

## TECHNICAL ARTICLE

# Expression of green fluorescent protein in human foreskin fibroblasts for use in 2D and 3D culture models

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**ABSTRACT**

The availability of fibroblasts that express green fluorescent protein (GFP) would be of interest for the monitoring of cell growth, migration, contraction, and other processes within the fibroblast-populated collagen matrix and other culture systems. A plasmid lentiviral vector-GFP (pLV-GFP) was utilized for gene delivery to produce primary human foreskin fibroblasts (HFFs) that stably express GFP. Cell morphology, cell migration, and collagen contraction were compared between nontransduced HFFs and transduced GFP-HFFs; no differences were observed. Immunocytochemical staining showed no differences in cell morphology between nontransduced and GFP-HFFs in both two-dimensional and three-dimensional culture systems. Furthermore, there was no significant difference in cellular population growth within the collagen matrix populated with nontransduced vs. GFP-HFFs. Within the limits of our assays, we conclude that transduction of GFP into HFFs did not alter the observed properties of HFFs compared with nontransduced fibroblasts. The GFP-HFFs may represent a new tool for the convenient monitoring of living primary fibroblast processes in two-dimensional or three-dimensional culture.

Since the 1980s, the fibroblast-populated three-dimensional (3D) collagen matrix (FPCM) culture system has been used to model wound contraction,<sup>1,2</sup> cellular migration/motility,<sup>1,3</sup> and other phenomena. The cells exert tension on the matrix<sup>4</sup> and in some cases develop a myofibroblast phenotype similar to that in some healing wounds.<sup>2,5</sup> Although the FPCM model may be more physiologically relevant than a two-dimensional (2D) cell culture model, use of the former has been associated with a number of technical issues, such as reagent molecule absorption to collagen, interference of the collagen with protein assays, and obfuscation of cellular morphology in living (unstained) samples.<sup>6</sup> This latter issue makes tracking of living cells within the collagen matrix difficult. The ability to track living cells within the 3D collagen matrix would facilitate the study of fibroblast migration and motility; such an ability would be relevant to chemotaxis, granulation tissue formation, wound contraction, and other healing-related phenomena.<sup>7,8</sup>

Fluorescent proteins have been used for live-cell imaging over the past decade.<sup>9</sup> Green fluorescent protein (GFP) has been used as a marker or gene activity and as a label for proteins and subcellular compartments within living cells.<sup>10</sup> In addition, GFP-labeled cells can be tracked in tissues and used in numerous GFP-based biochemical sensor applications.<sup>11,12</sup> The availability of stable GFP-expressing primary human fibroblasts would be useful for studying cell growth, migration, and contraction within the 3D collagen matrix model. The purpose of the present report was to determine whether

GFP expression affected select fibroblast functions and to investigate possible applications of GFP-expressing fibroblasts in 2D and 3D in vitro culture systems.

**METHODS****Reagents**

Fetal bovine serum (FBS), normal goat serum (NGS), Dulbecco's Modified Eagle Medium (DMEM; #12100-046),

DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
FPCM	Fibroblast-populated collagen matrix
GFP	Green fluorescent protein
HFF	Human foreskin fibroblasts
LV	Lentivirus
NGS	Normal goat serum
NMII	Nonmuscle myosin II
PBS	Phosphate buffered saline
PDGF	Platelet-derived growth factor
Pen Strep	Penicillin–streptomycin solution
RT	Room temperature
2D	Two-dimensional
3D	Three-dimensional

and 10X-MEM were from Life Technologies (Grand Island, NY). Amphotericin B (BP2645) and GlutaMax Supplement were obtained from Gibco (Grand Island, NY), and penicillin–streptomycin solution (Pen Strep, 15140-122) was obtained from Fisher Scientific (Pittsburgh, PA). PureCol type I bovine collagen (3 mg/mL) was obtained from Advanced Biomatrix (San Diego, CA). GFP (SC8334, rabbit) and  $\beta$ -actin (SC8432, mouse) antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX). (-)-Blebbistatin (B 0560) and human platelet-derived growth factor (PDGF, P8147) both were obtained from Sigma (St. Louis, MO).

