Serotype-Specific Transduction of Canine Joint Tissue Explants and Cultured Monolayers by Self-Complementary Adeno-Associated Viral Vectors

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Abstract

A formal screening of self-complementary adeno-associated virus (scAAV) vector serotypes in canine joint tissues has not been performed to date. Selecting appropriate serotypes is crucial for successful treatment due to their varying levels of tissue tropism. The objective of this study is to identify the most optimal scAAV vector serotype that maximizes transduction efficiencies in canine cell monolayer cultures (chondrocytes, synoviocytes, and mesenchymal stem cells) and tissue explant cultures (cartilage and synovium). Transduction efficiencies of scAAV serotypes 1, 2, 2.5, 3, 4, 5, 6, 8, and 9 were evaluated in each culture type in three different vector concentrations by encoding a green fluorescent protein. It was found that scAAV2 and 2.5 showed the overall highest transduction efficiency among serotypes with dose-response. Since possible immune response against conventional AAV2 was previously reported in dogs, the chimeric scAAV2.5 may be more suitable to use. Evaluation of the safety and efficacy of the scAAV2.5 vector with an appropriate therapeutic gene in vivo is indicated.

Introduction

Osteoarthritis (OA) is a leading cause of chronic disability, with a multifactorial etiology including age, obesity, trauma, instability, and systemic inflammation (1). Recent epidemiology studies revealed that 14 million people in the US and 250 million people worldwide are suffering from OA (2, 3). Dogs have been described as a valuable translational model for OA (4–6). The prevalence of OA in dogs has been reported to range from 2.5 to 20% (7–9). Canine OA can be severely debilitating, negatively impacts quality of life, and can lead to humane euthanasia due to uncontrolled multi-joint pain.

Current treatments for OA frequently include pain medications, joint supplements, intra-articular injection, surgical management, weight control, and physical therapy (10, 11). However, these treatments are limited in their ability to prevent or halt the progression of the disease. Therefore, new biotechnological therapeutic methods such as gene transfer and mesenchymal stem/stromal cell (MSC) therapy are being investigated (12). While MSC therapy is considered a promising treatment of OA, currently available research indicates that the treatment benefits are limited and are not long-term (13). Intra-articular gene transfer enables genetic modifications of the cells within articular tissues, such as chondrocytes and synoviocytes, for therapeutic purposes (14, 15). Such therapeutic strategies include the long-term release of anti-inflammatory mediators by the modified cells within the joint and therefore minimizes systemic effects and provides a long-lasting therapeutic effect. Gene therapy offers a clearly defined treatment approach (e.g., genetically inserting various DNA sequences to the vector) without the need to harvest donor tissues, the need for allogeneic donors, the morbidity associated with autologous harvesting. Another benefit is the off-the-shelf availability which reduces the cost of the product and convenience to the client/medical professional.

There are two main categories of gene transfer methods: viral and non-viral transfer (14). Viral gene transfer has been shown to be a more effective cellular transduction than non-viral gene transfer. It allows more efficient protein production from the genetically modified cells, faster transduction, and long-

term expression of the target protein (14). Among the various viral vectors, Adeno-associated virus (AAV) vector delivery has been shown as an effective and safe way for genetically modifying both dividing and nondividing cells (16). Gene transduction using conventional AAV vector has been applied in dogs for myotubular myopathy (17–19), hemophilia B (20–22), glycogen storage disease type Ia (23), retinal degeneration (24–27), and mucopolysaccharidosis VII (28). For targeting joints, only a single *in vivo* study is available to this date regarding canine intra-articular therapy research using AAV vectors (29), while non-viral naked DNA plasmid injection (30) and an *ex vivo* retroviral vector transduction (31) have been utilized.

Self-complimentary Adeno-associated virus (scAAV) is known to be 5- to 140-fold more effective than conventional AAV vectors within host cells because scAAV vectors can bypass the rate-limiting DNA synthesis step (32, 33). The scAAV vector delivery has been shown to be an effective and safe method for transducing joint tissues and MSCs with low immunogenicity and high efficiency in horses and humans (32–38). The scAAV vector has various serotypes with different tropisms depending on the tissue type (39), and scAAV serotype 2 is known as the most appropriate scAAV vector for joint tissues in horses (34).

It is imperative to identify an optimal serotype for successful intra-articular gene therapy. Since a formal screening of scAAV serotypes in canine joint tissues has not been performed to date, scAAV serotype screening study in a laboratory setting is necessary as the first step to minimize the use of live animals. Therefore, this study aims to identify the most optimal scAAV vector serotype that maximizes transduction efficiency in canine joint tissue explants and cell monolayers. Our null hypothesis was that there is no difference in transduction efficiency of various scAAV serotypes in canine cell monolayer cultures (chondrocytes, synoviocytes, and MSCs) and tissue explant cultures (cartilage and synovium).

