molecular pathways of some diseases including cancer, could lead to develop several interesting new treatments, such as gene therapy. But one of the major obstacles preventing this therapy from being used is the lack of specific and efficient delivery systems. The uptake of vectors by living cells depends on the degree of DNA condensation, thus we used a demonstrated condensing agent of nucleic acids: spermine. Nanoparticles based on DNA condensation by this natural polyamine were synthesized. In order to protect DNA against DNase degradation, these nanoparticles were coated with the positive charged polymers chitosan or polyethyleneimine (PEI). Folic acid was covalently bound to chitosan with the aim of enhance nanoparticle endocytosis via folate receptor, which is over-expressed in cancer