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Pseudotyped Adeno-Associated Viral Vectors for Gene Transfer in Dermal Fibroblasts: Implications for Wound Healing Applications

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Abstract

Background—Cell specific gene transfer and sustained transgene expression are goals of cutaneous gene therapy. Pseudotyping strategy with AAV vectors has the potential to confer unique cellular tropism and transduction efficiency. We hypothesize that pseudotyped AAV vectors have differential tropism and transduction efficiency under normal and wound conditions in dermal fibroblasts.

Materials and Methods—AAV2 genome with GFP reporter was packaged in capsids of other serotypes AAV5, AAV7, and AAV8, producing pseudotyped vectors AAV2/5, AAV2/7, and AAV2/8 respectively. Murine and human dermal fibroblasts were transduced by the different pseudotypes for 24 hours at multiplicities of infection (MOI) 10², 10³, 10⁴, and 10⁵. Transduction efficiency was assessed at days 3 and 7. Experiments were repeated in a simulated wound environment by adding 10ng/ml PDGF-B to culture media.

Results—Transduction efficiency of the pseudotyped AAV vectors is dose dependent. MOI 10⁵ results in significantly higher gene transfer. Under normal culture conditions, the pseudotyping strategy confers differential transduction of dermal fibroblasts, with significantly enhanced transduction of murine cells by AAV2/5 and AAV2/8 compared to AAV2/2. AAV2/8 is more efficacious in transducing human cells. Under wound conditions, transduction efficiency of AAV2/2, 2/5 and 2/8 is significantly lower in murine fibroblasts. At day 3 under wound conditions, all vectors demonstrate similar transduction efficiency, but by day 7 the three pseudotyped vectors transduce significantly more murine cells compared to AAV2/2. However, in human cells, there is no significant difference in transduction efficiency of each pseudotype between normal and wound conditions at both 3 and 7 days.

Conclusions—The AAV pseudotyping strategy represents a gene transfer technology that can result in differential transduction of dermal fibroblasts. The differences in transduction efficiency

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in murine and human dermal fibroblasts both in the normal as well as wound environment highlight issues with translatability of gene transfer techniques. These data provide a template for using pseudotyped AAV vectors in cutaneous applications.

Keywords

wound healing; gene therapy; adeno associated virus; pseudotyping; regenerative medicine

Introduction

Chronic non-healing wounds represent a significant cause of morbidity and accounts for significant public health care cost (1). These wounds are characterized by decreased levels of vulnerary growth factors and a hostile proteolytic environment (2-4). Supplementation of recombinant growth factors is a logical therapeutic strategy, but that has been proven to require large, repetitive dosing to achieve even modest improvements (5). An ideal delivery system for cytokines and growth factors to chronic wounds should result in local and sustained delivery, in order to correct the pathophysiologic wound impairments.

Gene therapy for cutaneous wound healing has the potential to provide local gene transfer and sustained transgene expression with lower risk of systemic toxicity, and has already been proven effective in various experimental wound healing models (3, 6-13). For successful translation of gene therapy to chronic wounds, the gene must be efficiently delivered to the specific target cell in which it must be expressed and sustained at therapeutic levels (7, 14, 15).

An important cellular target of gene therapy applications for wound healing is the dermal fibroblasts because they are the main effector cells in wound repair (16). Fibroblasts produce both pro-inflammatory and anti-inflammatory cytokines and matrix metalloproteases (17, 18). They are actively involved in the formation of the blood vessel network that takes place during wound healing, and are crucial to successful extracellular matrix remodeling and restoration of tissue strength and integrity (19-21). Transduction efficiency of dermal fibroblasts can be a major determinant of gene therapy efficacy in chronic wounds.