Materials And Methods

Canine joint tissues were collected aseptically from amputated limbs from client-owned dogs with permission at Colorado State University Veterinary Teaching Hospital (CSUVTH) and limbs that were part from a terminal teaching lab of College of Veterinary Medicine and Biomedical Science at Colorado State University (institutional ACUC approval protocol 17-7102A). Full-thickness cartilage and synovium were collected from grossly normal shoulders or stifles without signs of osteoarthritis and subsequently, adipose tissue was collected from subcutaneous fat using #10 or #15 blades. Tissues were placed in complete Dulbecco's modified eagle medium without phenol red (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Atlas Biologicals, Fort Collins, CO, USA) and 1U Penicillin-Streptomycin-Amphotericin B (Gibco, Grand Island, NY, USA) at 37°C and 5% CO2. Cartilage explants were prepared within 24 hours of collection using a 2 mm biopsy punch, and each explant was placed into a well of a 96 well culture plate and incubated for 24 hours. Noticeable fat was trimmed from synovial tissues. Synovial explants were prepared with a scalpel blade and each of the explants were placed in a well of a 96-well culture plate as described for the cartilage. Explants were weighed collectively and averaged to obtain an mg/explant amount. Based on previous tissue digestion procedures, we estimated that 1g of cartilage would yield $3.5x10^6$ cells, and 1g of synovium would yield $3.09x10^6$ cells. Cartilage and synovial explants had an average weight of 14 mg each. These values were used to determine the

dose needed for each scAAV-GFP construct. The viral dosages used for both explants and monolayer cultures were 10 000 virus particles per cell (vpc), 5 000 vpc, and 1 000 vpc. scAAV-GFP constructs were added to the explants in supplemented media for 24 hours. Viral media was then removed from the explants, and fresh media was added for 96 hours.

The chondrocytes, synoviocytes, and MSCs were cultured and expanded from the remaining cartilage and synovial tissues and collected adipose tissues as previously described (40). Chondrocytes were frozen immediately at a concentration of $10x10^6$ cells/ml in 95% fetal bovine serum and 5% dimethyl sulfoxide (American Type Culture Collection, Manassas, VA, USA) and used at passage 0 unless more cells were required. Passages above passage 1 were not used for our experiments. Synoviocytes and MSCs were brought to passage 2 and frozen at the above concentrations. For transduction experiments, cells were plated at a density of 60% in a 96 well culture plate (i.e., $1.2x10^5$ cells/cm^2 for chondrocytes and $4.5x10^4$ cells/cm^2 for synoviocytes and MSCs) in supplemented media. After 24 hours of incubation, scAAV-GFP constructs were added to the cell monolayers for 3 hours. Viral media was then removed from the monolayers, and fresh media was added for 96 hours.

The scAAV vectors (scAAV1, 2, 2.5, 3, 4, 5, 6, 8, and 9) used in this study were produced by the Gene Therapy Vector Core Facility (Chapel Hill, NC, USA). All scAAV vectors were encoded with the gene for GFP. GFP allowed visualizing transduced cells under fluorescent microscopy and quantifying the ratio of transduced and untransduced cells with flow cytometry. The construct of scAAV-GFP (pHpa-trs-SK) has been described previously (32), and cross-packaging of serotype capsids was done as described (41).

Since a previous equine study revealed that fluorescence appeared to reach maximal fluorescence several days after transduction (34), we evaluated the samples at day 4 post-transduction. Fluorescence micrographs of both tissue explants and cell monolayer were obtained using Olympus IX70 (Olympus, Center Valley, PA, USA) with a filter cube with excitation at 495 nm and emission at 521 nm. QCapture (QImaging, Surrey, BC, Canada) was used to capture images. The degree of GFP expression detected by fluorescence microscope was subjectively classified as high, moderate, low, and minimal. Then the cartilage and synovial explants were digested, and cell monolayers adhered to the wells were trypsinized. The transduction efficiency of digested explants and cell monolayers was quantified using flow cytometry (Attune flow cytometer, ThermoFisher Scientific, Waltham, MA, USA). The mean and standard deviation of percentages of cells transduced for each serotype concentration and culture type were calculated based on flow cytometry data. A mixed-model ANOVA was used with dogs as random variables, and comparisons were made between serotypes using Tukey's method. A value of $p < 0.05$ was considered significant. Statistical analysis was performed using a statistical software package (IBM SPSS Statistics for mac, SPSS Inc, Chicago, IL, USA).

Results

Joint tissue donor characteristics

We collected joint tissues from a total of 13 limbs from 13 different dogs. Ten dogs were client-owned animals that were treated with a limb amputation at CSUVTH. The median age was 8 years (range, 4–14 years), and the median body weight was 26.8 kg (range, 9.3–70.7 kg). Breeds include Australian shepherd (n = 1), Miniature Schnauzer (n = 1), Bernese Mountain dog (n = 1), Flat-coated Retriever (n = 1), Great Dane (n = 1), Staffordshire Bull Terrier (n = 1) and Mixed breed (n = 4). Five thoracic limb amputation cases had neoplasia in the distal limb (e.g. distal antebrachium, carpus, or digits), and cartilage, synovium, and adipose tissues were aseptically collected from the shoulder joint. Five pelvic limb amputation cases had neoplasia in the distal limb (e.g., distal crus, tarsi, or digits), pelvis, or abdomen, and tissues were aseptically collected from the stifle joint. The specific diagnoses were osteosarcoma (n = 4), soft tissue sarcoma (n = 1), lymphoma (n = 1), mast cell tumor (n = 1), chondrosarcoma (n = 1), fibrosarcoma (n = 1), and synovial cell sarcoma (n = 1). Explants and cell monolayers from two dogs were excluded due to potential contamination. History, age, breed, and body weight of dogs of the other three limbs, including two thoracic limbs and one pelvic limb, which were part of a terminal teaching lab, are unknown. Only cartilage was collected from these three limbs for supplementary experiments for cartilage explants. Joints for all 13 dogs appeared grossly normal without signs of osteoarthritis.

