Non-viral transfection methods optimized for miRNA delivery to human dermal fibroblasts

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Abstract. Fibroblasts are beneficial model cells for *in vitro* studies and are frequently used in tissue engineering. A number of transfection reagents have been employed to deliver microRNAs (miRNAs/miRs) into cells for genetic manipulation. The present study aimed to establish an effective method of transient miRNA mimic transfection into human dermal fibroblasts. The experimental conditions included three different methods: Physical/mechanical nucleofection, and two lipid-based methods, Viromer® Blue and INTERFERin®. To evaluate the impact of these methods, cell viability and cytotoxicity assays were performed. The silencing effect of miR-302b-3p was revealed to alter the expression levels of its target gene carnitine O-octanoyltransferase (*CROT*) by reverse transcription-quantitative PCR. The present study showed that all selected non-viral transient transfection systems exhibited good efficiency. It was also confirmed that nucleofection, for which a 21.4-fold decrease in the expression of the *CROT* gene was observed 4 h after 50 nM hsa-miR-302b-3p transfection, was the most effective method. However, these results indicated that lipid-based reagents can maintain the silencing effect of miRNAs up to 72 h after transfection. In summary, these results indicated that nucleofection may be the optimal method for the transport of small miRNA mimics. However, lipid-based methods allow for the use of lower concentrations of miRNA and maintain longer-lasting effects.

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Introduction

The last several years have seen marked progress in the understanding of the use of fibroblasts as an *in vitro* model of cell aging and in skin tissue engineering therapies, such as tissue repair and wound healing (1-3). Furthermore, one of the most common uses of fibroblasts is producing induced pluripotent stem cells (iPSCs) because of their accessibility and relatively high reprogramming efficiency (4). The iPSCs generated from dermal fibroblasts can be differentiated into key disease-affected cells *in vitro*. Generating patient-derived iPSCs has created opportunities for rare disease modeling, such as in fibrosis and osteoarthritis. Since these cells can simulate disease phenotypes, they are useful high-throughput drug screening platforms that may result in the reversal of these abnormal phenotypes (5,6). Moreover, human dermal fibroblasts (HDFs) have been used to assess skin fibrosis tendency in response to radiotherapy (7,8), some oxidative phosphorylation disorders (9,10), and to determine the presence or risk of metabolic diseases in patients (11,12). Fibroblasts have also been used to study neurodegenerative diseases, such as Parkinson's disease (13,14) and Alzheimer's disease (15,16). A further study has also shown the use of HDFs as a tool when assessing the biological mechanisms of major depression and antidepressant drug response (17). Fibroblast cultures have been successfully used for a range of tests because fibroblasts are easy to isolate, reliable and, most importantly, they grow rapidly and continuously (18). Despite their numerous advantages, primary cultures of fibroblasts are classified as difficult to transfect cells (19,20). The methods characterized by the highest transfection efficiency (>70%) of fibroblasts are systems based on viral vectors; however, due to the potential high toxicity of viral vectors, less toxic techniques, i.e., non-viral systems, have been extensively developed (21,22). Of all the non-viral systems, nucleofection is considered to be the most effective method of fibroblast transfection (20,23). Its advantages are considered to be the high level of transgene expression and cell viability post-transfection; however, the major disadvantage of

nucleofection is its high cost (24). Therefore, new non-viral transfection systems that can match nucleofection efficiency are constantly being tested. Among them, cationic lipids and cationic polymers are strongly recommended for their capacity to form particle-like complexes (called lipoplexes and polyplexes, respectively), which are readily able to enter cells (25).

MicroRNAs (miRNA/miRs) are short non-coding RNAs (~22 nucleotides) that post-transcriptionally regulate gene expression via binding to the 3' untranslated regions (3' UTRs) of target gene mRNA (26). Transient transfection of chemically synthesized miRNA mimics (synthetic double-stranded miRNA-like RNA molecules) or miRNA agomirs (artificial double-stranded miRNA mimics) has broad applications in genetic research (27). It is widely known that the administration of miRNA mimics or miRNA inhibitors leads to overexpressed or downregulated endogenous miRNAs (28). Furthermore, Paoletti *et al* (29) demonstrated that adult human cardiac fibroblasts can be directly reprogrammed into induced cardiomyocytes by transient transfection with four miRNA mimics (miR-1, 133, 208 and 499, termed 'miRcombo'). Due to their cytoplasmic activity, relatively small size and ability to be injected systemically or locally by nanoparticle-based supply systems, as well as avoiding the use of viral vectors, both mimics and agomirs possess considerable potential as therapeutic agents (30).