Several viral vectors have been used for gene therapy applications (22, 23). While adenoviral mediated gene transfer is promising, it does not offer sustained long term gene expression. Efficient and long-term gene transfer in murine skin has been demonstrated using HIV-based recombinant lentiviral vectors. However, lentiviruses are associated with an increased risk of oncogenic transformation due to insertional mutagenesis, limiting their therapeutic utility in near future (24). In contrast, adeno-associated viral (AAV) vectors have demonstrated an improved safety profile with a lower risk for insertion events (25). AAV has the ability to transduce dividing as well as non-dividing cells with extended long-term transgene expression. Further, their non-pathogenicity to humans enables them to be an attractive vector for clinical gene therapy and AAV serotype 2 has been widely used in preclinical and clinical trials (26-28). Several recent studies demonstrate that an AAV pseudotyping strategy, where the capsid of AAV2 is replaced with the capsid of another AAV serotype, can result in distinct transduction efficiency and tropism profiles. Previous studies have reported that pseudotype AAV2/5 has significantly greater transduction of muscle compared to AAV2/2. Additional pseudotypes AAV2/7 and AAV2/8 have demonstrated robust and tissue specific transduction profiles in liver, pancreas and cardiac tissue (7, 25, 28-31).

We have recently reported that pseudotyped AAV vectors delivered to murine wounds produce distinct tropism and efficiency profiles in the three cellular compartments of the skin (7). AAV2/5 and AAV2/8 result in significant enhancement of gene transfer to the cells

in the epidermis (keratinocytes) and dermal matrix (fibroblasts), when compared to AAV2/2. This data demonstrates that pseudotyping strategy can be used to tailor/control gene transfer efficiency to the desired target cells. In order to translate this work to human applications, it would be advantageous to understand how these pseudotyped AAV vectors transduce human cells, and particularly understand the effect of wound environment on transduction efficiency of dermal fibroblasts by the different pseudotypes.

Taken together, this has led us to hypothesize that pseudotyped AAV vectors can result in distinct tropism and transduction efficiency under normal and wound conditions in murine and human dermal fibroblasts. We characterized and compared cell specific transduction efficiencies of the AAV2/2 and pseudotyped AAV2/5, 2/7 and 2/8 vectors *in vitro* using primarily isolated murine and human dermal fibroblasts under normal and simulated wound conditions.

Methods

Adeno-associated virus production

All adeno-associated viral vectors (AAV) were obtained from Vector Core at the University of Pennsylvania). Vectors were produced as previously described (25). The AAV2/2 serotype was constructed by standard transfection protocols and purified by single step heparin chromatography. A pseudotyping strategy was used to produce AAV vectors packaged with the capsid proteins of AAV5, AAV7 and AAV8. Recombinant AAV genomes equipped with AAV2 inverted terminal repeats (ITRs) were packaged by triple transfection of 293 cells with cis-plasmid, adenovirus helper plasmid and a chimeric packaging construct where the AAV2 rep gene is fused with cap genes of other AAV serotypes. To create the chimeric packaging constructs, the XhoI site of p5E18 plasmid at 3,169 bp was ablated, and the modified plasmid was restricted with XbaI and XhoI in a complete digestion to remove the AAV2 cap gene and replace it with a 2,267-bp SpeI/XhoI fragment containing AAV5, AAV7 or AAV8 cap gene. For all AAV vectors, a cDNA bacterial green fluorescent protein (GFP) was inserted as a reporter and vectors were driven by a CMV promoter. Pseudotyped recombinant vectors were purified by the standard CsCl₂ or iodixanol sedimentation method. Genome copy (GC) titers of AAV vectors were determined by TaqMan (Applied Biosystems, Foster City, CA) analysis, using probes and primers targeting SV40 poly (A) region.

Cell Culture

Human dermal fibroblasts were obtained from Life Technologies (Grand Island, NY). Primary adult murine dermal fibroblasts were isolated in our laboratory from C57BL/6J mice (Jackson Labs, Bar Harbor, ME) using standard isolation protocols. The fibroblast culture was maintained in Dulbecco's modified Eagle's Media (DMEM) (GIBCO, Carlsbad, CA) supplemented with 10% bovine growth serum (BGS) (Hyclone, Logan, UT) and penicillin 100 units/ streptomycin 100 µg/ amphotericin B 0.25 µg (PSF) (Invitrogen, Carlsbad, CA) at 37 °C with 5% CO₂, and cells between passage 5-10 were used for experiments.