Fluorescence micrographs

Figure 1 shows representative images in 10 000 vpc from a dog. In chondrocyte monolayers, scAAV2 and 2.5 transduced at high efficiency, scAAV4 transduced at moderate efficiency, scAAV1, 5, 6, and 8 transduced at low efficiency, and scAAV 3 and 9 at minimal efficiency. In cartilage explants, scAAV2 and 2.5 transduced cartilage explants at low efficiency and the rest of serotypes showed minimal efficiency. GFP expression was only observed in the superficial layer of cartilage explants, not in the deeper layer, as presented in Fig. 2. In synoviocyte monolayers, scAAV2 and 2.5 transduced at high efficiency, scAAV4, 5, and 6 transduced at low efficiency, scAAV1, 3, 8, and 9 at minimal efficiency. In synovial explants, scAAV2.5 and 2 transduced synovial explants at high efficiency, scAAV4, 5, and 6 at low efficiency, and scAAV1, 3, 8, and 9 at minimal efficiency. In MSC monolayers, scAAV2 and 2.5 transduced at high efficiency, scAAV4 transduced at moderate efficiency, scAAV1, 5, and 6 transduced at low efficiency, and scAAV8 and 9 transduced at minimal efficiency.

Flow cytometry of vector transduction

Qualitative analysis using flow cytometry revealed scAAV2 and 2.5 to have the overall highest transduction efficiency among serotypes in all culture types. Both serotypes showed dose-dependent graphs in three different vector concentrations, and their transduction efficiencies were not significantly different within the same culture type (Fig. 3).

Transduction rates of chondrocyte monolayers were significantly higher than those of cartilage explants in both scAAV2 and 2.5 ($p < 0.001$), and there was no significant difference between synoviocyte monolayers and synovial explants ($p = 0.163$ and 0.421, respectively) (Fig. 4).

The AAV serotypes were divided into three classes based on their preference for transducing monolayers or explants: class I or higher efficiency in explants than monolayers, class II or similar efficiency in explants and monolayers, and class III or lower efficiency in explants than monolayers. In cartilage-derived culture, class I consisted of scAAV3; class II consisted of scAAV 9; class III consisted of scAAV1, 2, 2.5, 4, 5, 6, 8. In synovium-derived culture, class I consisted of scAAV3, 6 and 9; class II consisted of scAAV1, 4, and 8; class III consisted of scAAV2, 2.5, and 5 (Fig. 5). Table 1 reveals p-values of comparison of transduction efficiencies of tissue explants and cell monolayers.

Table 1
P-values of comparison of transduction efficiency of tissue explants and cell monolayers in 10 000 vpc.

	Cartilage explants vs. Chondrocytes	Synovial explants vs. Synoviocytes
scAAV1	0.930	0.689
scAAV2	< 0.001	0.163
scAAV2.5	< 0.001	0.421
scAAV3	0.037	0.066
scAAV4	0.061	0.924
scAAV5	0.015	0.372
scAAV6	0.003	0.060
scAAV8	0.030	0.872
scAAV9	0.623	0.010

Discussion

To develop effective gene therapy for OA in dogs, ideally the optimal scAAV vector serotype in joint tissues should be determined first. We therefore designed this *in vitro* study before applying scAAV vectors *in vivo* into canine joints. To our knowledge, this is the first study to compare transduction efficiencies of scAAV vectors in canine synoviocytes, chondrocytes, MSCs, cartilage and synovial explants. MSCs were also included in this experiment since we wanted to target joint cells and MSCs for treating OA. Our hypothesis is rejected since we revealed different transduction efficiencies to canine joint cell monolayers and explants among different scAAV vector serotypes. Based on the result of the current study, the most promising scAAV serotypes are scAAV2 and 2.5.

Previous canine joint gene therapy research described the use of various techniques and includes the encoding of genes of anti-inflammatory cytokines to modify the joint disease process *in vivo*. One study used an indirect gene delivery technique to inject cultured synovial fibroblasts, which were transduced *ex vivo* by a retroviral vector encoding human interleukin-1 receptor antagonist (IL-1ra), into cranial cruciate

ligament deficient canine stifles (31). The injection increased the local production of IL-1ra in the stifle joint and reduced the progression of OA (31). A more recent study used a plasmid DNA injection technique that directly delivers IL-10 gene into canine joints (30). This double-blind, prospective, randomized, placebo-controlled pilot study included 10 dogs with naturally occurring canine OA. Pain scores decreased based on subjective veterinary and owner questionnaires without any complications during the 8 weeks of the study period (30). The AAV vector system (e.g., conventional AAV and scAAV) in canine joints has also been investigated in laboratory settings and live animals. scAAV vectors package double-stranded genomes allowing faster transduction rate via bypassing the rate-limiting DNA synthesis step, while conventional AAV vectors package single-stranded genomes requiring synthesis of the complementary strand in the host cell (32, 33). Santangelo et al. revealed successful transduction of normal and osteoarthritic canine cartilage explants with scAAV2 and conventional AAV2 vectors (42). Our study is in agreement with these results, revealing good transduction efficiency with scAAV2 in dogs' joint cell monolayers and tissue explants.