The present study used hsa-miR-302b-3p, a molecule belonging to the miR-302b family and an important member of the miR-302/367 cluster, which is crucial in cellular stemness and a hallmark of diverse tumors (31,32). He *et al* (33) showed that miR-302b-3p is responsible for activation of the AKT pathway. As a target gene to test the effectiveness of transfection using miR-302b-3p, the carnitine O-octanoyltransferase (*CROT*) gene was selected. CROT is an enzyme involved in the transport of medium- and long-chain acyl-CoA out of peroxisomes (34). CROT has also been indicated as a new factor in stimulating vascular calcification by promoting fatty acid metabolism and mitochondrial dysfunction. Notably, inhibiting CROT is considered to have potential as an anti-fibrotic therapy (35).

The aim of the present study was to provide data to support the selection of an appropriate transient transfection method and experimental conditions within these methods for HDFs. Therefore, various delivery systems were investigated for miR-302b-3p transfection, including nucleofection and lipid-based transfection reagents.

Materials and methods

Cell culture. Normal HDFs (NHDFs) were purchased from PromoCell GmbH (NHDF-cryovial adult cell line, cat. no. C-12302) and were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Inc.) containing 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) and 1% Gibco® Antibiotic-Antimycotic Solution (10,000 U/ml penicillin, 10,000 μg/ml streptomycin and 25 μg/ml Gibco Amphotericin B; Gibco; Thermo Fisher Scientific, Inc.) in an incubator (5% CO_2, 37˚C).

Characteristics of miR-302b-3p. Hsa-miR-302b-3p (GenBank LM379226.1, chromosomal location 4q25, approved symbol MIR302B) was used to transfect NHDFs. The miRCURY LNA miRNA Mimic-5' FAM (miR sequence 5'-UAAGUG CUUCCAUGUUUUAGUAG-3') and its inhibitor (miRCURY LNA Inhibitor 5' TACTAAAACATGGAAGCACT-3') were provided by Qiagen GmbH. As a negative control, Negative Control 5 miRCURY LNA miRNA Mimic (Qiagen GmbH; cat. no. YM00479904-ADB; product no. 339173) was used. The guide strands of this control have no homology to any known miRNA or mRNA sequences in mice, rats or humans, and have been tested for adverse effects in multiple cell lines.

Transfection methods. Three non-viral transient transfection methods were employed for the transfection of NHDFs: Transfection with i) Nucleofector Amaxa™ 4D-Nucleofector™ System with X Unit apparatus (hereafter referred to as nucleofection) (Lonza Group Ltd.); ii) Viromer® Blue (Lipocalyx GmbH) and iii) INTERFERin® (Polyplus-transfection SA). As a physical/mechanical method of transfection, nucleofection is an electroporation-based transfection method that allows nucleic acids, such as DNA or RNA, to be transferred into cells by applying a specific voltage and using specific reagents (36). Techniques using Viromer Blue and INTERFERin are chemical transfection methods. According to the manufacturer's protocol, Viromer Blue is a polymer-based transfection reagent featuring a viral mechanism of membrane fusion. The reagent is capable of forming a complex with miRNA and transporting it into cells by endocytosis. By contrast, INTERFERin, a non-liposomal cationic amphiphilic lipid-based transfection reagent, is one of the most efficient reagents for small interfering RNA delivery. The transfection protocols are described hereafter. The reagent to nucleic acid ratio was consistent with the manufacturers' protocols. Different cell densities were used for the three transfection methods because all reagents were used in the cell density range recommended by the manufacturer.

Transfection efficiency was assessed using a fluorescence microscope (Leica DM IL LED; Leica Microsystems, Inc.) by counting the total number of observed cells and the number of cells that express fluorescence of a FAM™ dye-labeled synthetic miRNA.

Nucleofection. Briefly, for nucleofection, cells were passaged 2 days before transfection. Cells ($1x10^5$) were seeded in 96-well plates. The cells were transfected upon reaching a confluence of 90%. The cells were washed with phosphate-buffered saline and dissociated with 0.25% Trypsin-EDTA solution at 37˚C for 5 min. The enzyme was neutralized with DMEM containing 10% FBS. Aliquots of the cells were taken and cell density was determined, and then the required number of cells was resuspended in 20 μl 4D-Nucleofector™ Solution; 5 and 50 nM miRNA mimic and negative control (10% of final sample volume) was added to each sample. For transfection, the P2 Primary Cell 4D-Nucleofactor™ X Kit for Human Dermal Fibroblasts Basic Nucleofector Kit (Lonza Group Ltd.) and 4D-Nucleofector System apparatus (Lonza Group Ltd.) were used according to the manufacturer's protocol. After transfection, the Nucleocuvette™ was incubated at room temperature for 10 min and resuspended in pre-warmed medium to a total volume 100 μl. Next, 25 μl of each sample was transferred to a 96-well culture plate with 175 μl medium in each well (four wells for each sample and negative control). The cells were

then harvested after 4, 24, 48 and 72 h and gene expression was measured.