### Cell culture

The use of primary dermal fibroblasts derived from discarded human neonatal circumcision specimens was approved by the Research and Development Committee of the Omaha VA Medical Center and by the Institutional Review Board of the University of Nebraska Medical Center. Fibroblasts were cultured from explants of human neonatal foreskins as previously described.<sup>13</sup> Human foreskin fibroblasts (HFFs) were maintained in T75 flasks (Fisher Scientific) with 10% FBS in DMEM (supplemented with 20 mM 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES, Sigma), 44 mM sodium bicarbonate, 1% Pen Strep, 1% GlutaMax, and 750  $\mu$ g/L amphotericin B; final pH = 7.2). HFFs were stored at passage 3–7 (P3-7) in liquid nitrogen. To perform an experiment, a vial of P3-7 HFFs was thawed, plated, and passaged upon confluence. Most experiments were repeated using fibroblasts at passage 10–15 from five different donors.

### Fibroblast populated collagen matrix (FPCM)

The collagen matrix model was utilized as previously described.<sup>13,14</sup> Final matrix parameters were volume = 0.2 mL; diameter = 12 mm; collagen concentration = 1.5 mg/mL; cell concentration =  $1.0 \times 10^6$  cells/mL. Matrices were established in 24-well plates (BD #353047) and incubated in the attached state with 5% FBS in DMEM (supplemented with 50  $\mu$ g/mL ascorbic acid) for approximately 48 hours prior to the initiation of an experiment.

### Lentiviral transduction of primary HFFs

Early passage primary HFFs were transduced with plasmid lentiviral vector-GFP (pLV-GFP, LV-GB-10, Biosettia Inc., San Diego, CA) according to the manufacturer's instructions. In brief, P3-4 primary HFFs were seeded in a 24-well plate ( $1 \times 10^4$  cell/well) and cultured in 1 mL 10% FBS in DMEM. After 2 days, the medium was removed and replaced with 1 mL fresh medium and 8  $\mu$ g/mL polybrene. After gently mixing with a pipette tip, 100  $\mu$ L of LV solution ( $10^7$  IU/mL) was added to each well, followed by gentle swirling and incubation at 37 °C and 5% CO<sub>2</sub> for 24 hours. Following incubation, transduction medium was replaced with fresh medium, and cells then were cultured at 37 °C and 5% CO<sub>2</sub> until >50% confluence was reached (usually 2–3 days). Next, the transduced cells were selected by blasticidin, which eliminated the uninfected cells. The medium was replaced with 1 mL 10% FBS in DMEM and 40  $\mu$ g/mL blasticidin; the cells

then were incubated at 37 °C and 5% CO<sub>2</sub> for 24 hours. The cells subsequently were washed twice with 1 mL of 10% FBS in DMEM. Purity of culture was estimated using a fluorescent microscope (fluorescence microscope, EVOS FL, Bothell, WA). Pure, transduced HFF cultures were expanded and/or stored in liquid nitrogen as needed. Nontransduced HFFs were defined as cells not exposed to the LV or other transduction reagents.

### Immunoblotting

Immunoblotting was utilized as previously described,<sup>13</sup> with minor modifications. FPCMs were collected from culture dishes, washed twice with phosphate buffered saline (PBS), and HFFs were lysed using the mammalian cell lysis kit (MCL1-1KT, Sigma-Aldrich, St. Louis, MO) following manufacturer instructions. Western blots were probed with antibodies recognizing GFP and  $\beta$ -actin (1 : 10,000). Secondary antibodies were goat antimouse or anti-rabbit immunoglobulin G (IgG) conjugated to alkaline phosphatase (1 : 5,000). Signals were detected by chemiluminescence (SuperSignal West Dura Chemiluminescent Substrate, Thermo Scientific, Waltham, MA). Each Western blot was repeated in three different donors. A single representative immunoblot is shown in the Figures.