At the time of writing, our literature search reveals only one study in live dogs describing intra-articular gene therapy using conventional AAV and none using scAAV. That study has evaluated the effect of conventional AAV2 and 5 encoding hyaluronic acid synthase-2 gene in canine stifle joints without a formal screening of AAV serotypes (29). Interestingly, it revealed that conventional AAV5 had consistent gene transfer in a dose-dependent manner, while conventional AAV2 had inconsistent gene transfer with a lack of dose-response effect (29). This result is worth noting since conventional AAV2, which showed successful transduction to canine joint cells *in vitro* (42), did not show consistent transduction *in vivo*. Similar mismatched gene transduction efficiency between *in vitro* and *in vivo* studies has been reported in other species. Watson et al. compared the transduction rate of conventional AAV1, 2, 5, 8, and 9 encoding human IL-1ra in equine synovial fibroblast culture and live horses' joints (43). While serotype 1, 2, and 5 showed superior IL-1ra production in cell culture, serotype 2, 5, and 8 showed better IL-1ra production in joints (43). There is no clear explanation for why the serotypes act differently *in vitro* and *in vivo*; however, cell surface glycan receptor expression (36) and unforeseen immunity (38) are possible reasons for these differences.

Since there is scarce information regarding behaviors of scAAV serotypes in canine joints, this *in vitro* scAAV serotype screening study was necessary as the first step to minimize the use of live animals for our next *in vivo* study due to various serotypes, even with an understanding of which those serotypes can act differently *in vitro* and *in vivo*. Consistently good transduction rates of scAAV2, 2.5 and 5 both *in vitro* and *in vivo* in horses justify the use of *in vitro* serotype screening experiments in dogs for selecting serotypes for *in vivo* experiments (37, 43).

For successful transduction of scAAV vectors, cell surface glycans are the crucial first step for the vectors to attach to the surface of target cells. It has been shown that AAV2, 3, and 6 bind to heparan sulfate as the primary receptor, AAV1, 4, 5, and 6 bind to sialic acid, AAV8 binds to laminin receptor in the basement membranes of many tissues, and AAV9 binds to glycans with terminal galactose (44–46). Cell surface receptor expression can be affected by various factors, including oxygen concentration, growth medium,

culture system, or cell density (47), even by arthritic conditions (48). We classified the scAAV vectors into three classes based on different levels of transduction efficiency between cell monolayers and tissue explants as previously established from our work (36). There may be different expressions of cell surface receptors in joint cells in the native environment. Cell surface receptor analysis using flow cytometry with receptor antibodies (43) or comparison of transduction with enzymatic digestion of cell surface receptor (36) can be considered to identify the receptor expression in each condition for further investigation.

Another factor that can affect transduction efficiency *in vivo* is the innate and acquired immunity of live animals towards scAAV vectors. Serum neutralizing antibodies, which can make injected scAAV vectors ineffective, can exist naturally or can be formed with repeated injections. Immune response regarding scAAV vectors has not been investigated in dogs, but it may have similar response to that of conventional AAV vectors due to same capsid structure. The most prevalent neutralizing antibody in dog serum is against AAV6 (49–51). Antibodies against AAV5, 8, and 9 have not been reported in dogs (49, 51). However, lower concentrations of canine antibodies against AAV1(49, 50) and AAV2 (50), compared to those of AAV6, have been reported. In addition, a strong humoral and cellular immune response has been revealed *in vivo* application of conventional AAV2 into canine muscle (52). It is unknown whether the same immune response would occur intra-articularly. However, the immune response against AAV2 may be related to the inconsistent transduction rate of AAV2 in Kyostio-Moore et al.'s experiment (29). Immune response regarding AAV2.5 has not been reported to this date.

scAAV2.5 is an enhanced chimeric vector, combining characteristics of serotype 1 and 2 (53). scAAV2.5 is designed to bind to heparan sulfate to have an affinity to tissue well like scAAV2, but also have the ability to avoid immune response seen against scAAV2 via features of scAAV1 (53). Goodrich et al. revealed scAAV2.5 produced encoded protein over a more prolonged period than scAAV2 (i.e., 6 months and 23 days in scAAV2.5 and 2, respectively) in horses, while both scAAV2 and 2.5 showed effective transduction with high levels of protein concentration intra-articularly during those periods (37). Therefore, scAAV2.5 may be the more suitable vector for *in vivo* application than scAAV2 in dogs.

