A New Method for Improved Gene Transfer into Bone Marrow Stromal Cells

*Kuroda, S; *Sumner, D R; +*Virdi, A S
+*Rush Presbyterian St. Luke’s Medical Center, Chicago, IL

Introduction

Use of commercially available transfection reagents for gene transfer into bone marrow stromal cells has shown limited success. Efficient transfer of genes, for genetic labeling and growth factor delivery, into these cells would prove useful in investigating the fate and role of marrow stromal cells in tissue regeneration and repair. In this study, we report a new method for gene transfer in rat bone marrow stromal cells in vitro using naked plasmid and APES-coated cover glasses.

Methods

A mammalian expression vector (pEGFP-N1; Invitrogen) containing the coding region for a green fluorescent protein was prepared to high purity. Cover glasses (Ø18 mm) were coated with 3-aminopropyl triethoxysilane (APES; Sigma), which provides a positively charged glass surface and attracts negatively charged sites in tissue sections or on the surfaces of whole cells. pEGFP-N1 (10 μg) was placed on APES treated cover glass and allowed to air-dry overnight. Rat bone marrow cells (passage 1 to 3) or UMR-106 cells were cultured in a 24-well plate culture plate in D-MEM (supplemented with 10% FBS) at a concentration of 5 x 10⁴/ml/well, and an hour later, was followed by placing the treated (APES +/- plasmid DNA) cover glass in each well. The cells were cultured for 7 days and the fluorescence was monitored daily through a microscope.

For quantitative assessment of fluorescence, rat bone marrow stromal cells were cultured and transfected in the same way as described above. The cells were trypsinized and collected at day 1, 2, 3, 4, 5, and 7 post-transfection. The expressed protein, EGFP, was extracted from the cell lysates by sonic and freeze/thaw techniques. The level of EGFP fluorescence was measured directly with a fluorometer (Ex 509nm, Em 488nm) at each time point.

For comparison, pEGFP-N1 was transferred into rat bone marrow and UMR-106 cells by lipofection (LipofectAmine; Invitrogen), where the cells had been seeded at 1 x 10⁵/ml/well 24 hrs before the transfection according to the manufacturer’s instructions.

Essential Results

APES-coated cover glass seemed to provide both rat bone marrow cells and UMR-106 with much less initial uptake of the plasmid than LipofectAmine as assessed by microscopy. However, the cells cultured with the APES-coated cover glass over a longer period of time constantly express the fluorescent protein for up to a week; while LipofectAmine gave the maximum signal at day 2 and the intensity of the fluorescence decreased gradually thereafter (data not shown). Using LipofectAmine, UMR-106 exhibited greater transfection efficiency than the rat bone marrow cells. This discrepancy was reversed with APES mediated plasmid transfer. When compared with LipofectAmine, APES-coated cover glass resulted in visibly more efficient transfection in the rat bone marrow cells compared to UMR-106.

Quantitatively, rat bone marrow stromal cells cultured with APES-coated cover glass exhibit lower average initial fluorescence than when cultured with LipofectAmine (Figure 1, black diamond vs. red triangle). However, the cells cultured with APES-coated cover glass expressed the fluorescent protein constantly for up to a period of one week and the level was significantly higher than control cultures (APES, no plasmid; Figure 1, black diamond vs. black square). LipofectAmine, on the other hand, gave the maximum signal at day 2 and the intensity of the fluorescence decreased gradually thereafter (Figure 1, red triangle) but was significantly higher than control (LipofectAmine, no plasmid) at all time points (Figure 1, red cross). After the transfection by APES method, the proliferation of rat bone marrow stromal cells during the exponential period was similar to control cells (APES but no plasmid) (Figure 2, black diamond vs. black square). But the proliferation rate of the same cells was adversely affected by the LipofectAmine mediated transfection of the plasmid (Figure 2, red triangle vs. red cross).

Discussion

In this method, APES serves as a holder for plasmid DNA on the surface of glass and allows the cells to take up the pEGFP-N1 plasmid during cell division. This system provides the cells with a constant supply of plasmid DNA carrying reporter gene or other genes of interest. While the average expression signal is low during the initial culture period, a sustained, higher level of expression was observed at latter stages. In contrast, although an initially very high EGFP expression is prominent at day 2 in LipofectAmine mediated transfection, there is a continual decline in the fluorescence signal thereafter. This high level of EGFP expression may be responsible for the suppression of cell proliferation observed in these cultures. In comparison, the lower level of EGFP expression in APES mediated transfection has no effect on cell proliferation.

In conclusion, this new method of APES mediated transfer of plasmid DNA into bone marrow stromal cells provides a useful technique for optimizing tissue engineering protocols for cell labeling and growth factor delivery.

Supported by:
University Committee on Research (RUSH) & Grainger Foundation.