Viromer blue. The standard complexation protocol was employed for Viromer® Blue. Briefly, $8x10^4$ cells were seeded 1 day before transfection in one well of a 24-well plate in 0.5 ml DMEM. The medium was changed to fresh medium of the same type immediately before transfection. Cells were transfected upon reaching a confluence of 60-80%. Next, the transfection complex was prepared: 45 μl Viromer solution mixed with 5 μl of 5 and 50 nM miRNA mimic. Then, 50 μl of the transfection complex was added to the cells in each well. Effects were monitored 24, 48 and 72 h post-transfection.

INTERFERin. Briefly, ~$2.5x10^4$ cells were seeded 1 day before transfection in a 24-well plate in 1 ml DMEM. The cells were transfected upon reaching a confluence of 30-50%. Three different miRNA mimic concentrations were tested: 1, 3 and 7 nM. Each concentration of miRNA was diluted in 100 μl medium without FBS and mixed with 2 μl INTERFERin. Afterwards, the DMEM was removed, and 0.5 ml fresh medium was added to each well, followed by 100 μl of the transfection mix. *CROT* gene silencing was assessed between 24 and 72 h.

Cell viability and cytotoxicity assays. Metabolic activity and the viability of transfected cells were measured via mitochondrial dehydrogenases, by means of the colorimetric MTT assay (Invitrogen™ CyQUANT™ MTT Cell Viability Assay; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The absorbance was measured at a wavelength of 570 nm using an Infinite M200 Pro Microplate Reader (Tecan Group Ltd.).

To investigate the cytotoxic effects of transfection, the CyQUANT lactate dehydrogenase (LDH) Cytotoxicity Assay (Thermo Fisher Scientific, Inc.) was used. The assay measures the LDH released from cells with a damaged membrane, whereby the amount of LDH released into the medium is quantified via the level of formazan formation. For this purpose, transfection with INTERFERin® and Viromer® Blue was compared with non-transfected NHDFs. A total of 24 h after transfection, the CyQUANT LDH cytotoxicity assay was performed according to the manufacturer's instructions. A sample of Triton-X-100 was used as the positive control for 100% cytotoxicity.

Reverse transcription-quantitative PCR (RT-qPCR) detection of CROT expression. RNA was isolated from NHDFs using the Total RNA Mini Plus (A&A Biotechnology) according to the manufacturer's protocol. The quality of isolated RNA was assessed via electrophoresis using a 1.5% agarose gel (MilliporeSigma) and it was quantified on a NanoDrop 2000c (Thermo Fisher Scientific, Inc.). RT was performed using 0.5 μg total RNA for each sample using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's recommendations. *CROT* (GenBank accession no. NM_021151) was analyzed as a target gene of hsa-miR-302b-3p, according to miRBase (https://mirbase.org). The expression of *CROT* was normalized to the housekeeping genes *GAPDH* (GenBank accession no. NM_002046.3) and 18S ribosomal RNA (*18S*; GenBank accession no. X03205.1). Target and housekeeping gene probes were provided by Applied Biosystems (Thermo Fisher Scientific, Inc.) as ready to use assays: *CROT* TaqMan™ Gene Expression Assay (FAM), Assay ID: Hs00221733_m1; *GAPDH* Human GAPD (GAPDH) Endogenous Control (FAM™/MGB probe, non-primer limited), Assay ID: Hs99999905_m1; *18S* Eukaryotic 18S rRNA Endogenous Control (FAM™/MGB probe, non-primer limited), Assay ID: Hs99999901_s1. qPCR reaction was performed using