In vitro comparison of transduction efficiencies of AAV and pseudotyped vectors

Human or mouse fibroblasts were transduced with AAV pseudotyped vectors using an *in vitro* culture system. 7000 cells were seeded per chamber in 4-chamber slides in normal culture media. Cells were allowed to attach overnight and then transduced with AAV2/2 and pseudotyped AAV2/5, 2/7 and 2/8 GFP vectors for 24 hours. AAV vector preparations were added at different multiplicities of infection (MOI: 100, 1000, 10000, 100000) to cells under reduced serum conditions (media containing 2% BGS). After 24 hours the AAV

preparations were removed and chambers slides were rinsed. The cells were cultured in normal media (media containing 10% BGS) for up to 7 days. GFP positive cells were counted at day 3 and 7 using Nikon 80I microscope and Nikon Elements analysis software (Melville, NY). A total of 150-200 cells were counted in 6-10 20x magnification fields per AAV preparation. The mean of percentage of transduced cells per AAV preparation are reported. Each experiment was repeated three times.

In order to stimulate wound environment and test the efficiency of the different pseudotyped vectors to transduce fibroblasts in a cutaneous wound, 10 ng/ml PDGF recombinant protein (murine or human, species specific) was added to the culture media and the above experiments were repeated.

Statistical Analysis

All data are presented as mean±standard deviation. Two-way analysis of variance (ANOVA) followed by Bonferroni test was used for comparing data between different AAV vectors. For comparing different doses or time points Student's t-test and one-way ANOVA followed by Tukey's multiple comparison tests were used. Probability values of $p<0.05$ were interpreted to denote statistical significance.

Results

Dose dependent transduction efficiency of different AAV vectors

To compare tropism and transduction efficiency profiles of the pseudotyped AAV vectors, we first need to define the titer at which highest gene transfer efficiency is achieved. To assess this, cells were transduced with different multiplicities of infection of the vectors for 24 hours under normal culture conditions. The transduction efficiency of the pseudotyped AAV vectors and the levels of transgene delivery in the fibroblasts are influenced by the AAV titers. MOI 10^5 results in significantly higher gene transfer compared to other MOIs ($p<0.05$) in both murine and human fibroblasts at both early (day 3) and later (day 7) time points (Figure 1). The results further demonstrate that at day 3, AAV2/5 and AAV2/8 can be used at much lower titers of MOI $10^2 - 10^3$ to achieve at least 40% transduction (arbitrarily chosen), as compared to > 2 -log higher titers of AAV2/2 and AAV2/7 to achieve similar transduction efficiency. There is no evidence of vector toxicity at high MOI of 10^5 in any culture. Based on this data, all further experiments were performed at MOI 10^5 .

Species Specific Tropism and Transduction Efficiency of AAV2/2

Under normal cell culture conditions, AAV2/2 administration (at MOI 10^5) results in a lower rate of gene transfer to both murine ($43.9\% \pm 11.1$) and human ($51.0\% \pm 9.9$) fibroblasts at day 3 post transduction. However, at the day 7 time point, AAV transduction efficiency significantly increases in both cell types ($p<0.05$). Compared to murine cells ($80.9\% \pm 5.3$), human fibroblasts ($97.7\% \pm 3.1$) demonstrate increased transduction efficiency by AAV2/2 at day 7 (Table 1).

Tropism and Transduction Efficiency of Pseudotyped AAV Vectors

Under normal culture conditions, the pseudotyping strategy confers differential transduction of dermal fibroblasts. At day 3 post transduction, in murine fibroblasts, AAV2/5 has maximal gene transfer ($66.6\% \pm 15.7$), followed by AAV2/8 ($53.8\% \pm 10.3$) and AAV2/2 ($43.9\% \pm 11.1$). Both AAV2/5 and AAV2/8 demonstrate significantly higher transduction efficiencies compared to AAV2/7 ($30.7\% \pm 9.0$) (Figure 2A, Table 1, 2). Human fibroblasts are also differentially transduced by the AAV pseudotyped vectors. AAV2/8 has maximal transduction efficiency in human fibroblasts ($80.1\% \pm 9.3$), followed by AAV2/5 ($61.0\% \pm 14.8$). Similar to murine transduction efficiencies, both AAV2/5 and AAV2/8 transduce

significantly more human fibroblasts, compared to AAV2/2 ($51\% \pm 9.9$) and AAV2/7 ($53.1\% \pm 10.3$) (Figure 3A, Table 1, 2).

