

Analysis of Novel Nonviral Gene Transfer Systems for Gene Delivery to Cells of the Musculoskeletal System

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Abstract The aim of the present study was to evaluate the efficacy of novel nonviral gene delivery systems in cells of musculoskeletal origin. Primary cultures of lapine skeletal muscle cells, lapine articular chondrocytes, human cells from fibrous dysplasia and cell lines established from human osteosarcoma (SAOS-2), chondrosarcoma (CS-1), murine skeletal myoblasts (L8) and fibroblasts (NIH 3T3) were transfected with the *P. pyralis luc* or the *E. coli lacZ* genes using Nanofectin 1 and 2, Superfect, JetPEI, Gene-Jammer, Effectene, TransPass D2, FuGENE 6, Lipofectamine 2000, Dreamfect, Metafectene, Escort III, and calcium phosphate. Maximal transfection efficiency in lapine skeletal muscle cells was of $60.8 \pm 21.2\%$ using Dreamfect, $38.9 \pm 5.0\%$ in articular chondrocytes using Gene Jammer, $5.2 \pm 8.0\%$ in human cells from fibrous dysplasia using Lipofectamine 2000, $12.7 \pm 16.2\%$ in SAOS-2 cells using FuGENE 6, $29.9 \pm 3.5\%$ in CS-1 cells using Lipofectamine 2000, $70.7 \pm 8.6\%$ in L8 cells using FuGENE 6, and $48.9 \pm 13.0\%$ in NIH 3T3 cells using Metafectene. When the cells were transfected with a human IGF-I gene, significant amounts of the IGF-I protein were secreted. These results indicate that relatively high levels of transfection can be achieved using novel nonviral gene transfer methods.

Keywords Gene transfer · Nonviral · Musculoskeletal cells · IGF-I · Transfection efficiency

Introduction

Gene transfer to cells that constitute the musculoskeletal system is a valuable tool for functional studies and possible therapeutic applications such as gene-modified tissue regeneration. Although it has been shown that these cells can be transduced with viral vectors at high efficiencies [1], these vectors carry the risk of insertional mutagenesis (retroviral vectors), immunogenicity (adenoviral vectors) and acquisition of replication competence [1]. Nonviral gene delivery systems are potent alternatives that may overcome such crucial issues [2]. They usually do not provoke an immune response, are relatively easy to manipulate and show no limitation in the size of DNA to be delivered. Transfection of articular chondrocytes and skeletal muscle cells has been already reported using typical transfection methods [3, 4]. Recently, however, novel nonviral gene delivery systems have been developed [2], including activated-dendrimer [5], linear polyethylenimine [6], lipid- [7] and nanoparticle-based compounds [8]. Although these new systems hold promises to achieve high-transfection efficiencies, a systematic evaluation in a large variety of cells has not, to our knowledge, been performed to date.

The aim of this study was to comparatively examine a panel of novel nonviral compounds in both primary cells and established cell lines from musculoskeletal origin. We tested the hypothesis that, under optimized conditions, these gene transfer systems are capable of yielding high transfection efficiencies. We chose 13 systems that are based on nanoparticles, activated-dendrimers, linear polyethylenimines and several lipid-based compounds and cationic liposomes. All of them were tested in primary cultures of skeletal muscle cells, articular chondrocytes, cells from fibrous dysplasia and cell lines from osteosarcoma (SAOS-2), chondrosarcoma (CS-1), myoblasts (L8) and fibroblasts (NIH 3T3). For each

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cell type, the transfection system promoting the highest efficiency was next applied, in order to monitor the secretion levels of the transgene product. For this purpose, a human insulin-like growth factor I (IGF-I) plasmid expression vector was used.