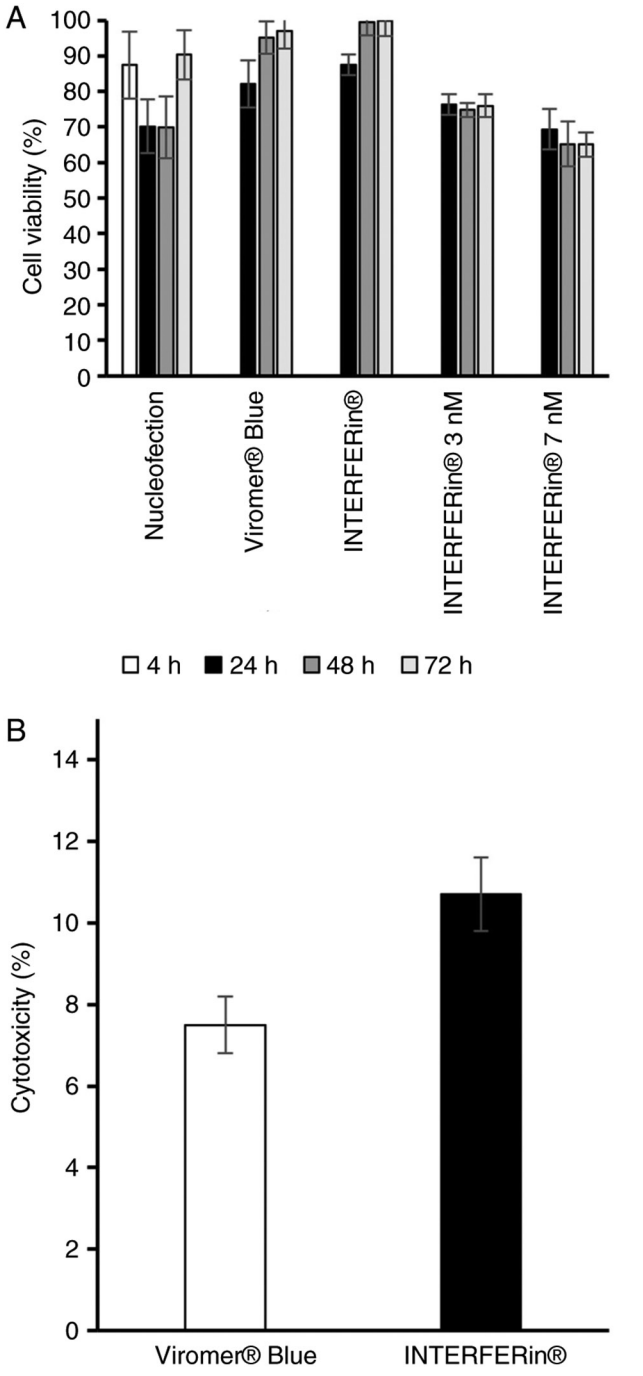

Figure 1. Estimation of cell viability and cytotoxicity. (A) Analyses of cell viability 4, 24, 48 and 72 h after transfection. (B) Analyses of the cytotoxicity of Viromer® Blue and INTERFERin®. Determination of cytotoxicity in non-sense negative control microRNA-transfected fibroblasts. Values are represented as the mean ± SD.

