Lipofection does not require the removal of serum

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Most cationic liposome-mediated cellular transfections are performed in the absence of serum for an initial period of at least several hours (1,2). The ability to transfect cells in the continued presence of serum would be advantageous for several reasons: 1) transfections would be easier and less time consuming, 2) decreased requirements for media and serum would lower the cost of experiments, 3) one would be able to avoid depriving cells of serum, which may alter cellular function and viability. We have found that cationic liposomes can produce transfection levels that are at least as high in the presence of serum, as achieved using a standard, serum-free protocol.

Specifically, we transiently transfected an adherent simian kidney cell line, CV-1 (Figure), as well as the suspension cells, MEL (meurine erythroleukemia) cells using a Rous Sarcoma virus (RSV)-choramphenicol acteyltransferase (CAT) expression plasmid complexed to each of four different cationic liposome formulations. In each case, CAT gene expression was comparable or better when the cells were transfected in the presence of 10% serum than when transfected in serum-free medium for an initial 4 hour period (figure and data not shown).

Liposomes composed of the cationic lipid DOTMA (1), provided by Syntex, and dioleoylphosphatidylethanolamine (DOPE) in a 1:1 molar ratio were prepared as described (1) and are commercially available as Lipofectin (Gibco BRL). We also tested liposomes composed of DOTMA and cholesterol (1:1), the cationic lipid DDAB (2) and DOPE (1:2), commercially available as TransfectACE (Gibco BRL) and the cationic lipids lysinyl phosphatidylethanolamine (L-PE) and the cholesterol ester of β-alanine (CEβA) (6:4), provided by Liposome Technology, Inc (3). 1.2×10^6 CV-1 cells were plated on 100 mm dishes in 5 ml of Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS). Eighteen hours later, cells were transfected with pRSV-CAT complexed with cationic liposomes, as follows. The liposomes were added to pRSV-CAT and gently mixed. For transfections performed in the absence of serum, 1 ml of serum-free DMEM was then added, and the mixture was incubated at 20°C for 30 minutes. Concurrently, the cells were washed 3 times with 3 ml of serum-free DMEM, 5 ml of serum-free DMEM were added followed by addition of the plasmid-liposome solution. The cells were then incubated for 4 hours at 37°C in a 7% CO_2 incubator. For transfections performed in the presence of serum, DNA and liposomes were mixed as described, and 1 ml of DMEM with 10% FCS was added to the complex. The solution was incubated at 20°C for 30 minutes, and transferred directly to the cells without removal or replacement of the medium in the dish, and the cells incubated for 4 hours at 37°C. After the 4 hour incubation, all plates were washed 3 times with 3 ml of serum-free DMEM, followed by the addition of 7 ml of DMEM with 10% FCS, (omitting this washing step does not affect transfections performed in the presence of serum (data not shown)). All cells were harvested 48 hours after transfection, and cellular extracts were prepared and assayed for CAT activity as described previously (4).

For each of the four cationic liposome formulations tested, transfections performed in serum-containing medium were at least as efficient as transfections performed in serum-free medium. Differences in the lipid:DNA ratio, number of cells treated and amount of cationic lipid used may explain differences between these results and prior studies (1). These findings have led us to routinely perform transfections by simply adding the plasmid-liposome complexes to preplated cells in serum-containing medium without further manipulation until harvesting.

Transfections performed in medium with serum are thus convenient, time saving, less costly and in some cases, may improve transgene expression levels.

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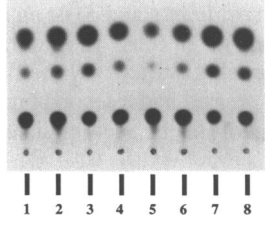

1.2×10^6 CV-1 cells were transfected with 5 μg pRSV-CAT complexed with liposomes containing a total of 10 nanomoles of cationic lipid (#1-4) or 20 nanomoles of cationic lipid (#5-8). 1) DOTMA:DOPE 1:1, serum-free; 2) DOTMA:DOPE 1:1, 10% serum; 3) DOTMA:chol 1:1, serum-free; 4) DOTMA:chol 1:1, 10% serum; 5) DDAB:DOPE 1:2, serum-free; 6) DDAB:DOPE 1:2, 10% serum; 7) L-PE:CEβA 6:4, serum-free; 8) L-PE:CEβA 6:4, 10% serum.

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