Materials and Methods

Materials

Reagents were from Invitrogen (Karlsruhe, Germany) unless otherwise indicated. Plasticware was from Falcon (Beckton Dickinson, Pont de Claix, France). For transfections, Nanofectin 1 and 2 (PAA, Pasching, Austria), Superfect (Qiagen, Hilden, Germany), JetPEI (PolyPlus Transfection, Illkirch, France), Escort III (Sigma, Taufkirchen, Germany), Gene Jammer (Stratagene, La Jolla, CA), Effectene (Qiagen), TransPass D2 (New England Biolabs, Ipswich, MA), FuGENE 6 (Roche, Mannheim, Germany), Lipofectamine 2000 (Invitrogen), Metafectene (Biontex, Munich, Germany), and Dreamfect (Oz Bioscience, Marseille, France) were used.

Cell Culture

Primary cells used in this study included lapine skeletal muscle cells and articular chondrocytes and human cells from fibrous dysplasia. Established cell lines were SAOS-2 (human osteosarcoma), CS-1 (human chondrosarcoma), L8 (murine skeletal myoblasts), and NIH 3T3 cells (murine fibroblasts). Cells were cultured at 37°C in a humidified atmosphere with 5% CO₂. Lapine articular chondrocytes were obtained from cartilage of the knee joints of male Chinchilla bastard rabbits as previously described [9]. Lapine muscle was obtained from the quadriceps muscle of male Chinchilla bastard rabbits. Pieces of 0.5 cm³ were removed after sacrifice and transferred to a spinner bottle containing DMEM with 50 µg/ml ascorbic acid, 100 U/ml penicillin G and 100 ml/ml streptomycin (basal medium) with collagenase (0.05%) for 20 h. Cells were filtered through a 40-µm cell strainer to remove undigested matrix. Cells from human fibrous dysplasia were obtained from tissue removed during tumor surgery of the proximal femur from a 23-year-old patient after digestion for 16 h in basal medium containing 0.2% collagenase. All cells were placed in monolayer culture in DMEM with 10% FBS (growth medium) in 24-well plates. Transfections were performed at 60–70% cell confluence.

Plasmid DNA

Plasmid expression vectors carried either the *Photinus pyralis* luciferase gene (pCMVLuc), the *Escherichia coli*

lacZ gene (pCMVlacZ) or the human IGF-I gene (pCMVhIGF-I) under the control of the CMV-IE promoter/enhancer. Vectors were propagated in competent *E. coli* cells (TOP 10 One Shot, Invitrogen). Ultrapure endotoxin-free plasmid DNA was prepared using the Qiagen Endofree Plasmid Maxi Kit according to the manufacturer's instructions. Plasmid DNA was diluted in sterile water to a final concentration of 1 µg/µl DNA.

Nonviral Gene Transfer

Prior to transfections monolayer cultures of cells in 24-well plates were rinsed with phosphate-buffered saline (PBS, PAA) and fresh growth medium was added to the cells. For transfections with Escort III, basal medium was added instead of growth medium. For transfections with TransPass D2, growth medium was removed and the transfection complexes were directly added to the cells. All transfections were performed according to the manufacturer's instructions. Reagents were complexed to plasmid DNA in sterile polypropylene tubes at room temperature.

Nanofectin 1 and 2

Nanofectin 1 and 2 are positively charged polymers with DNA-binding capacity that are embedded into a porous nanoparticle. For transfections, 1 µg DNA in 50 µl NaCl (0.9%) was complexed with 3.2 µl Nanofectin 1 or 2 in 50 µl NaCl (0.9%) and added to the cells after 25 min. Two hours later, 500 µl growth medium was added to the complexes.

Superfect

This activated-dendrimer transfection reagent has a spherical architecture with branches radiating from a central core and terminating at positively charged amino groups. About 1 µg DNA was diluted in Opti-MEM to a total volume of 60 µl. Superfect transfection reagent (5 µl) was added. Complexes were allowed to form for 10 min and added to the cells. Two and a half hours later, the cells were washed with PBS and fresh growth medium was added.