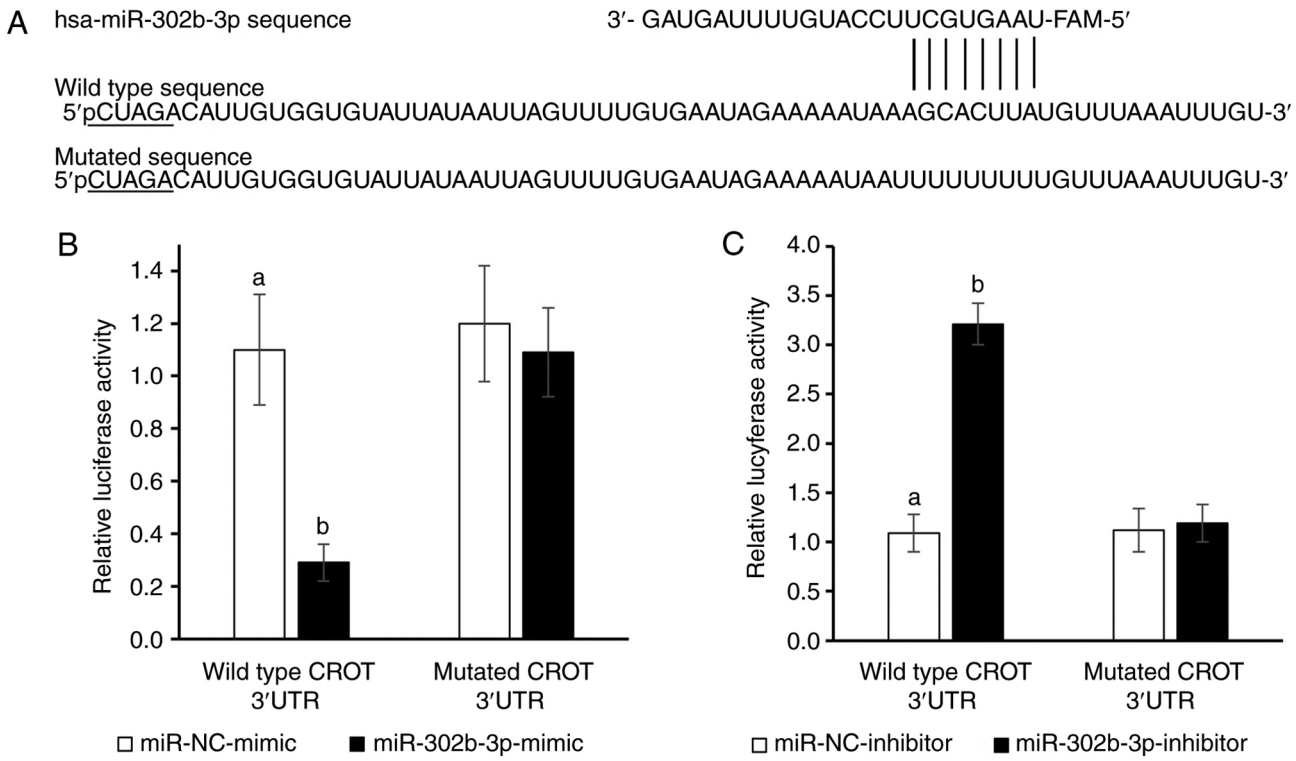

Figure 2. *CROT* is a direct target of hsa-miR-302b-3p in human dermal fibroblasts. (A) Sequences of hsa-miR-302b-3p, and the wild type or mutated 3'-UTR of *CROT*. Relative luciferase activity of human dermal fibroblasts cells transfected with (B) miR-302b-3p mimics or miR-NC mimics, or with (C) miR-302b-3p inhibitor or miR-NC inhibitor and wild type or mutated 3'-UTR of *CROT*. [a,b]P<0.05, mean values marked with different letters are significantly different. Values are presented as the mean ± SD. 3'-UTR, 3'-untranslated region; *CROT*, carnitine O-octanoyltransferase; miR, microRNA; NC, negative control.

TaqMan® Gene Expression Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) as recommended by the manufacturer: 50˚C for 5 min and 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 1 min. Gene expression was assessed using the StepOnePlus™ Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.).

Dual luciferase reporter (DLR) assay. The prediction of downstream target gene sequences of miR-302b-3p was performed using the miRDB website (https://mirdb.org). pmirGLO reporter vectors (Promega Corporation) comprising *CROT*-3'UTR wild type (WT) and *CROT*-3'UTR mutant (MUT) were transfected by nucleofection into NHDF cells along with a miR-302b-3p inhibitor, miR-302b-3p mimics or a miRNA negative control. The Dual-Luciferase® Reporter Assay System (Promega Corporation) was used for the luminescence intensity determination 48 h after transfection. Comparison with *Renilla* luciferase activity was used as a method of normalization.

Statistical analysis. The individual gene expression level was calculated by relative quantitative analysis and the Pfaffl model, including the reaction efficiency for individual genes (37). The normality of the gene expression distribution of all analyzed variables was assessed using the Shapiro-Wilk test. To compare experimental groups, one-way analysis of variance (ANOVA) was used. The significant differences between the analyzed groups were calculated using the Tukey-Kramer HSD post-hoc comparison in Statistica version 10 (StatSoft, Inc.). The results are presented as the mean ± standard deviation of three experimental repeats. P<0.05 was considered to indicate a statistically significant difference.

Results

Cytotoxicity and cell viability. The MTT assay revealed that NHDFs transfected with hsa-miR-302b-3p mimics delivered by INTERFERin, Viromer Blue or nucleofection exhibited lower but not significantly reduced viability when compared with non-transfected control cells (Fig. 1A).

The cytotoxicity assay results demonstrated that, after a 24-h incubation, both of the investigated miRNA delivery agents (Viromer Blue and INTERFERin) had relatively low toxic effects on NHDFs, i.e., 7.5 and 10.7% for Viromer Blue and INTERFERin, respectively (Fig. 1B).