A major difference between the current research from our previous equine research was overall transduction efficiency. Previously reported transduction efficiencies of scAAV2, 3, 5 and 6 in 4 000 vpc at day 7 in equine chondrocytes and synoviocytes were more than 95% and 85%, respectively (34). Conversely, for scAAV2, 2.5, 3, 5 and 6 in 10 000 vpc at day 4 they were approximately 75%, 70%, 4%, 11%, and 20% in canine chondrocytes and 47%, 38%, 2%, 12%, and 3% in synoviocytes, respectively. Even though we are comparing canine results on day 4 and equine results on day 7 post-transduction, there still appears to be a substantial difference between the two species. Similarly, Watson et al. revealed that equine joint cell monolayers showed higher transduction efficiency than human joint cell monolayers (43). Interestingly, heparan sulfate glycan expression level was significantly higher in equine joint cells than in humans, and 20-fold or more of the viral genome was detected within equine joint cells than in humans for the same viral particles per cell (43). As there is an innate difference between equine and human cells, there may be a similar difference between canine and equine cells.

Transduction efficiencies of cell monolayers appeared to be superior to those of tissue explants from the current study. Possible reasons include different expression levels of cell surface receptors, various amounts of extracellular matrix (ECM), and the need of diffusion of scAAV vectors into tissue explants. Given the small size (~ 20 um) of the scAAV particle, the latter rationalization seems less likely. Further investigation with enzymatic digestion of cell surface receptor or ECM would allow to understand the cause of the difference between cell monolayers and tissue explants.

Another interesting finding of this study is that GFP expression was observed only from the superficial zone of cartilage explants, not the deeper zones. This could potentially be a reason why transduction efficiency of cartilage explants was substantially lower than other culture types in flow cytometry. According to Santangelo et al., while normal cartilage showed uniform GFP expression in partial or full-thickness cartilage, osteoarthritic cartilage showed GFP expression only in the superficial zone (e.g., tangential and transitional zone) of cartilage, not the deeper zones (42). Our results differ from these findings however, we used normal cartilage only. It has been suggested that chondrocytes in the superficial layers are metabolically more active to repair damaged matrix if needed (54). Chondrocytes in the deeper zone may have unique characteristics that inhibit AAV vector transduction or have a slower metabolic rate that cannot achieve transduction. Further comparison between normal and osteoarthritic cartilage is warranted on scAAV transduction.

There are several limitations of this study. First, due to the *in vitro* nature of the study, these results may not represent gene transfer in live dogs' joints. This study design is helpful to screen many serotypes without using live animals, but they do not necessarily exactly reflect the native environment of joints. In this respect, the application of these vectors *in vivo* may provide more representative information on the transduction efficiency of variant AAV serotypes. Second, a small number of samples were used for analysis, which could potentially cause a Type I statistical error. Third, the age of the tissue donor and the type of joints were not controlled. Lastly, even though the neoplasia was not at the location we harvested tissue, the collected tissues may not represent normal healthy tissue since some were harvested from dogs had neoplasia.

Conclusion

To our knowledge, this is the first study to compare serotype efficiency of scAAV in canine synoviocytes, chondrocytes, MSCs, synovial explants, and cartilage explants. Our results revealed that scAAV2 and scAAV2.5 showed the highest transduction efficiencies in a laboratory setting. Given the previous reports regarding immune response against conventional AAV2 in dogs, the chimeric scAAV2.5 may be more suitable to use. Further screening of the scAAV2.5 vector with an appropriate therapeutic gene in live dogs joints to evaluate the safety and efficacy is indicated.

Declarations

Data Availability

All data are available upon reasonable request.

Acknowledgements

N.A.

Author Contributions

L.G. managed the project. L.G., J.P., F.D., and A.K. conceived and designed the experiments. J.P. and A.K. performed most of the experiments and data analysis. A.K. wrote the manuscript. L.G., R.S., J.G., and F.D. provided feedback on the manuscript.

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Ethical Approval

Ethical approval was not required since live animals are not involved in this study.

Competing Interests

The authors (A.K., F.D., J.P., R.J., and L.G) do not have any conflicts of interest in the publication of this manuscript.