JetPEI

JetPEI consists of a linear polyethylenimine and compacts the DNA into positively charged particles. Intracellularly, it protects the DNA from degradation in endolysosomes by buffering the endosomal pH. The DNA (1 µg) and 2 µl of the JetPEI reagent were complexed in 50 µl NaCl (0.9%), left to incubate for 25 min and added to the cells.

Escort III

Escort III is a cationic liposome suspension in water, composed of a polycationic lipid and a neutral lipid compound. The DNA (0.3 µg) and the liposome suspension (0.6 µl) were diluted in 20 µl Opti-MEM, combined, allowed to form complexes for 30 min and then added to the wells. After 6 h, basal medium containing 20% fetal bovine serum (FBS) was added which was replaced by growth medium after 18 h.

Gene Jammer

This formulation of polyamine and other components in 80% ethanol was diluted in 100 µl Opti-MEM and was complexed with 1 µg DNA. Three hours after adding these complexes, cells were washed and incubated with growth medium. For transfections of lapine articular chondrocytes, the volume of Gene Jammer reagent was varied between 3 and 12 µl (vector/DNA ratios of 3:1, 6:1, 9:1 and 12:1). Exposure times tested ranged from 3 to 7 h.

Effectene

Effectene is a transfection system based on a non-liposomal lipid. As a first step, 0.2 µg of the DNA was condensed by interaction with a specific enhancer (1.6 µl) in a defined buffer at a total volume of 60 µl. Afterwards, a volume of 5 µl of the Effectene reagent was added, complexes were allowed to form for 5 min and added to the cells.

TransPass D2

TransPass D2 is based on a non-lipid cationic polymer. DNA (0.3 µg) was complexed with 0.8 µl TransPass D2 in 300 µl Opti-MEM. Growth medium was removed from the cells and the polymer/DNA complexes were added after an incubation period of 25 min. Two and a half hours later, the transfection solution was replaced by growth medium.

Metafectene

Metafectene is a polycationic transfection reagent based on a liposome that enables destabilization of the lipid membrane within the acidic endosomal environment by repulsive electrostatic forces. In 30 µl Opti-MEM each, 0.5 µg plasmid DNA and 2.5 µl Metafectene were diluted, combined, and then the complexes obtained were added to the cells after 20 min. For *lacZ* transfections of NIH 3T3 cells,

in order to evaluate the optimal transfection conditions, the volume of Metafectene ranged from 1 to 4 µl (vector/DNA ratios of 2:1, 5:1 or 8:1).

FuGENE 6

FuGENE 6 is a proprietary, non-liposomal mixture of lipids. FuGENE 6 and 1 µg of DNA were allowed to form complexes in 90 µl Opti-MEM 15 min prior to addition to the cells. For optimization studies, the volume of FuGENE 6 per µg of DNA was varied from 1 to 4 µl (exposure times were of 24, 48 and 72 h).

Lipofectamine 2000

Lipofectamine 2000 is a novel proprietary cationic lipid formulation. DNA (0.8 µg) and Lipofectamine 2000 (2 µl) were diluted in 50 µl Opti-MEM each. After combining these solutions, the complexes were allowed to form for 20 min and next added to the cells. Lipofectamine 2000 was optimized for transfections of cells from fibrous dysplasia and CS-1 cells. The volume of transfection reagent varied between 1 and 3 µl. Vector/DNA ratios were of 1.25:1, 2.5:1, and 3.75:1.

Dreamfect

Dreamfect combines cationic lipids and polymers, resulting in lipopolyamines, which contain a lipophilic part and a charged polyamine moiety. Plasmid DNA (0.5 µg) was complexed with 2 µl Dreamfect, diluted in 50 µl Opti-MEM, incubated for 20 min, and added to the cells. The transfection of lapine skeletal muscle cells was optimized using Dreamfect. Dreamfect volumes were of 2, 4, and 6 µl (vector/DNA ratios of 4:1, 8:1 and 12:1).