At 7 days after AAV administration, the percentage of transduced cells by the pseudotyped vectors increase significantly compared to day 3. Interestingly, AAV2/2 results in doubling of the percent transduced cells at all MOIs from day 3 to day 7. Notably in murine fibroblasts, AAV2/5 ($97.3\% \pm 2.8$) demonstrates a higher transduction efficiency compared to AAV2/2 ($80.9\% \pm 5.3$), AAV2/7 ($66.4\% \pm 9.0$), and AAV2/8 ($82.0\% \pm 5.1$) (Figure 2B, Table 1,2). AAV2/8 appears to be more efficacious in transducing human cells at both time points. At day 7, AAV2/2 demonstrates increased transduction similar to AAV2/8 ($p=ns$). In human fibroblasts, AAV2/2 ($97.7\% \pm 3.1$) and AAV2/8 ($94.6\% \pm 6.3$) result in significantly higher gene transfer rates compared to AAV2/5 ($75.4\% \pm 11.4$) and AAV2/7 ($77.0\% \pm 10.6$) (Figure 3B, Table 1,2).

Comparison of AAV2/2 and pseudotyped AAV Vectors transduction efficacy under normal and wound conditions

Simulating wound conditions (with increased PDGF expression in the cell culture media) results in significantly lower gene transfer by each of the AAV 2/2, 2/5, 2/7 and 2/8 vectors in murine fibroblasts as compared to gene transfer under normal (control) conditions, at both 3 and 7 days ($p < 0.01$). In murine fibroblasts, at 3 days under wound conditions, there is no difference in transduction efficiency between the different pseudotypes (Day 3: AAV2/2 $24.3\% \pm 3.9$, AAV2/5 $23.9\% \pm 5.4$, AAV2/7 $35.5\% \pm 14.3$, AAV2/8 $31.0\% \pm 9.4$; $p=ns$) (Figure 2A). Even under wound conditions, by day 7 all pseudotyped vectors transduce significantly more murine cells ($p < 0.005$). However, there were no differences in transduction efficiency between the different pseudotypes (Day 7: AAV2/2 $63.6\% \pm 7.2$ vs. AAV2/5 $76.4\% \pm 9.0$, AAV2/7 $73.5\% \pm 7.8$, AAV2/8 $65.6\% \pm 5.5$; $p=ns$) (Figure 2B).

Notably, in human cells, there is no significant difference in transduction efficiency of each pseudotype between normal and wound conditions at either day 3 or 7. At 3 days under wound conditions, AAV2/8 ($79.3\% \pm 7.6$) appears to transduce more human dermal fibroblasts compared to AAV2/2 ($69.6\% \pm 12.9$), AAV2/5 ($47.8\% \pm 7.7$) and AAV2/7 ($67.2\% \pm 18.7$) (Figure 3A). Interestingly, under wound conditions, there is no significant increase at day 7 in the transduction efficiencies of any of the vectors as compared to day 3. At 7 days under wound conditions, there are no differences in transduction efficiencies among the different pseudotypes (Day 7: AAV2/2 $79.4\% \pm 12.0$, AAV2/5 $71.0\% \pm 12.8$, AAV2/7 $80.3\% \pm 3.0$, AAV2/8 $85.1\% \pm 3.6$; $p=ns$) (Figure 3B).

Discussion

The goal of this study is to determine if a pseudotyping strategy with AAV vectors can result in differential transduction of the primary dermal fibroblasts under normal versus wound conditions for the individual AAV vectors. *In vitro* transduction of human and mouse primary fibroblasts demonstrate that AAV2/5 and AAV2/8 had higher transduction efficiency rates compared to AAV2/2 or AAV2/7. These results are consistent with our previously reported transduction efficiencies in murine wound model (7). Similar to our previous *in vivo* murine wound data, AAV2/5 maximally transduced mouse fibroblasts in the *in vitro* culture experiments. Notably, AAV2/8 more efficiently facilitates gene transfer to human cells, as compared to the other pseudotyped AAV vectors. Interestingly AAV2/8 transduces about 75% cells within 3 days after transduction, and continues to increase in transduction efficiency. This suggests that depending on the nature of the wounds and the requirements of transgene expression, AAV2/8 is optimal for delivery in gene transfer applications that require early transgene expression, as well as for sustained long term transgene expression. However, by day 7, AAV2/2 demonstrates similarly high transduction

efficacy, suggesting that AAV2/2 can be used for therapy where a later onset of expression of targeted genes/ factors are required. This leads us to the novel concept to improve the overall efficacy of gene transfer by tailoring the AAV vectors, using a pseudotyping strategy, based on temporal and total expression needs for the application for which it is intended. This concept can be utilized for all the debilitating chronic wounds conditions, such as diabetic ulcers, pressure ulcers and epidermolysis bullosa etc., where growth factors expression or gene transfer requirements vary in different phases of the wound healing continuum.