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Figures

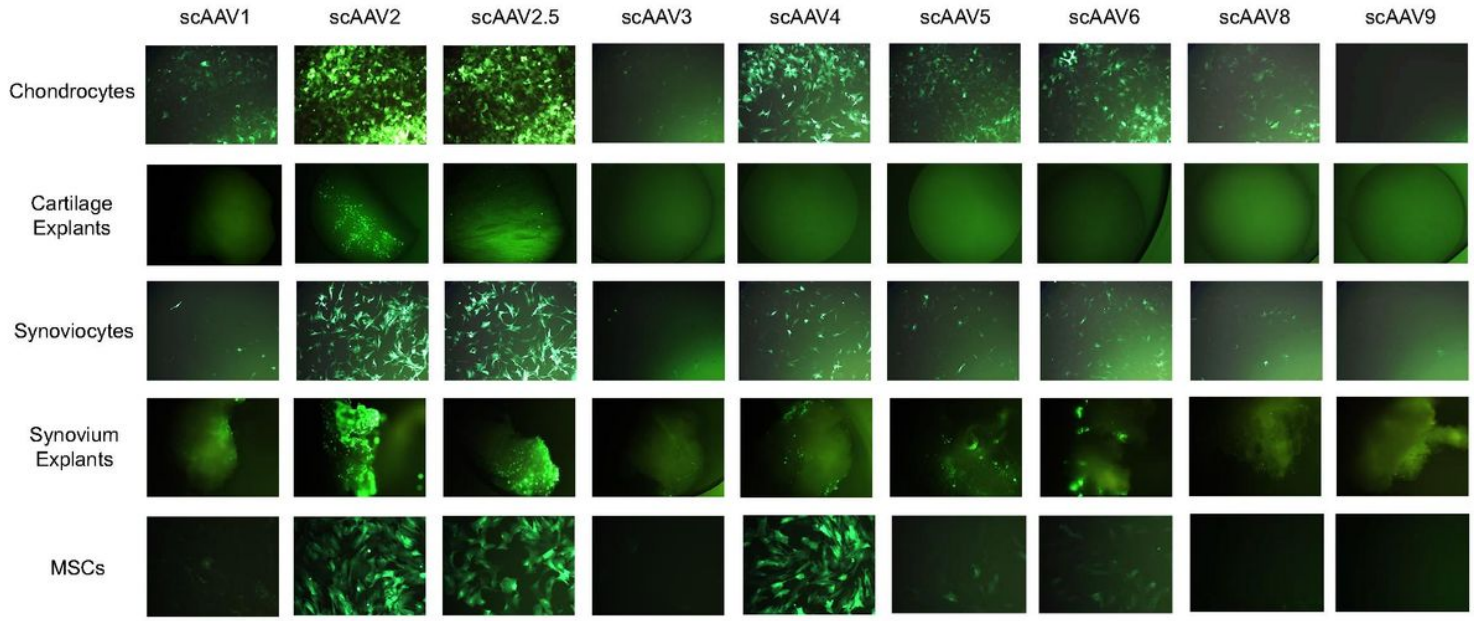


Figure 1

Representative fluorescence micrographs of cell monolayers and tissue explants showing the presence or absence of scAAVGFP transduction with the vectors tested. Pictures taken four days following transduction in 10 000 vpc in a dog.

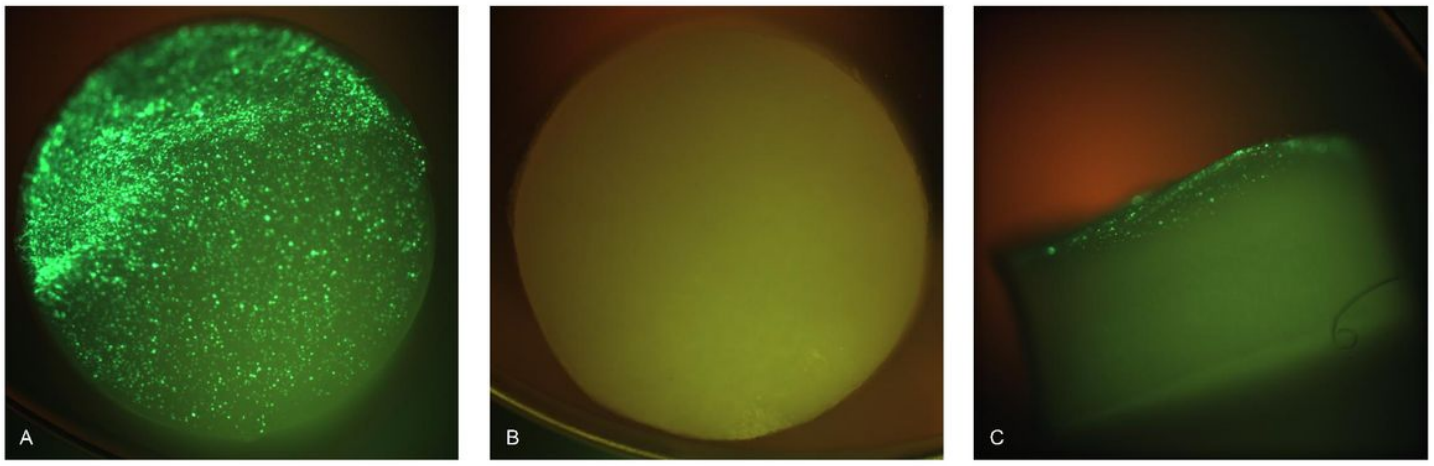


Figure 2

Representative fluorescence micrographs of cartilage explants with scAAV2 in 10 000 vpc on day 4 post-transduction. scAAV2-GFP successfully transduced the superficial zone of the explant (A) but not the deeper zone of the explant (B). Observation of the explant in cross section (C) also shows GFP expression only on the superficial zone of the explant.

