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HYPERGRAVITY ENHANCES LIPOFECTAMINE-MEDIATED TRANSFECTION OF NIH/3T3 CELLS

Abstract

Gene therapy has become an important technique in medicine and tissue engineering. Viral vectors, like adenovirus, adeno-associated virus or retrovirus, have been exploited even in clinical trials. However, some side effects, including adverse immunoresponse and infection, could represent important limiting factors. At this aim, the development of non-viral vectors for practical gene therapy is highly explored, as well as alternative approaches to enhance transfection efficiency [1]. Magnetofection, for example, represents a simple and effective method that exploits magnetic fields to concentrate particles containing nucleic acid towards the target cells. Sonoporation exploits ultrasonic frequencies to modify cell membrane permeability, in order to allow DNA up-take. Acustic cavitation or microbubble-assisted sonoporation can further enhance gene delivery inside the cell. It is well known as hypergravity could represent an active physical stimulus towards cells and tissues [2]. Here, we propose a cell treatment at higher g values as a positive stimulus for an enhanced Lipofectamine-mediated cell transfection. NIH/3T3 fibroblasts have been cultured on collagen-treated coverslips (12 mm in diameter) and allowed to reach 80% of confluence. Afterwards, they underwent transfection with Lipofectamine (Invitrogen) following the manufacturer's protocol for 2 h, at both 1 g (control) and 150 g, with the help of a bench centrifuge (Hettich); pDNA coding for green fluorescence protein (GFP) was used as reporter gene (0.8 μg of DNA for each coverslip was used in each experiment). At 24 h and 72 h, cell transfection was qualitatively assessed with fluorescence microscopy, while a quantitative evaluation of transfection efficiency was performed with a plate fluorimeter. Results demonstrated a significant increment of transfected cells following hypergravity treatment, that reached about 15% at the third day after the stimulation. Further studies will be performed at different g values and focused on understanding the mechanism of this effect, that we believe could be highly beneficial in biotechnology and regenerative medicine applications.

1. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. Adv Biomed Res. 2012;1:27.
2. Ciofani G, Ricotti L, Rigosa J, Menciassi A, Mattoli V, Monici M. Hypergravity effects on myoblast proliferation and differentiation. J Biosci Bioeng. 2012;113:258.