Calcium Phosphate Co-precipitates

Calcium phosphate co-precipitates were prepared by combining 0.8 µg plasmid DNA in 22 µl 0.1 × TE with 25 µl of 2 × HEPES buffered saline [10]. CaCl₂ (3.1 µl, 2 M) was carefully added to this solution and the resulting complexes were applied to the cells after 25 min of incubation.

Detection of Transgene Expression

Transgene Expression was detected at a standardized representative time point of 48 h after transfection. Luciferase activity using all 13 transfection systems was measured by

chemiluminescence (Luciferase Assay System; Promega, Madison, WI) using a TECAN GENios microplate reader (TECAN, Crailsheim, Germany). Sample light units (relative light units, RLU) were recorded in duplicates within 5 s after addition of the substrate. Transfections using the *E. coli lacZ* and the human IGF-I gene were carried out with the most effective transfection system per cell type as determined by luciferase. Efficiencies were determined by X-gal staining for 5 h at 37°C of *lacZ* transfected and control cells (β -Gal Staining Set; Roche) using a standard procedure as previously described [3]. In order to determine the production of IGF-I protein, the medium was replaced by basal medium 1 day after transfection. After 24 h, the conditioned medium was collected and subjected to ELISA (detection limit: 0.03 ng/ml; R&D Systems, Minneapolis, MN).

Statistical Analysis

Data are expressed as mean \pm standard deviation (s.d.) of separate experiments. Luciferase transfections were independently performed three to four times ($n = 3$ wells per transfection reagent) for each cell type. Sample light units (RLU) per well were recorded in duplicate. For *lacZ* transfections, 3–5 independent optimization experiments were carried out ($n = 3$ wells per condition). The IGF-I transfection experiments were performed in duplicate ($n = 2$ wells per condition). ANOVA was applied to determine the significance of differences among groups. P values less than 0.05 were considered significant.

Results

Primary Lapine Skeletal Muscle Cells

Out of the 13 transfection systems tested, Dreamfect and Metafectene promoted maximal transgene expression as determined by chemiluminescence ($13,013 \pm 593$ and $12,519 \pm 1,428$ RLU, respectively; $P = 0.73$) (Fig. 1) vis-a-vis all other reagents tested. In contrast, Escort III mediated the minimal transgene expression; 30-fold lower than Dreamfect (435 ± 21 RLU). Maximal gene expression was seen with $0.5 \mu\text{g}$ DNA at a (v/w) Dreamfect/DNA ratio of 4:1, corresponding to a mean transfection efficiency of $60.8 \pm 21.2\%$. Increasing the lipid/DNA ratio (8:1; 12:1) led to decreased efficiencies. IGF-I expression levels in IGF-I-transfected cells using Dreamfect were of $142.3 \pm 7.0 \text{ ng}/10^6 \text{ cells}/24 \text{ h}$, 285-fold higher than in non-transfected cells (Table 1).

Primary Lapine Articular Chondrocytes

Gene Jammer, JetPEI and calcium phosphate co-precipitates mediated the highest luciferase expression ($22,806 \pm 8,373$,