### Immunocytochemistry and immunohistochemistry

For the 2D model, HFFs were plated in 24-well plates ( $1 \times 10^4$  cell/well) and cultured in 10% FBS in DMEM for 2 days. Collagen matrices were prepared as described earlier and cultured in 5% FBS in DMEM for 2 days. Monolayer HFFs or attached FPCMs were fixed with 4% paraformaldehyde in PBS at 4 °C overnight. Fixed samples were permeabilized for 30 minutes at room temperature (RT) with 0.3% Triton X-100 (Sigma-Aldrich) in PBS. Permeabilized samples were blocked with 10% NGS in 0.3% Triton X-100/PBS at RT for 2 hours. Blocked samples were incubated with primary antibodies (rabbit anti-GFP, 1 : 250; mouse anti-Actin, 1 : 250) in 10% NGS/0.3% Triton X-100/PBS at 4 °C overnight. The samples then were washed three times with PBS and treated with donkey anti-rabbit (conjugated to Alexa-Fluor 488, Life Technologies) and donkey antimouse (conjugated to Alexa-Fluor 576, Life Technologies) secondary antibodies for 2 hours at RT. After three PBS washes, samples were mounted with mounting solution (Prolong Gold antifade reagent with DAPI; P36931 Life Technologies). Slides were examined with an EVOS FL fluorescence microscope.

### Gel contraction assay

FPCM contraction was determined by the floating matrix contraction assay.<sup>15</sup> Briefly, matrices were polymerized, covered with 2 mL of 5% FBS in DMEM, released from the culture well with a sterile spatula, and then incubated at 37 °C. Matrices were fixed at different time points after release with 4% paraformaldehyde in PBS at 4 °C overnight and scanned with a desktop flatbed scanner. Matrix area was measured using software ImageJ ([rsbweb.nih.gov/ij/](http://rsbweb.nih.gov/ij/)) and was expressed as a ratio of released matrix area to starting attached matrix area.

### Measurement of cell number per matrix

Cell number per matrix for the FPCM model was determined using a Scepter Cell Counter (EMD Millipore, Billerica, MA, USA). Cells were retrieved from the collagen matrix, as previously described.<sup>16</sup> Briefly, each retrieved matrix was digested with 400  $\mu$ L collagenase type I solution (Life Technologies; 5 mg/mL in 130 mM NaCl, 10 mM Ca acetate, 20 mM HEPES, pH 7.2) and 200  $\mu$ L 0.05% trypsin/Ethylenediaminetetraacetic acid (EDTA, Life Technologies) at 37 °C with intermittent vortexing every 5 minutes until digestion was complete. Digestion was stopped with the addition of 10% FBS in DMEM. Cells were collected by centrifugation at 600  $\times$  *g* for 4 minutes and then resuspended in 500  $\mu$ L PBS for Scepter counting. The means shown in the figures represent data from five experiments each performed on a different cell strain.