Transfection efficiency. In NHDFs, ~77.8 and 68.3% efficiencies were achieved with nucleofection of 50 nM hsa-miR-302b-3p, after 4 and 24 h, respectively (Fig. S1A). The transfection efficiency with Viromer Blue after 48 h was ~67.8% (50 nM) and 57.9% (5 nM) (Fig. S1B). Efficiencies of ~61.5% (7 nM), 58.2% (3 nM) and 53.3% (1 nM) were achieved with INTERFERin 24 h after transfection. The percentage of transfection was maintained up to 48 h after transfection but decreased after 72 h (Fig. S1C).

CROT is the direct target gene of miR-302b-3p. To confirm that *CROT* is a target of hsa-miR-302b-3p, *CROT* 3'UTR luciferase reporter plasmids containing WT or MUT potential binding sites for miR-302b-3p were designed

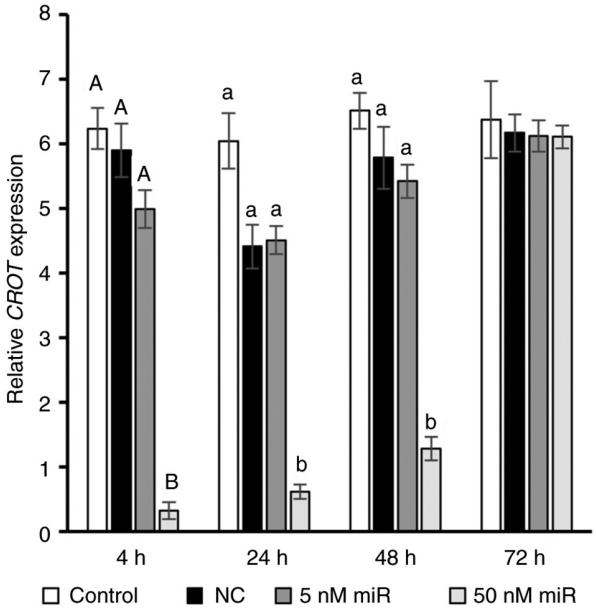

Figure 3. Relative mRNA expression levels of *CROT* in human dermal fibroblasts after nucleofection, as detected by quantitative PCR. [A,B]P<0.01; [a,b]P<0.05, mean values marked with different letters within each timepoint are significantly different. Values are presented as the mean ± SD. *CROT*, carnitine O-octanoyltransferase; miR, microRNA; NC, negative control.

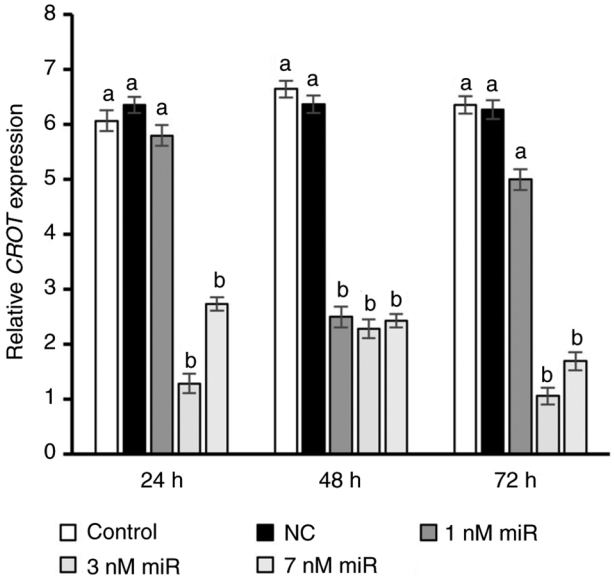

Figure 5. Relative mRNA expression levels of *CROT* in human dermal fibroblasts after chemical transfection with INTERFERin®, as detected by quantitative PCR. [a,b]P<0.05, mean values marked with different letters within each timepoint are significantly different. Values are presented as the mean ± SD. *CROT*, carnitine O-octanoyltransferase; miR, microRNA; NC, negative control.