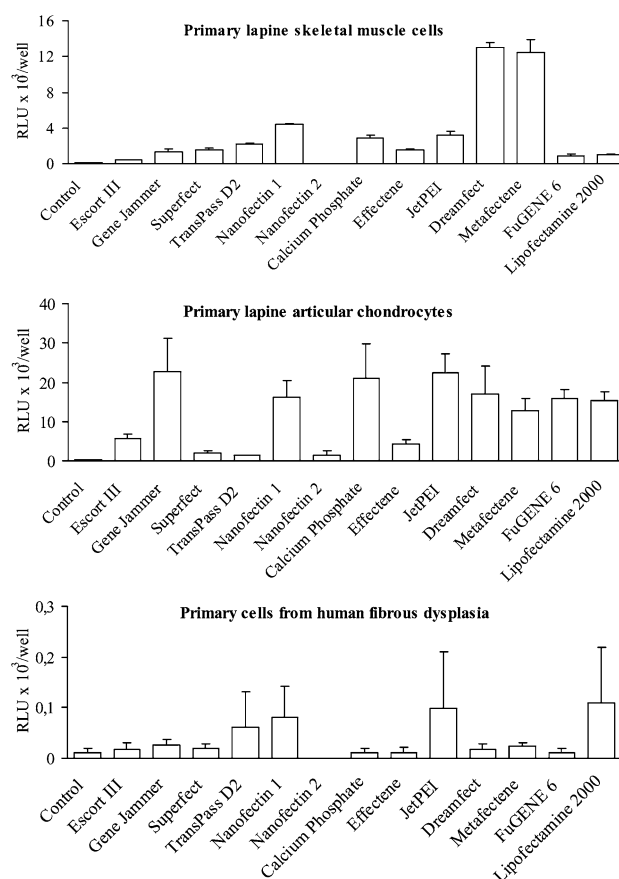


Fig. 1 Comparative analysis of transgene expression for primary cells. Data are expressed as mean luciferase activity (relative light units, RLU) per well \pm standard deviation from at least three independent experiments

$22,347 \pm 4,865$ and $21,047 \pm 8,653$ RLU; $P = 0.26$) (Fig. 1). Transfection with Nanofectin 2 resulted in the lowest efficiency ($1,296 \pm 1,214$ RLU). Maximal transgene activity was obtained with $1 \mu\text{g}$ DNA at a (v/w) Gene Jammer/DNA ratio of 3:1, corresponding to a transfection efficiency of $38.9 \pm 5.9\%$. Increasing the lipid/DNA ratio (6:1; 9:1; 12:1) led to decreased transfection efficiencies. Secretion of IGF-I in Gene Jammer-transfected chondrocytes was of $61.3 \pm 12.0 \text{ ng}/10^6 \text{ cells}/24 \text{ h}$, 77-fold higher than in non-transfected cells ($0.8 \pm 0.2 \text{ ng}/10^6 \text{ cells}/24 \text{ h}$).

Primary Cells from Human Fibrous Dysplasia

Lipofectamine 2000, JetPEI, Nanofectin 1, and TransPass D2 achieved highest transgene expression (109 ± 111 , 98 ± 111 , 81 ± 63 , 60 ± 71 RLU, respectively; $P = 0.24$). Maximal transfection efficiency was $5.2 \pm 8.0\%$ (Lipofectamine 2000; lipid/DNA ratio: 2.5:1). Other (3.75:1, 1.25:1) lipid/DNA ratios did not further increase

Table 1 Comparative analysis of luciferase activity and IGF-I secretion levels of different cell types

Cells	Murine skeletal myoblasts (L8)	Primary skeletal muscle cells	Primary lapine articular chondrocytes	Human chondrosarcoma cells (CS-1)	Human osteosarcoma cells (SAOS-2)	Primary cells from human fibrous dysplasia
Transfection system	FuGENE 6	Dreamfect	Gene Jammer	Lipofectamine 2000	FuGENE 6	Lipofectamine 2000
Lipid/DNA ratio	2:1	4:1	3:1	2.5:1	2:1	2.5:1
Luciferase activity (RLU $\times 10^3$ /well)	14,490 \pm 5,937	13,013 \pm 593	22,806 \pm 8,373	8,470 \pm 6,204	11,430 \pm 9,480	98 \pm 111
IGF-I production (transfected cells) (ng/10 ⁶ cells/24 h)	179.2 \pm 5.7	142.3 \pm 7.0	61.3 \pm 12.0	37.9 \pm 8.8	27.3 \pm 7.1	0.9 \pm 0.2
IGF-I production (non-transfected cells) (ng/10 ⁶ cells/24 h)	0.9 \pm 0.2	0.5 \pm 0.1	0.6 \pm 0.2	1.3 \pm 0.3	1.2 \pm 0.2	0.4 \pm 0.1