### In vitro scratch assay

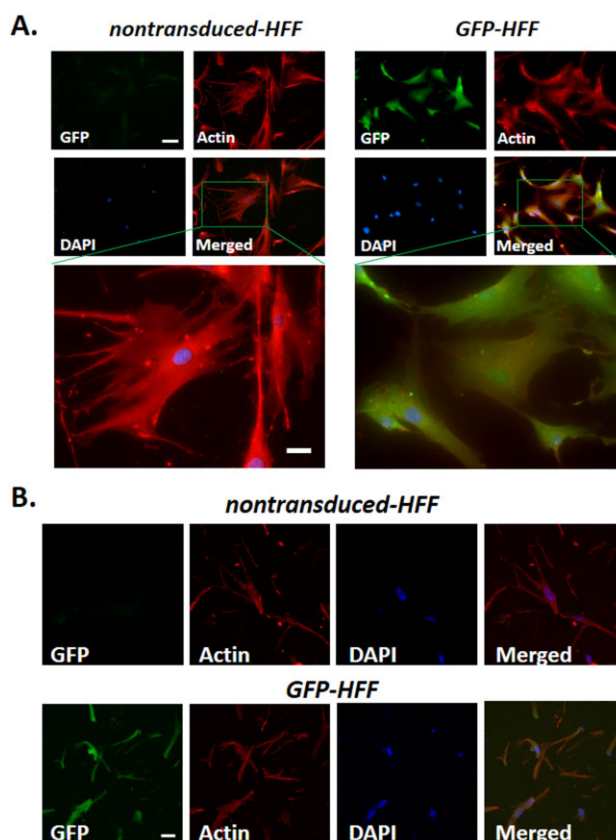
Cell migration in a 2D culture system was evaluated using an in vitro scratch assay.<sup>17</sup> Briefly, HFFs were seeded into 24-well plates ( $1 \times 10^5$  cell/well) and cultured in 10% FBS in DMEM for 24 hours, which resulted in confluence of ~70–80%. Using a sterile 200  $\mu$ L pipette tip, a line was scratched in the monolayer across the center of the well, keeping the pipette tip perpendicular to the plate. A second straight line then was scratched perpendicular to the first line to create a cross-shaped gap that was devoid of cells and about 800  $\mu$ m wide. Each well was washed twice with 1 mL DMEM to remove any non-adherent cells. Digital images of the cell gap were captured at set intervals, and the gap width was measured using ImageJ. Each experiment was repeated with five different cell strains.

### Cell migration

The nested collagen matrix model was utilized to quantify cell migration in a 3D matrix as previously described,<sup>8</sup> with some modifications. For the attached nested assay, an FPCM was incubated in the attached state for 48 hours prior to removal from the culture dish and embedding in a fresh acellular collagen matrix, which then was allowed to polymerize for 1 hour. For the released nested assay, an FPCM was incubated in the attached state for 48 hours, then released for 24 hours, and embedded in a fresh acellular collagen matrix, which then was allowed to polymerize for 1 hour. Each a cellular neomatrix remained attached to the culture dish for the duration of each experiment. Cellular migration out of the nested matrix and into the cell-free matrix was imaged with a fluorescent microscope in unfixed samples. To quantify cell migration, digital images were captured from five microscopic fields (constant size) randomly selected at the edge of the embedded matrix, at 1 and 24 hours after embedding. Cells that had migrated out from the edge of the embedded matrix were counted manually. Each experiment was repeated with five different cell strains.

### Statistics

Data are expressed as mean  $\pm$  standard error of the mean. Unpaired numerical data were compared using the unpaired *t*



**Figure 1.** Morphology of nontransduced human foreskin fibroblasts (HFFs) and green fluorescent protein (GFP)-HFFs in two-dimensional and three-dimensional culture. (A) Representative images of monolayer nontransduced HFFs vs. GFP-HFFs after immunocytochemistry for GFP and actin, with merged images. Bars: main = 100  $\mu$ m; inset = 50  $\mu$ m. (B) Similar comparative images obtained from the intact, attached collagen matrix. Bar = 20  $\mu$ m.

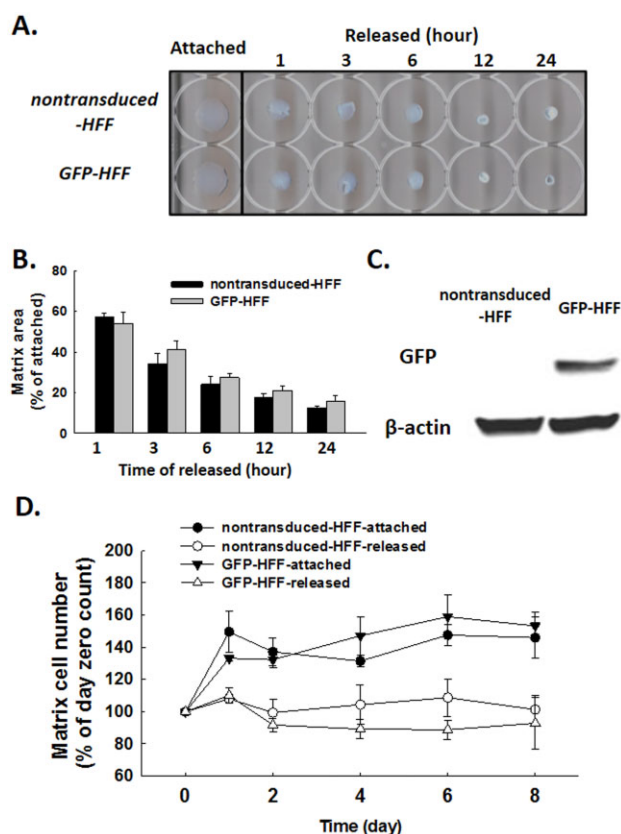
test (two groups) or ANOVA (more than two groups), with statistical significance set at  $p < 0.05$ .