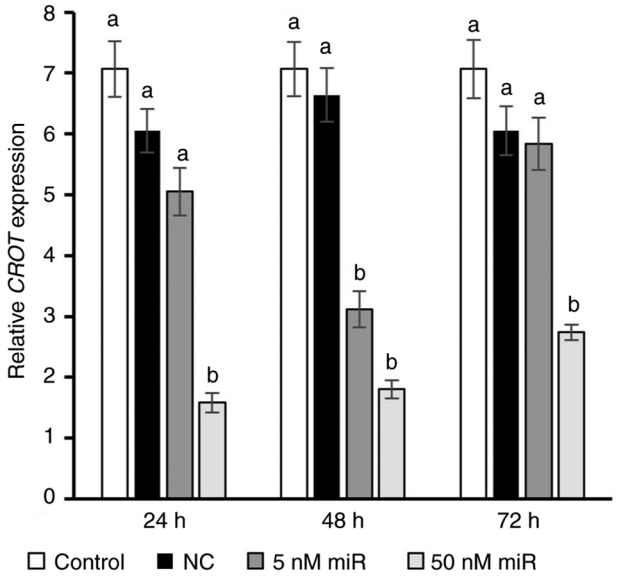

Figure 4. Relative mRNA expression levels of *CROT* in human dermal fibroblasts after chemical transfection with Viromer® Blue, as detected by quantitative PCR. [a,b]P<0.05, mean values marked with different letters within each timepoint are significantly different. Values are presented as the mean ± SD. *CROT*, carnitine O-octanoyltransferase; miR, microRNA; NC, negative control.

(Fig. 2A) and a DLR assay was performed (Fig. 2B and C). Co-transfection of NHDFs with the WT *CROT* 3'-UTR construct and hsa-miR-302b-3p mimic resulted in a significant decrease in cellular luciferase activity compared with cells transfected with the control mimic (P<0.05; Fig. 2B). Overexpression of miR-302b-3p did not affect the luciferase activity of MUT CROT 3'-UTR (Fig. 2B). By contrast, transfection of NHDFs with the miR-302b-3p inhibitor increased the luciferase activity of the WT *CROT* 3'-UTR construct (P<0.05, Fig. 2C), whereas it had no impact on MUT *CROT* 3'-UTR. Overall, CROT was revealed to be a target gene of miR-302b-3p.

Effect of hsa-miR-302b-3p on the expression levels of the CROT gene. Using RT-qPCR, the mRNA expression levels of *CROT* were detected following transfection with hsa-miR-302b-3p. As expected, 50 nM hsa-miR-302b-3p nucleofection caused a decrease in the expression levels of the *CROT* gene in NHDFs; the greatest decline (21.4-fold; P<0.01) was observed after only 4 h. Furthermore, 24 and 48 h after nucleofection, *CROT* transcript expression was 7.5- and 4.7-fold lower compared with the negative control (P<0.05). After 72 h, the expression of the *CROT* gene reached the same level as the negative control. However, transfection with 5 nM miRNA mimic had no effect on *CROT* (Fig. 3).

Viromer Blue-mediated transfection with 50 nM hsa-miR-302b-3p also resulted in a significant decrease in *CROT* gene expression, i.e., 3.8-, 3.7- and 2.2-fold at 24, 48 and 72 h, respectively. In the 5 nM group, a decrease in target gene expression was only demonstrated after 48 h (P<0.05, Fig. 4).

Transfection using INTERFERin was tested at three different concentrations of hsa-miR-302b-3p (1, 3 and 7 nM). Notably, a significant 2.7-fold decrease in *CROT* expression was observed only after 48 h in the 1 nM group, whereas higher concentrations of hsa-miR-302b-3p (3 and 7 nM) decreased target gene expression at all measured time points compared with in the control group (P<0.05; Fig. 5). Moreover, 4.7- and 6-fold lower *CROT* transcript levels were detected in the 3 nM group at 24 and 72 h post-transfection, respectively (P<0.05). Unexpectedly, a weaker effect of 3 nM hsa-miR-302b-3p on *CROT* expression (3-fold decrease) was observed after 48 h (P<0.05). The impact of

3 nM hsa-miR-302b-3p on *CROT* expression was strongest (6-fold decrease) after 72 h ($P<0.05$).

Discussion

Transient transfection can effectively deliver miRNA mimics into *in vitro* cultured mammalian cells, and has been adopted as a fast, easy and economical way to examine the functions and mechanisms of action of endogenous miRNAs (38,39). A range of transfection reagents have been employed to transfect miRNAs into cells in order to achieve genetic manipulation. Non-viral gene delivery methods can be generally divided into chemical and physical approaches; however, there is no single non-viral miRNA deliver strategy that is appropriate for all cell types (24). Therefore, the aim of the present study was to estimate the cytotoxic effects and transfection efficiencies of nucleofection and two lipid-based transfection reagents: Viromer Blue and INTERFERin, on NHDFs.

