Poster P116

fDISCO evaluation of AAV-mediated gene expression upon different routes of administration

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ABSTRACT

Detection methods to assess gene transfer-mediated expression of reporter proteins in tissue requires one to dissect or slice the sample, during which the three-dimensional (3D) structure is lost. On the other hand, live imaging often does not provide sufficient resolution to identify reporter protein expression on a cellular level. Here, we have tested whether fDISCO (3D imaging of solvent-cleared organs with superior fluorescence-preserving capability) could overcome these limitations. To this end, DBA/1 mice were injected intraarticularly (IA), intramuscularly (IM) or intravenously (IV) with AAV-encoding fluorochrome mGreenLantern (mGL). Hindlimbs and livers were isolated three weeks after administration, followed by fDISCO tissue clearing. Cleared tissues were imaged by a light-sheet fluorescence microscope, followed by image stitching to create the 3D structure of the sample. The fluorescent signal of mGL was detectable at the cellular level in the tissue. The highest number of mGL expressing cells were found in the injected muscles (gastrocnemius or quadriceps) of IM groups. The IA group showed visible mGL expression in muscles around joint. The IV group showed the lowest number of mGL expressing cells in the muscles throughout the entire hindlimb. All routes of administration resulted in mGL expression in liver correlating with the total vector dose injected per animal. In conclusion, fDISCO is able to capture the complete histological information of fluorescent reporter signal, thus being a suitable method to study the AAV-mediated reporter expression on a cellular level in 3D tissue samples.

1. Background

Detection methods to assess gene transfer-mediated expression of reporter proteins in tissue requires one to dissect or slice the sample, during which the three-dimensional (3D) structure is lost. On the other hand, live imaging often does not provide sufficient resolution to identify reporter protein expression on a cellular level. Here, we have tested whether fDISCO (3D imaging of solvent-cleared organs with superior fluorescence-preserving capability) could overcome these limitations. In addition, the AAV mediated reporter gene expression after different routes of administration was evaluated using the fDISCO in murine hindlimbs.

2. Method

DBA/1 mice were injected intraarticularly (IA), intramuscularly (IM) or intravenously (IV) with AAV-encoding fluorochrome mGreenLantern (mGL) or green fluorescent protein (GFP). In addition, animals without any treatment were included to serve as the naïve group (Figure 1).

Hindlimbs and livers were collected three weeks after administration, followed by fDISCO tissue treatment for light-sheet imaging (Figure 2). Briefly, after fixation in paraformaldehyde (PFA), the tissues were dehydrated in 50%, 70%, 80%, and 100% tetrahydrofuran (THF) solutions. After dehydration, tissues were cleared and stored in dibenzyl ether (DBE). The cleared tissues were imaged by a light-sheet fluorescence microscope, followed by image stitching to create the 3D structure of the sample.

Group	Route of administration	Injection site	Dose
AAV-GFP	IA	Knee joints	7.5 x 10^9 VG
AAV-mGL	IA	Knee joints	7.5 x 10^9 VG
AAV-mGL	IM	Gastrocnemius	5.0 x 10^{10} VG
AAV-mGL	IM	Quadriceps	5.0 x 10^{10} VG
AAV-mGL	IV	Tail	1.0 x 10^{13} VG/Kg (1.4 – 1.7 x 10^{12} VG)
Naïve	N/A	N/A	N/A

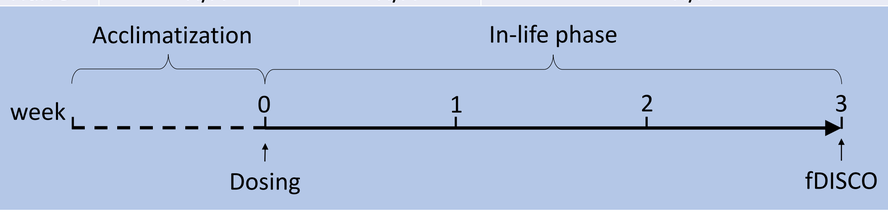

Figure 1. Study design. IA: intraarticular injection, IM: intramuscular injection, IV: intravenous injection, mGL: mGreenLantern, GFP: green fluorescent protein.