## RESULTS

### Similar morphology between nontransduced HFFs and GFP-HFFs

The morphology of nontransduced HFFs vs. GFP-HFFs in 2D and 3D culture is shown in Figure 1A–B, respectively. Cells were double-immunostained for GFP and actin. With visual inspection, all lentiviral-infected cells in both 2D and 3D culture expressed GFP, which localized to the cytoplasm (merged images, Figure 1A). There were no qualitative differences in fibroblast morphology between the nontransduced HFFs and the GFP-HFFs in either 2D or 3D culture. The presence of visible GFP expression throughout the lentiviral-infected cell populations indicated that the transduction process was qualitatively efficient.





**Figure 2.** Matrix contraction and cell number in the attached vs. released fibroblast-populated collagen matrix (FPCM) using nontransduced human foreskin fibroblasts (HFFs) vs. green fluorescent protein (GFP)-HFFs. (A) Representative images showing attached and released matrices after timed contraction of the FPCM with nontransduced HFFs vs. GFP-HFFs. (B) Time course of matrix diameter after release of the FPCM with nontransduced HFFs vs. GFP-HFFs (mean  $\pm$  standard error of the mean [SEM] of five separate experiments). (C) Representative immunoblots showing expression of GFP in nontransduced HFFs vs. GFP-HFFs in the FPCM. (D) Matrix cell number (expressed as a percent of the day zero count) in the attached vs. released FPCM populated with nontransduced HFFs vs. GFP-HFFs. Each data point represents the mean  $\pm$  SEM from five separate experiments.

### Similar matrix contraction in the 3D matrix populated with nontransduced HFFs vs. GFP-HFFs

Matrix contraction is a commonly employed assay involving the FPCM, so we wanted to determine if GFP expression would affect floating matrix contraction.<sup>15</sup> There were no gross differences in matrix area with nontransduced HFFs vs. GFP-HFFs (Figure 2A). The ratio of released to attached matrix area at 0–24 hours showed no significant differences between nontransduced HFF and GFP-HFF matrices (Figure 2B). Western blot analysis confirmed the presence and absence of GFP in GFP-HFFs and nontransduced HFFs, respectively (Figure 2C).