Maximal IGF-I secretion following transfection of pCMVhIGF-I was achieved in L8 murine skeletal myoblasts, significantly higher than all other cell types evaluated ($P < 0.001$). IGF-I protein production in primary lapine skeletal muscle cells was significantly higher than in NIH 3T3 fibroblasts ($P < 0.001$). No significant difference was observed between NIH 3T3 fibroblasts and primary lapine articular chondrocytes ($P = 0.07$). IGF-I levels in all cells were significantly higher than in non-transfected cells from the same origin ($P < 0.05$)

efficiencies. IGF-I secretion (Lipofectamine 2000) was 2.5-fold higher than of non-transfected cells ($P < 0.05$).

Human Osteosarcoma Cells (SAOS-2)

Gene expression was maximal with FuGENE 6, JetPEI, Gene Jammer, Metafectene, Dreamfect and calcium phosphate co-precipitates ($11,430 \pm 9,480$, $9,426 \pm 7,887$, $9,156 \pm 6,606$, $8,165 \pm 4,692$, $6,159 \pm 4,360$ and $5,924 \pm 3,466$ RLU, respectively; $P = 0.08$) (Fig. 2). Highest efficiency of $12.7 \pm 16.2\%$ was seen at a FuGENE 6/DNA ratio of 2:1. Ratios of 1:1, 3:1, and 4:1 did not led to further increases. IGF-I secretion was 27.3 ± 7.1 ng/10⁶ cells/24 h, 23-fold higher than in non-transfected cells (1.2 ± 0.2 ng/10⁶ cells/24 h).

Human Chondrosarcoma Cells (CS-1)

Lipofectamine 2000, Gene Jammer, Nanofectin 1, and FuGENE 6 led to the highest transgene expression ($8,470 \pm 6,204$, $7,482 \pm 4,575$, $5,354 \pm 1,188$, $5,197 \pm 2,647$ RLU, respectively; $P = 0.12$) (Fig. 2). Maximal transfection efficiency was $30.0 \pm 3.5\%$ at a (v/w) Lipofectamine/DNA ratio of 2.5:1. Higher (3.75:1) and lower (1.25:1) ratios did not result in higher transfection efficiencies. IGF-I expression levels were of 37.9 ± 8.8 ng/10⁶ cells/24 h, 29-fold higher than in non-transfected cells (1.3 ± 0.3 ng/10⁶ cells/24 h).

Murine Skeletal Myoblasts (L8)

FuGENE 6, TransPass D2 and Nanofectin 1 produced maximal transgene expression ($38,882 \pm 16,729$, $14,490 \pm 5,937$, $11,271 \pm 527$ RLU, respectively; $P = 0.07$) (Fig. 2) with an efficiency of $64.6 \pm 14.2\%$ (FuGENE 6). Secretion of IGF-I was 179.2 ± 5.7 ng/10⁶ cells/24 h, almost 200-fold higher than in non-transfected cells (Table 1).

Murine Fibroblasts (NIH 3T3)

Luciferase expression was the highest with Metafectene and Nanofectin 1 ($45,370 \pm 25,958$ and $44,065 \pm 17,745$ RLU, respectively; $P = 0.84$) (Fig. 2). Maximal efficiency was $48.9 \pm 13.0\%$ (Metafectene; lipid/DNA ratio: 5:1). Lower (2:1) or higher lipid/DNA ratios (8:1) led to decreased efficiencies. IGF-I expression levels were 84.2 ± 16.9 ng/10⁶ cells/24 h, 70-fold higher than in non-transfected cells (1.2 ± 0.1 ng/10⁶ cells/24 h) (Table 1).