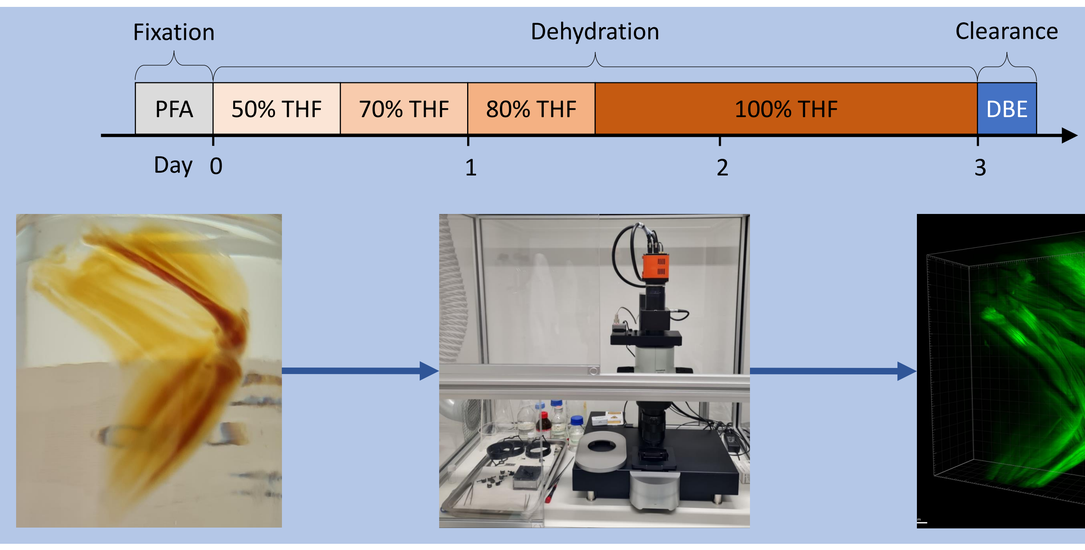

Figure 2. fDISCO and light-sheet imaging. PFA: paraformaldehyde, THF: tetrahydrofuran, DBE: dibenzyl ether, fDISCO: 3D imaging of solvent-cleared organs with superior fluorescence-preserving capability. Imaged at O|2 microscope core facility, VU University Medical Center Amsterdam.

3. mGL and GFP signal after fDISCO

AAV-mGL (IA) hindlimb showed specific cellular green fluorescent signal in the shape of clear, punctured and granulated dots (Figure 3D). AAV-GFP (IA) Hindlimb showed only autofluorescence (Figure 3C) that was similar to naïve Hindlimb (Figure 3B).

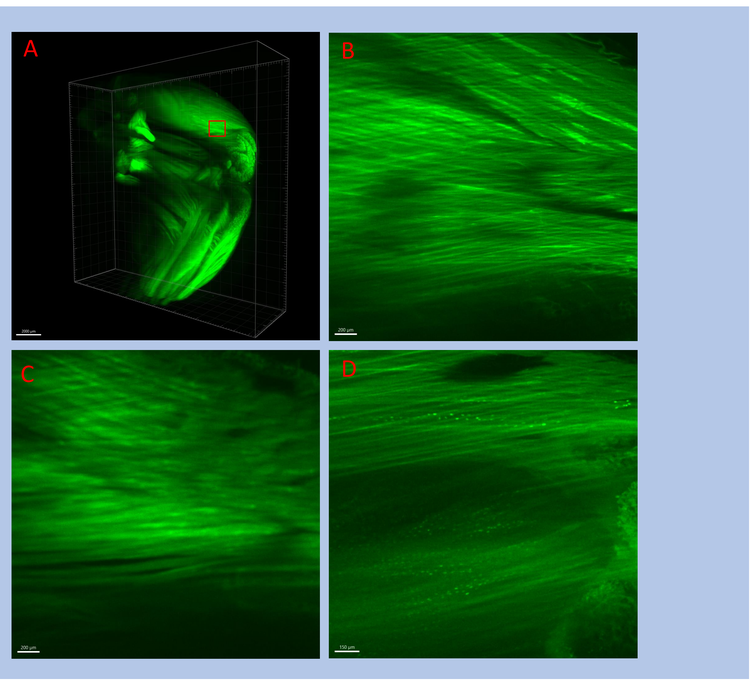

Figure 3. mGL and GFP signal in hindlimbs after fDISCO clearance. A: light-sheet imaging of the whole hindlimb (Naïve), red square indicates the zoom-in area of B, C and D. B: Naïve, C: GFP (IA), D: mGL (IA).