### Similar cell population growth in the 3D matrix populated with nontransduced HFFs vs. GFP-HFFs

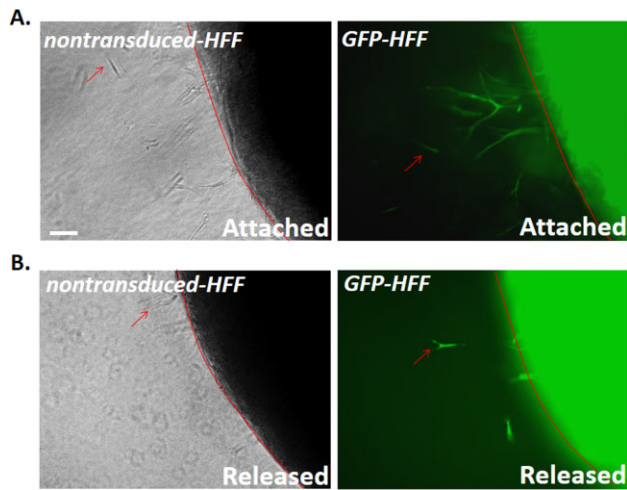
Previous work showed that fibroblast population within the collagen matrix increases in the attached state and decreases in the released state through combined effects on cellular proliferation and survival.<sup>14,18</sup> So, we next wanted to determine whether GFP expression in the fibroblast would affect expected changes in population growth within the collagen matrix. Fibroblasts were retrieved from attached and released matrices at various time points, and matrix cell number was quantified. Matrix cell number was expressed as a percent of the day zero count for each matrix type. As expected, matrix cell number gradually increased in the attached state with both nontransduced HFFs and GFP-HFFs during the 8-day period, with no significant differences between the two cell types (Figure 2D;  $p > 0.05$ , ANOVA). Also as expected, cell population growth remained flat in the released state with both nontransduced HFFs and GFP-HFFs, with no significant differences between the two cell types (Figure 2D;  $p > 0.05$ , ANOVA).

### Similar 3D cell migration ability with nontransduced HFFs vs. GFP-HFFs

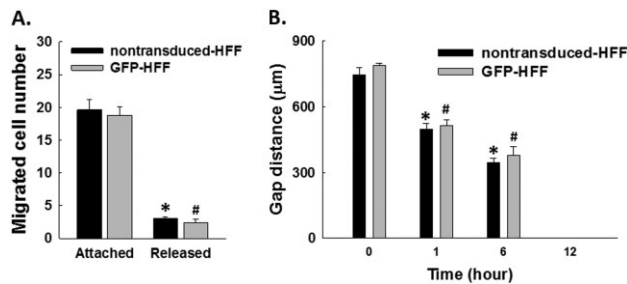
A recently described 3D assay of cellular motility involves the migration of fibroblasts out of their native collagen matrix and into an adjacent acellular matrix.<sup>8</sup> This is accomplished by embedding an established FPCM into a fresh, acellular collagen matrix. In the presence of serum or growth factors, the fibroblasts will migrate out of the populated (“nested”) collagen matrix and into the enveloping, acellular collagen matrix. We compared the ability of nontransduced HFFs vs. GFP-HFFs to migrate out of either attached nested or released nested (Figure 3) collagen matrices. After 24 hours of embedding in a fresh acellular collagen matrix, there were no significant differences in the number of cells that had migrated out of the nested FPCM between nontransduced HFFs vs. GFP-HFFs in either the attached or released states (Figure 4A). As expected, fibroblasts in the presence of serum migrated in much greater numbers out of the attached nested matrix compared with the released nested matrix, but the fibroblast type (nontransduced vs. GFP-HFF) did not influence this migration.

### Similar 2D cell migration ability with nontransduced HFFs vs. GFP-HFFs

A more traditional 2D migration assay for fibroblasts (and other adherent cells) is the monolayer wound healing assay also known as scratch assay.<sup>17</sup> This assay tests the ability of monolayer cells to migrate across a defined gap (typically on tissue culture plastic). We wanted to determine whether lentiviral transduction and GFP expression would affect the fibroblast’s ability to migrate on a 2D surface. The scratch gap distance (in microns) was compared between nontransduced HFFs and GFP-HFFs in the presence of serum (Figure 4B). As expected, the fibroblasts migrated to close the gap, but there was no significant difference in gap distance at 0, 6, or 12 hours between the nontransduced vs. GFP-HFFs ( $p > 0.05$ , unpaired  $t$  test).