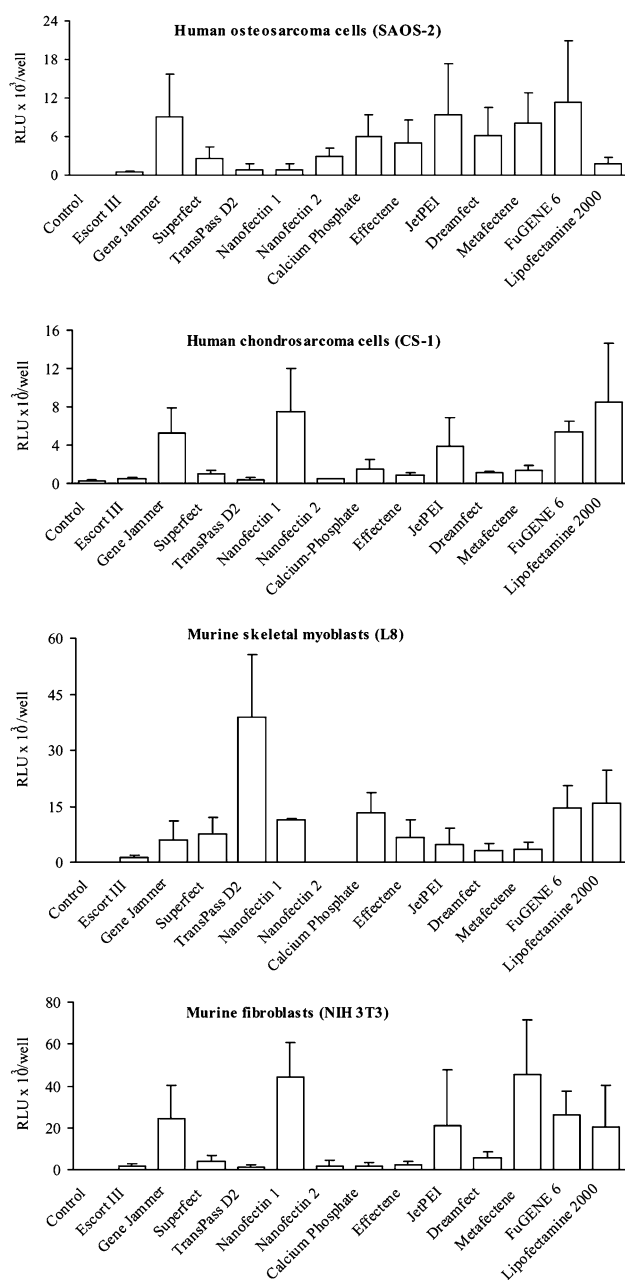


Fig. 2 Comparative analysis of transgene expression for established cell lines. Data are expressed as mean luciferase activity (relative light units, RLU) per well \pm standard deviation

Discussion

The present study comparatively analyzed the efficacy of transfection of 13 novel nonviral transfection systems in cells of musculoskeletal origin. The data indicate that relatively high levels of transfection can be achieved in primary cultures of lapine skeletal muscle cells and articular chondrocytes, as well as in established cell lines such as L8 (muscle cells), CS-1 (chondrosarcoma) and NIH 3T3 cells

(fibroblasts). The data further demonstrates that human osteosarcoma (SAOS-2) cells and cells from human fibrous dysplasia can be transfected at lower efficiencies. Finally, the data suggest that significant amounts of human IGF-I, a potential therapeutic candidate, can be produced in primary lapine muscle cells and chondrocytes, L8, and NIH 3T3 cells. The results obtained using three genes (*luc*, *lacZ*, IGF-I) indicate that the nature of the cell itself is a critical factor that determines the level of gene expression following transfection. Therefore, it is important to carefully characterize the most suitable transfection system for each cell type prior to functional studies in order to achieve maximal transgene expression.

Transfection efficiencies for muscle cells have been previously reported to be in the range of 0–70%, e.g., using LipofectAmine and DOSPER in human myoblast clones [11], DAC-40 or Effectene in human skeletal muscle cells [12], complexes of adenovirus particles with cationic lipids and polymers in murine muscle cells [13] or Lipofectamine and DOTAP in primary cultures of murine myoblasts [14]. In the present study, efficiencies in lapine skeletal muscle cells were 61% using Dreamfect and 65% in the myoblast cell line L8 using FuGENE 6. Comparable high levels of transgene expression in a myoblast cell line have been achieved to date only using high-titer retroviral vectors [15].