The present study demonstrated that all of the chosen non-viral transfection systems decreased the expression of a target gene of miR-302b-3p with good efficiencies. However, nucleofection resulted in the highest silencing effect of miR-302b-3p on *CROT* gene expression among all delivery methods; however, its effect lasted for only 48 h. This result is in line with the current state of knowledge. Nucleofection remains the most effective non-viral system for transferring cargo molecules into cells (24). This method has been successfully employed in the transfection of primary HDFs (20,23), rat dermal fibroblasts (23), and mouse and pig embryonic fibroblasts (23,40).

If lipid-mediated transfection is the only method available to alter the expression levels of the target gene, then it is necessary to optimize the transfection conditions by assessing the cytotoxicity of the cargo molecule, the miRNA concentration and transfection time. The cytotoxicity results of the present study indicated that INTERFERin caused a 1.4-fold higher level of toxicity compared with Viromer Blue. These results were also reflected in the viability assay. Carrying out a LDH assay solely for chemical methods may be a limitation of the study. A 1.5-fold higher, but not significant, level of toxicity has also been observed in hepatocytes transfected with INTERFERin compared with L-iMAX, which is based on the Lipofectamine chemical transfection system (41). Cationic lipids may interfere with membrane function, and the integrity of the cell or subcellular compartments, and cause toxicity (42). The most evident difference between cationic lipids and cationic polymers is that polymers do not possess a hydrophobic moiety and are completely soluble in water. They form positively charged complexes with negatively charged phosphate groups in nucleic acids, interact with negatively charged proteoglycans on the cell surface, and enter the cell by endocytosis (43). In contrast to cationic liposomes, they pack the nucleotide molecule into a relatively small space, which may be essential for increasing transfection efficiency, as a smaller particle size may be preferable (42).

Additionally, the concentration of miRNA mimics is another parameter that should be examined with caution. Depending on the transient transfection method, different miRNA mimic concentrations were used, i.e., 5 and 50 nM for nucleofection, 5 and 50 nM for Viromer Blue, and 1, 3 or 7 nM for INTERFERin according to the manufacturers' protocols. Jin *et al* (44) reported that transient transfection of miRNA mimics at high concentrations (>100 nM) could alter gene expression in a non-specific manner. Furthermore, this previous study suggested that miRNA mimics should be introduced into cells at transfection concentrations much lower than the 25 or 100 nM that are commonly used. In this context, the best results were observed for INTERFERin. It is worth adding that the ratio of reagent to nucleic acid may be important, particularly in the case of chemical transfection with the use of cationic lipids or cationic polymers. This is mainly due to the ratio of positive charge contributed by the cationic lipid component of the transfection reagent and negative charge contributed by the phosphates of nucleic acid (45).

Another factor that may have an impact on cytotoxicity or gene expression is cell density. However, such analysis was not part of the present study as the protocols of the three transfection systems were based on the cell density range recommended by the individual reagent manufacturers.

Transfection is one of the procedures used in medical research on fibroblasts. It is widely employed in investigations related to gene therapies, wound healing, skin aging or to obtain pluripotent cells through the reprogramming process, for which fibroblasts are commonly used. Transfection takes advantage of some desirable characteristics of fibroblasts, such as the ease of isolation, rapid cell growth and a high degree of robustness. On the other hand, primary fibroblasts are considered difficult to transfect, mainly due to the low efficiency of transporting cargo molecules into these cells (21,22). Intensive efforts carried out in recent years have led to an increase in the efficiency of the transfer of particles, mainly DNA, to fibroblasts, both in the case of viral (transduction) and non-viral (transfection) systems (24). However, techniques based on viral systems remain the most effective way of transporting cargo molecules into cells. With these methods, a high efficiency of fibroblast transduction has been achieved. Unfortunately, the safety risk of such vectors remains an obstacle.

In conclusion, despite the difficulty in transfecting primary cells, progenitor cells and stem cells, there has been considerable enthusiasm for further improvement of chemical vectors in the hope of achieving efficacies and efficiencies that could potentially mimic viral vectors. Based on the observations of the present study, several recommendations can be made for non-viral transfection systems. Firstly, nucleofection remains the preferred method of choice, especially when using smaller cargo molecules such as miRNA. However, lipid-based transfection systems require lower miRNA concentrations and have longer-lasting effects. The quality of these non-viral systems continues to be improved and they will become an increasingly competitive and low-cost option to consider.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

EO, MK conceived and designed the experiments. MK, PM and EO performed the experiments. EO and MK confirm the authenticity of all the raw data. MK, PM, SB, HM, MM, AS, DZP and EO analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The requirement for ethics approval was waived by the Bioethics Committee of the Medical University of Silesia (Katowice, Poland).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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