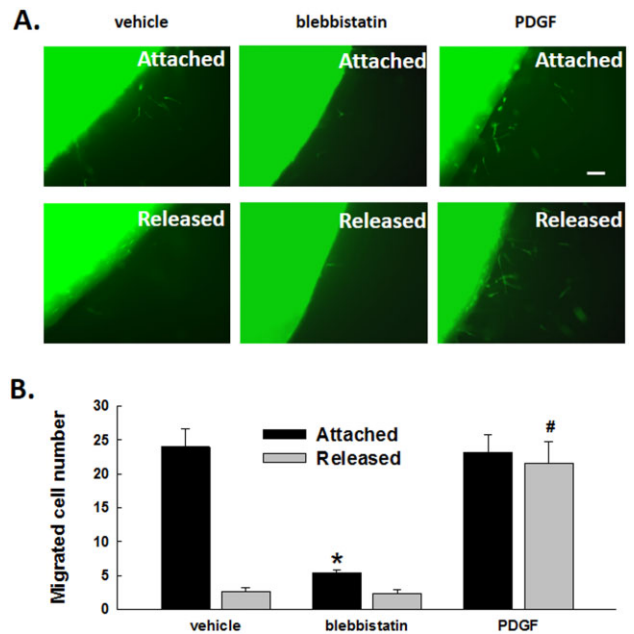
**Figure 3.** Migration of nontransduced human foreskin fibroblasts (HFFs) vs. green fluorescent protein (GFP)-HFFs out of the nested fibroblast-populated collagen matrix (FPCM). (A) Representative images showing nontransduced HFF and GFP-HFF migration out of an attached FPCM nested into a cell-free attached matrix. The interface between each nested populated matrix and the enveloping acellular matrix is indicated with a curved red line. Each arrow indicates a typical fibroblast migrating out of the nested matrix. (B) Representative images showing nontransduced HFF and GFP-HFF migration out of a released nested FPCM into a cell-free attached matrix. Bars: 100  $\mu\text{m}$ .



**Figure 4.** Migration of nontransduced human foreskin fibroblasts (HFFs) vs. green fluorescent protein (GFP)-HFFs in three-dimensional (3D) and two-dimensional (2D) assays. (A) Migrated cell number of nontransduced HFFs vs. GFP-HFFs out of either the attached nested fibroblast-populated collagen matrix (FPCM) or the released nested FPCM (Figure 3). Each bar represents the mean  $\pm$  standard error of the mean (SEM) of five separate experiments; \* $p < 0.05$  vs. nontransduced attached; # $p < 0.05$  vs. GFP attached (unpaired  $t$  tests). (B) Scratch gap distance (2D migration assay) of nontransduced HFFs vs. GFP-HFFs; each bar represents the mean  $\pm$  SEM of five separate experiments; \* $p < 0.05$  vs. nontransduced time zero; # $p < 0.05$  vs. GFP time zero (ANOVA and unpaired  $t$  tests).

**Utility of GFP-HFFs in the study of fibroblast migration**

The mechanism by which fibroblast migration out of the released nested matrix is decreased with respect to the attached nested matrix (refer to Figures 3–4) is not clear. As an example of how the GFP-HFFs might be used to study this phenomenon, the effects of some soluble mediators on serum-stimulated fibroblast migration in the nested matrix assay are shown in Figure 5. GFP-HFF migration out of the attached nested matrix was decreased by blebbistatin (an inhibitor of nonmuscle myosin II [NMII]<sup>10</sup>). The role of NMII in fibroblast migration is a complex and evolving story, but current evidence suggests that NMII is essential in fibroblast motility through stiffer substrates<sup>10</sup> (e.g., an attached FPCM with established stress). Treatment with PDGF increased GFP-HFF migration out of the released nested matrix; this result is consistent with previous observations that PDGF stimulates fibroblast migration out of nonstressed collagen matrices.<sup>8</sup> These data are presented here to show the utility of the GFP-HFFs in probing specific biologic phenomena. A detailed dissection of the migration pathways operating in the attached nested vs. released nested matrix, however, is beyond the scope of this paper.



**Figure 5.** Effect of blebbistatin or platelet-derived growth factor (PDGF) on fibroblast migration out of the nested fibroblast-populated collagen matrix (FPCM). (A) Representative images showing green fluorescent protein (GFP)-human foreskin fibroblasts (HFFs) migration out of the nested FPCM in the presence of blebbistatin (20  $\mu\text{M}$ ), PDGF (20 ng/mL) or vehicle. (B) Migrated cell number of GFP-HFFs out of the attached nested or released nested FPCM. Each bar represents the mean  $\pm$  standard error of the mean (SEM) of five separate experiments; \* $p < 0.05$  vs. attached nested matrix treated with vehicle; # $p < 0.05$  vs. released nested FPCM treated with vehicle (ANOVA and unpaired  $t$  tests).

