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Cationic star homo- and co-polymers for gene delivery

Theoni K. Georgiou[1,*], Mark A. Ward[1], Phillip Knight[1], Maria D. Rikkou[2], Maria Vamvakaki[3,4], Edna N. Yamasaki[5], Leonidas A. Phylactou[6], Costas S. Patrickios[2]

[1] *Department of Chemistry, The University of Hull, HU6 7RX, Hull, UK*

[2] *Department of Chemistry, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus*

[3] *Department of Materials Science and Technology, University of Crete, P.O. Box 2208, 710 03 Heraklion, Crete, Greece*

[4] *Foundation for Research and Technology, Institute of Electronic Structure and Laser, P.O. Box 1527, 711 10, Heraklion, Crete, Greece*

[5] *Department of Life and Health Sciences, School of Sciences, University of Nicosia, 46 Makedonitissas Ave, 1700 Nicosia, Cyprus*

[6] *Cyprus Institute of Neurology and Genetics, P.O. Box 23462, 1683 Nicosia, Cyprus*

*Corresponding author.
E-mail: T.Georgiou@hull.ac.uk (T.K. Georgiou).

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

L. Chopinet*, L. Wasungu, M.P. Rols
Institut de Pharmacologie et de Bilogie Structurale, 205 route de narbonne, 31077 Toulouse Cedex, France

*Corresponding author.
E-mail: chopinet@ipbs.fr (L. Chopinet).

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*

Luc Wasungu[1,*], Anne-Laure Marty[2,3], Michel Francis Bureau[2,3], Michel Bessodes[2,3], Justin Teissie[1], Daniel Scherman[2], Marie-Pierre Rols[1], Nathalie Mignet[2,3]

[1] *CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), 205 route de Narbonne, F-31077, Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France*

[2] *Unité de Pharmacologie Chimique et Génétique; CNRS, UMR 8151, Inserm U 640, Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, F-75270 Cedex, France*

[3] *ENSCP, Paris, F-75231 Cedex, France*

*Corresponding author.
E-mail: luc.wasungu@ipbs.fr (L. Wasungu).

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.