To establish a clinical relevance of gene therapy findings from animal wound healing models, translational studies using *in vitro* human cell culture are routinely employed. The experimental conditions of the *in vitro* experiments should mimic the *in vivo* environment. In order to fully appreciate the benefits of the AAV pseudotyping strategy for cutaneous wound gene therapy, PDGF-B was selected as an *in vitro* wound stimulatory cytokine. PDGF-B is one of the first factors released during platelet degranulation, a key early element of the wound-healing cascade. PDGF-B is a potent mitogen for dermal fibroblasts and induces cell proliferation (32). In addition, PDGF-B has been shown to stimulate fibroblast production of vulnerary growth factors and cytokines during the wound-healing process. Further, PDGF-B stimulates the extracellular matrix production by fibroblasts, specifically the differential regulation of hyaluronan synthesis (by hyaluronan synthases) and degradation (by hyaluronidases) (33). Hyaluronan is an important glycosaminoglycan found in both the dermis and epidermis, contributing to the structural and functional integrity of skin. Taken together, this supports the use of PDGF-B at levels reported in literature (34) to simulate the wound microenvironment. Our *in vitro* gene transfer results are consistent with our previously reported gene transfer rates *in vivo* (7), suggesting that PDGF-B is an appropriate single choice for *in vitro* wound simulation assay. Moreover, future studies can be performed *in vitro* using this model to determine optimal gene transfer conditions. In literature, both, *in vivo* and *in vitro* models have been used for evaluating transduction efficiency of AAV vectors. The transduction efficiency exhibited by several pseudotyped AAV vectors for many cultured cells such as lung epithelium, skeletal muscle, gastrointestinal tract cells, etc. was found comparable to the levels of delivered-transgene *in vivo* suggesting that *in vitro* models can provide useful information regarding the efficacy of AAV vectors (35, 36). Additionally, *in vitro* models are economical, easily manageable and allow precise control of experimental conditions, as well as determine optimal conditions for all the groups.

Altering gene transfer efficiencies and tropism profiles by pseudotyping or mutating AAV vector capsids have been previously reported (37, 38), but AAV2/5, 2/7, and 2/8 have not been examined in dermal fibroblasts. Our previous results suggest that the transduction of cells in an excisional wound with AAV2/2 and these pseudotyped AAV vectors have no deleterious effects on the wound healing process, which is consistent with AAV vector administration in other applications of wound repair. This is an advantage over adenoviral based vectors, which can impair wound healing in the absence of a vulnerary transgene due to the augmented inflammatory response to the adenovirus (25). The differential transduction efficiency profiles exhibited by the pseudotyped AAV vectors are favorable for wound healing. For example, at day 3 of wounding, if the goal of the cutaneous gene therapy is to achieve at least 40-50% gene transduction rate, 2-fold lower titers of AAV2/5 and 2/8 can be administered, as compared to AAV2/2 or 2/7, which will theoretically result in lower vector associated toxicity.

Proof of concept of the effectiveness of AAV-mediated gene therapy has been demonstrated in work by other groups (39). AAV2 mediated gene transfer to the skin produces stable transgene expression limited to the panniculus carnosus (PC) in rodents and the epidermis in

porcine skin (40). An AAV construct carrying the vascular endothelial growth factor (VEGF) transgene was administered to diabetic mice wounds and resulted in significantly improved wound neovascularization and accelerated wound healing (41). While these results in diabetic mice were promising, the authors conceded that the VEGF transgene expression was limited to the muscular panniculus carnosus. This tissue acted as a platform for transgene expression in the bed of the wound. This restricted distribution limits the clinical applicability of AAV2 vectors in human chronic wounds that frequently lack an epidermis and do not have a muscle “platform” (panniculus carnosus) to facilitate growth factor production in the wound bed. As fibroblasts are the major effector cells in chronic wound healing in humans, we have used fibroblasts in our experiments.