The reported efficiencies in lapine articular chondrocytes of 39% using the cationic liposome formulation Gene Jammer correspond well to the 40% transfection efficiencies that were previously reported [3]. Since, the data from this study were obtained after additional treatment of the cells with hyaluronidase prior to and during transfection, it is possible that the reported transfection efficiencies may be further increased by such enzymatic treatment aimed at partially removing the extracellular matrix [3].

Transfection of primary cells from human fibrous dysplasia was difficult to achieve. Maximal efficiency was of 5.2%, obtained with Lipofectamine 2000. Yet, even such relatively low efficiencies may result in possible therapeutic effects, since studies overexpressing growth factors in the arterial wall showed that efficiencies of less than 1% were sufficient to achieve a biological effect in vivo [16]. Osteosarcoma (SAOS-2) cells were transfected with an efficiency of 13% using FuGENE 6 following optimization. Nonviral transfection of SAOS-2 cells was reported only rarely [17] and transfection efficiencies have not yet been reported to date. For human chondrosarcoma CS-1 cells, the cationic liposome Lipofectamine 2000 mediated the highest efficiencies. Comparable transfection efficiencies (30%) using non-viral systems for this cell line and other chondrosarcoma cells have, to our best knowledge, not been demonstrated to

date. High transfection efficiency is a basis for successful application of gene-based treatments of musculoskeletal tumors.

NIH 3T3 cells have been previously transfected with various nonviral transfection reagents, including Lipofectamine and DOSPER [18], Effectene and Superfect [19], LipofectAMINE PLUS [20] or FuGENE 6 [21]. While transfection efficiencies were less than 30% in these studies, the present data indicate that transfection efficiencies of almost 50% can be achieved with Metafectene, a cationic liposome formulation.

Interestingly, the calcium phosphate co-precipitation method promoted high transfection efficiencies in primary lapine articular chondrocytes and SAOS-2 cells, statistically not different from those mediated by commercially available reagents. With regard to its low cost and simple and reproducible production, the calcium phosphate transfection thus represents an attractive alternative.

We have selected the human IGF-I gene as an additional candidate because of potential applications in the therapy of musculoskeletal diseases. For example, IGF-I prevents muscle atrophy [22], stimulates cutaneous wound healing [23] and enhances articular cartilage repair [9, 24]. In the present study, IGF-I expression levels were between 27 and 240 ng/10⁶ cells/24 h, depending on the cell type, except for cells of fibrous dysplasia. Transfections of lapine articular chondrocytes resulted in IGF-I production of 61 ng/10⁶ cells/24 h, i.e. 7-fold higher than the 8.3 ng/10⁶ cells/24 h previously reported for bovine articular chondrocytes [25]. In L8 cells, FuGENE 6 mediated the highest transfection efficiency (65%) and maximal IGF-I production (179.2 ± 5.7 ng/10⁶ cells/24 h) among all the transfection systems used. A bovine mammary epithelial cell line secreted 9.2 ng IGF-I/10⁶ cells/24 h following selection for cells carrying the transgene [26]. Retroviral gene transfer and selection of transduced cells resulted in 56 ng IGF-I/10⁶ keratinocytes/24 h [27]. These data show that cells from different musculoskeletal origin can be transfected with optimized systems to produce significant amounts of a secreted recombinant protein that are sufficient to mediate a therapeutic effect [9, 24, 25].

Taken together, our results indicate that novel lipid-based methods can be sufficiently optimized to offer a feasible approach for gene delivery into a wide range of cells. The described techniques might be of potential value for functional gene analyses and for gene therapy approaches in tissue engineering and the treatment of musculoskeletal disorders.

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