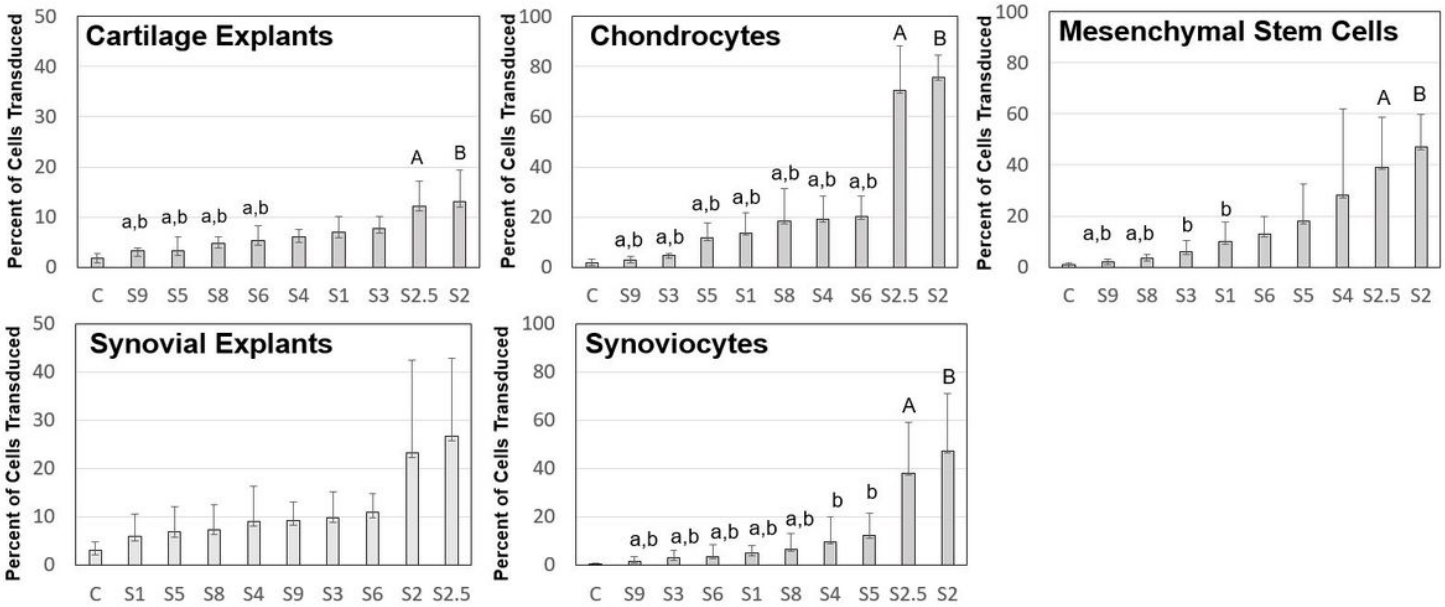
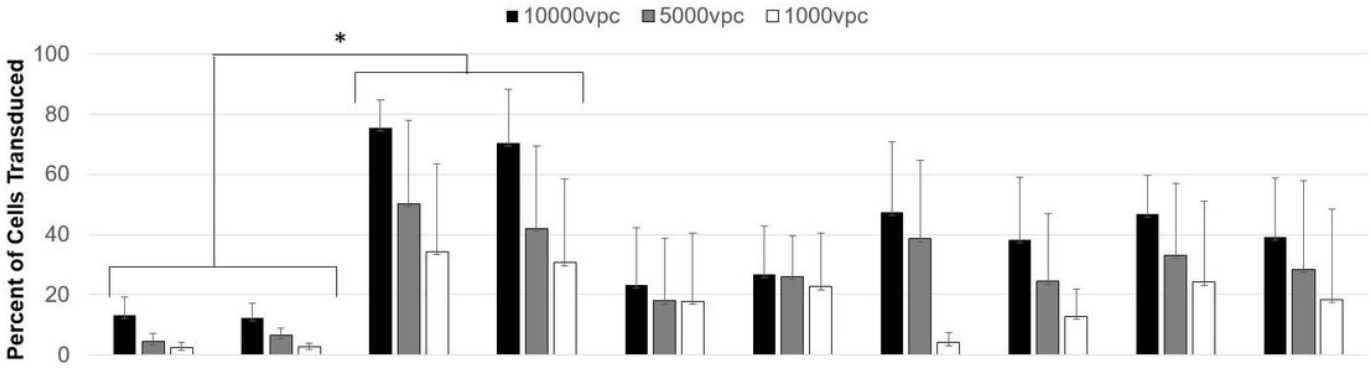


Figure 3

Ranking of scAAV transduction efficiency as the percent of cells transduced in cartilage explants (n=6), chondrocytes (n=4), MSCs (n=4), synovial explants (n=4), and synoviocytes (n=4) in 10 000 vpc. Columns indicate mean percent and bars indicate standard deviation of the mean. P-values less than 0.05 are considered significant. Capitalized letters denote statistically significant differences from their uncapitalized respective letters.