The transduction efficiency of AAV vectors varies greatly in different cell types. AAV transduction efficiency of murine cells in general has been reported to be low (18), with the exceptions of muscle and brain tissues (10, 12, 17, 41). This problem has been attributed to the obstacles that AAV must overcome. First, AAV must bind to both a cellular receptor as well as a co-receptor for successful entry into the target cell (42-45), and second, because AAV is a single-stranded DNA-containing virus, the target cell must allow for the conversion of the single-stranded viral genome to a transcriptionally active double-stranded intermediate (46, 47). A recently reported third obstacle is impaired intracellular trafficking into the nucleus, which AAV must also overcome prior to enabling high-efficiency transduction (48). For example while AAV2 binds to HeLa and KB cells more efficiently than to 293 cells, AAV-mediated transduction of HeLa and KB cells is significantly lower than that of 293 cells. Similarly, AAV2 binds to murine NIH 3T3 fibroblast cells efficiently but little transgene expression occurs (48, 49). The authors of this study concluded that AAV2 transduction efficiency among permissive human and murine cells correlates with the efficient intracellular trafficking and translocation to the nucleus.

Recent studies have further identified that several cellular growth factor receptors also function as co-receptors for transduction by adeno-associated viruses. In a study by Pasquale *et al*, a significant correlation was observed between expression of the platelet-derived growth factor receptor (PDGFR- α) and AAV5 transduction and tropism (50). Qing *et al*, reported that transduction efficiency of AAV vectors varies greatly in different cells and tissues *in vitro* and *in vivo*; and further demonstrated that cell surface expression of heparan sulfate proteoglycan (HSPG), ubiquitous AAV2 receptor alone is insufficient for AAV2 transduction and that AAV2 also required human fibroblast growth factor receptor 1 (FGFR1) as a co-receptor for successful viral entry into the host cell (44). In a different study, Ling *et al* hypothesized that AAV serotype 3 uses hepatocyte growth factor receptor (HGFR) as a cellular co-receptor for viral entry. Their data not only corroborated their hypothesis, but also identified species specificity as an important factor in AAV3 transduction efficiency, where only human-HGFR but not murine-HGFR functions as a cellular co-receptor for transduction (51). These data taken in concert with the current findings suggest that the vulnerary growth factors in the wound milieu have the potential to impact gene transfer efficiency rates. Therefore the *in vitro* fibroblast assay can be utilized to study the viral cellular interactions in order to understand the mechanisms of the efficacy of pseudotyping strategy. It will also permit the study of the inherent differences in murine and human cells to facilitate clinical translation of this work.

In conclusion, we have demonstrated that pseudotyped recombinant AAV vectors produce distinct transduction efficiency profiles in murine and human dermal fibroblasts *in vitro* under normal and simulated wound conditions. Gene expression is dose and pseudotype dependent. This study provides a solid foundation for future work using the pseudotyping strategy of AAV vectors for preclinical wound gene therapy studies, as well as other gene transfer applications.

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Abbreviations

AAV	Adeno-associated virus
BGS	Bovine growth serum
DMEM	Dulbecco's modified Eagle's Media
CMV	Cytomegalovirus
FGFR1	Fibroblast growth factor receptor 1
GC	Genome copies
GPF	Greene fluorescent protein
HGFR	Hepatocyte growth factor receptor
HSPG	Heparan sulfate proteoglycan
MOI	Multiplicities of infection
PDGF	Platelet derived growth factor
PDGFR-α	Platelet-derived growth factor receptor- α
VEGF	Vascular endothelial growth factor

Figure 1.

Comparison of Dose dependent transduction efficiencies of different AAV2/2 and pseudotyped AAV vectors AAV2/5, AAV2/7 and AAV2/8. *In vitro* cultures of murine and human fibroblasts were transduced with different multiplicities of infection (MOI 10^2 , 10^3 , 10^4 , and 10^5) of the vectors for 24 hours under normal culture conditions. Gene transfer was measured by counting GFP positive cells at day 3 and day 7 post vector administrations. MOI 10^5 resulted in maximal gene transfer to both human and murine dermal fibroblasts at 3 and 7 days in all vector preparations. x axis - different MOIs; y axis - % of cells transduced.