## DISCUSSION

In this study, we demonstrated the feasibility of stable GFP expression in HFFs transduced with a lentiviral vector for use in 2D- or 3D-culture systems. There were no differences in cellular morphology, matrix contraction ability, cellular migration, and cellular population growth between the nontransduced HFFs and GFP-HFFs. As the relative abundance of collagen within the FPCM model can interfere with morphological, molecular, and functional assays, GFP labeling of cells offers the advantage of visualizing living cells cultured in a 3D matrix. Moreover, it can facilitate the tracking of cells in real time (in both 2D and 3D) as they participate in various processes.<sup>19</sup> Such advantages should facilitate the in vitro study of fibroblast physiology in real time within living 3D culture systems.

Adenoviral, retroviral, and lentiviral vectors commonly are used to deliver genetic material into cells. Adenoviral DNA does not integrate into the genome, and is not replicated during cell division. Retroviruses have the ability to integrate into the host genome in a stable fashion and have been used in gene therapy.<sup>20</sup> LVs are a subclass of retroviruses. Unlike retroviral systems, an LV can integrate into the genome of nondividing cells, which makes lentiviral integration independent of the cell cycle.<sup>21</sup> So, we elected to use the lentiviral system to deliver GFP to HFFs. A potential downside of using the lentiviral system is disruption of cellular genes and activation of oncogenes. Studies in both CD34<sup>+</sup> cell lines and in mice, however, have shown that lentiviral vectors have a lower tendency to cause mutation than gamma-retroviral vectors.<sup>21,22</sup> In addition, other investigators have found that lentiviral transduction did not adversely affect mesenchymal stem cell biology.<sup>23</sup>

In the present study, we did not find any significant effect of GFP expression on morphology, contraction, migration, and population growth in HFFs. It is likely that increasing the degree of GFP expression within HFFs will, at some point, produce detectable effects on cellular physiology. Moreover, increasing the sensitivity of select assays or utilizing different assays might have detected an effect of GFP expression in our study. There were numerous other endpoints that we could have assayed in this study, including gene transcription, protein synthesis, and cytokine release. In our study, the use of functional endpoints such as migration, contraction, and cellular population growth requires coordination of multiple cellular processes (not reviewed here) and presumably would be among the endpoints utilized in studies with GFP-expressing fibroblasts. It does not appear, however, that the multiple processes involved with these endpoints were perturbed enough to influence the endpoints. So, within the limits of our assays and the degree of GFP expression that we obtained (which we did not quantify), an effect of GFP transduction was not observed on HFFs.

An incidental finding in this study was that the motility of fibroblasts out of the nested released matrix was decreased compared to the nested attached matrix. It has been shown that matrix release induces expressional change of proteins related to both apoptosis and proliferation, including the RAS-RAF-MEK-MAPK axis,<sup>24</sup> the  $\beta$ 1-integrin-FAK-PI3-kinase-Akt axis,<sup>25</sup> as well as phosphatase and tensin homolog deleted on chromosome 10 (PTEN/MMAC1)<sup>26</sup> and integrin-linked kinase.<sup>27</sup> Whether the effect of matrix release on fibroblast migration in the nested model is secondary to a direct

mechanical effect,<sup>28</sup> a change in gene transcription or some other mechanism would require further investigation (refer to Figure 5).

In conclusion, lentiviral delivery of GFP to primary human fibroblasts was an effective method of producing stable expression of GFP in HFFs. This GFP expression did not appear to have a major effect on fibroblast phenotype other than the fluorescence. The availability of GFP-HFFs may facilitate the study of cellular and gene functions of HFFs in 2D and 3D culture.

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*Conflicts of Interest:* The authors declare no competing financial interests.

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