Mean%(SD)	Cartilage Explants		Chondrocytes		Synovial Explants		Synoviocytes		MSCs	
	S2	S2.5	S2	S2.5	S2	S2.5	S2	S2.5	S2	S2.5
10 000vpc	13.0 (6.2)	12.1 (5.1)	75.5 (9.1)	70.4 (17.8)	23.1 (19.1)	26.7 (16.0)	47.3 (23.6)	38.1 (20.8)	46.9 (12.6)	39.2 (19.5)
5 000vpc	4.3 (2.6)	6.3 (2.5)	50.2 (27.7)	42.0 (27.3)	17.9 (20.6)	26.1 (13.4)	38.6 (26.0)	24.3 (22.6)	33.0 (23.9)	28.4 (29.4)
1 000vpc	2.4 (1.7)	2.7 (1.2)	34.3 (29.2)	30.5 (28.0	17.7(22.6)	22.6 (17.7)	4.0 (3.2)	12.7 (9.2)	24.1 (26.8)	18.3(29.9)

Figure 4

Transduction efficiencies of scAAV2 and 2.5 in three different vector concentrations (1 000/5 000/10 000 vpc) in cartilage explants (n=6), chondrocytes (n=4), MSCs (n=4), synovial explants (n=4), and synoviocytes (n=4). Columns indicate mean percent and bars indicate standard deviation of the mean. Numbers in the chart indicate mean % (standard deviation). P-values less than 0.05 are considered significant. Asterisks denote statistical differences between same serotype in different culture type.

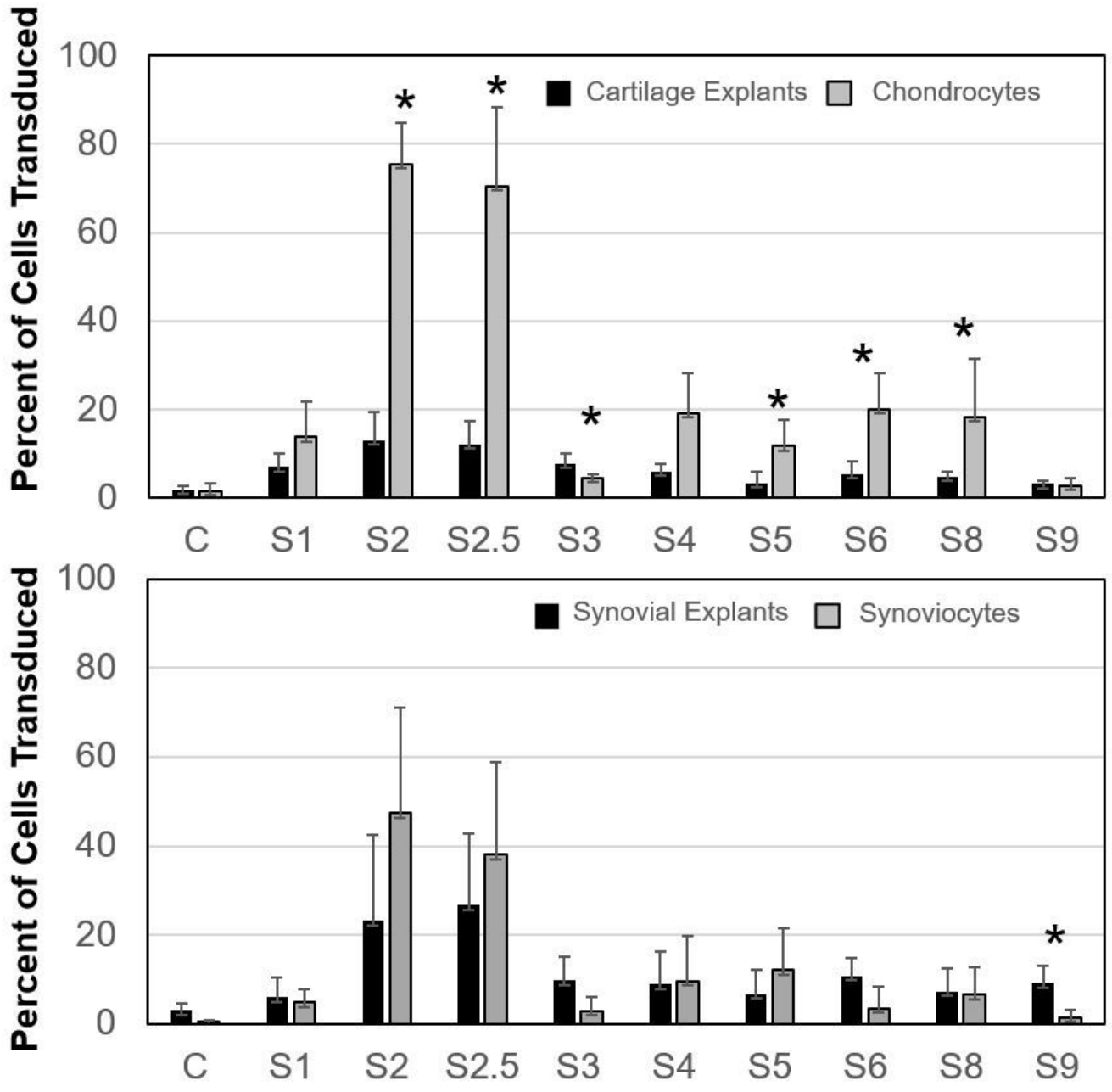


Figure 5

Comparison of transduction efficiency within different culture types but same tissue of origin, including cartilage explants (n=6), chondrocytes (n=4), synovial explants (n=4), and synoviocytes (n=4) in 10 000 vpc. Columns indicate mean percent of cells transduced and bars indicate standard deviation of mean. Asterisk means a significant difference between explants and cell monolayers. P-values less than 0.05 are considered significant.