Figure 2.

The pseudotyping strategy results in differential transduction efficiencies of murine dermal fibroblasts. (A) Under normal (control) cell culture conditions, at day 3 post transduction with MOI 10^5 , AAV2/2 results in a lower rate of gene transfer to murine dermal fibroblasts, compared to AAV2/5 (that resulted in maximal gene transfer) and AAV2/8. Simulating wound conditions (with increased PDGF expression in the cell culture media) results in significantly lower gene transfer by each of the AAV 2/2, 2/5, 2/7 and 2/8 vectors in murine fibroblasts as compared to gene transfer under normal (control) conditions. There is no difference in transduction efficiency between the different pseudotypes under wound conditions. (B) At the day 7 time point, AAV transduction efficiency significantly increases in all vector preparations in both control and wound (PDGF) conditions compared to day 3. Under control conditions, AAV2/5 demonstrates higher transduction efficiency followed by AAV2/8, AAV2/2, and AAV2/7. Simulating wound conditions results in significantly lower gene transfer by each of the AAV vectors, compared to control. However, there were no differences in transduction efficiency between the different pseudotypes. Solid fill bars – normal (control) conditions, gradient fill bars – wound (PDGF) conditions. * $p < 0.05$, ** $p < 0.01$

Figure 3.

Human fibroblasts are also differentially transduced by the AAV pseudotyped vectors. (A) Under normal (control) cell culture conditions, at day 3 post transduction with MOI 10^5 , AAV2/8 had maximal transduction efficiency in human fibroblasts followed by AAV2/5, AAV2/2 and AAV2/7. However, simulating wound conditions (with increased PDGF expression in the cell culture media) did not result in differences in transduction efficiency either between the different pseudotypes or when compared to normal (control) conditions. (B) At the day 7 time point, AAV transduction efficiency significantly increases in all vector preparations under control conditions compared to day 3. Under control conditions, AAV2/2 demonstrates increased transduction similar to AAV2/8 (p=ns). AAV2/2 and AAV2/8 result in significantly higher gene transfer rates compared to AAV2/5 and AAV2/7. Under wound conditions, there is no significant increase at day 7 in the transduction efficiency of any vector when compared to day 3, as well as, there are no differences in the transduction efficiencies between the different pseudotypes. Solid fill bars – normal (control) conditions, gradient fill bars – wound (PDGF) conditions. * $p < 0.05$, ** $p < 0.01$, p=ns (no significance).

Table 1

Pseudotyping AAV Vectors have differential transduction efficiency

% GFP-positive cells (average±SD)	MOI 10 ⁵	AAV2/2		AAV2/5		AAV2/7		AAV2/8	
		Control	PDGF	Control	PDGF	Control	PDGF	Control	PDGF
@ day 3 post transduction	Murine	43.9±11.1	24.3±3.9	66.6±15.7	23.9±5.4	30.7±9.0	35.5±14.3	53.8±10.3	31.0±9.4
	Human	51±9.9	69.6±12.9	61.0±14.8	47.8±7.7	53.1±10.3	67.2±18.7	80.1±9.3	79.3±7.6
@ day 7 post transduction	Murine	80.9±5.3	63.6±7.2	97.3±2.8	76.4±9.0	66.4±9.0	73.5±7.8	82.0±5.1	65.6±5.5
	Human	97.7±3.1	79.4±12.0	75.4±11.4	71.0±12.8	77.0±10.6	80.3±3.0	94.6±6.3	85.1±3.6

Table 2

Comparison of transduction efficiencies by pseudotyped AAV vectors under normal (control) and wound (PDGF supplementation) conditions.

	AAV Transduction in normal conditions (Control)	AAV Transduction in wound conditions (PDGF)
Murine dFB	AAV2/5 $\gg$ AAV2/8 $\gg$ AAV2/2 $\gg$ AAV2/7	AAV2/2 $\approx$ AAV2/5 $\approx$ AAV2/7 $\approx$ AAV2/8
Human dFB	AAV2/8 $\gg$ AAV2/2 $\gg$ AAV2/5 $\gg$ AAV2/7	AAV2/8 $>$ AAV2/2 $\approx$ AAV2/5 $\approx$ AAV2/7