4. mGL signal in liver after different routes of administration

Specific mGL green fluorescent signal (punctured and granulated green dots) were found in livers of animals injected IA, IM and IV with AAV-mGL (Figure 4). IV showed the highest number of mGL expressing cells in liver, and IA showed the lowest number of mGL expressing cells (Figure 4).

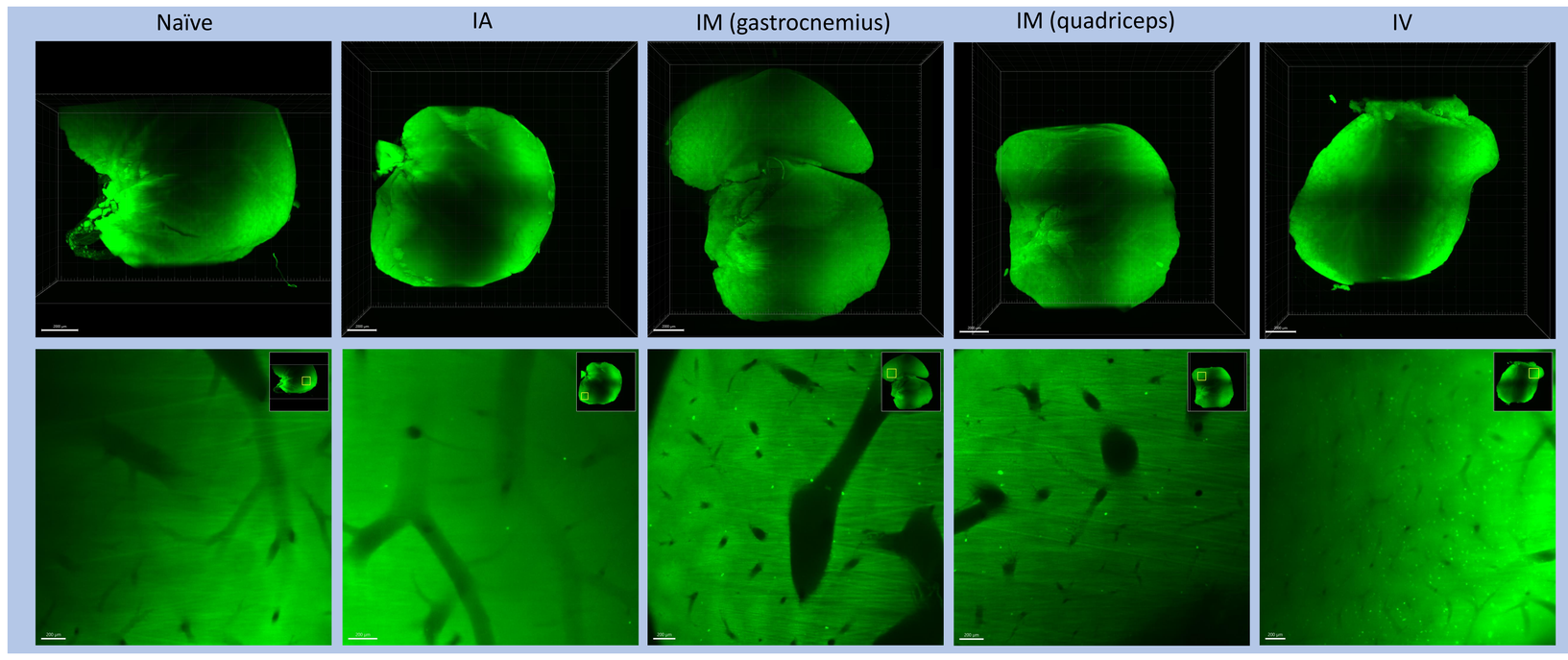

Figure 4. mGL signal in liver after different routes of administration. IA: intraarticular injection, IM: intramuscular injection, IV: intravenous injection

5. Reporter expression in hindlimb after different routes of administration

The highest density of mGL expression cells were found near the site of injection after IA or IM administrations. Cells more distant from the injection site only showed non-specific autofluorescence. IV administration showed the lowest density of mGL expression cells, which were scattered throughout the entire hindlimb muscles.

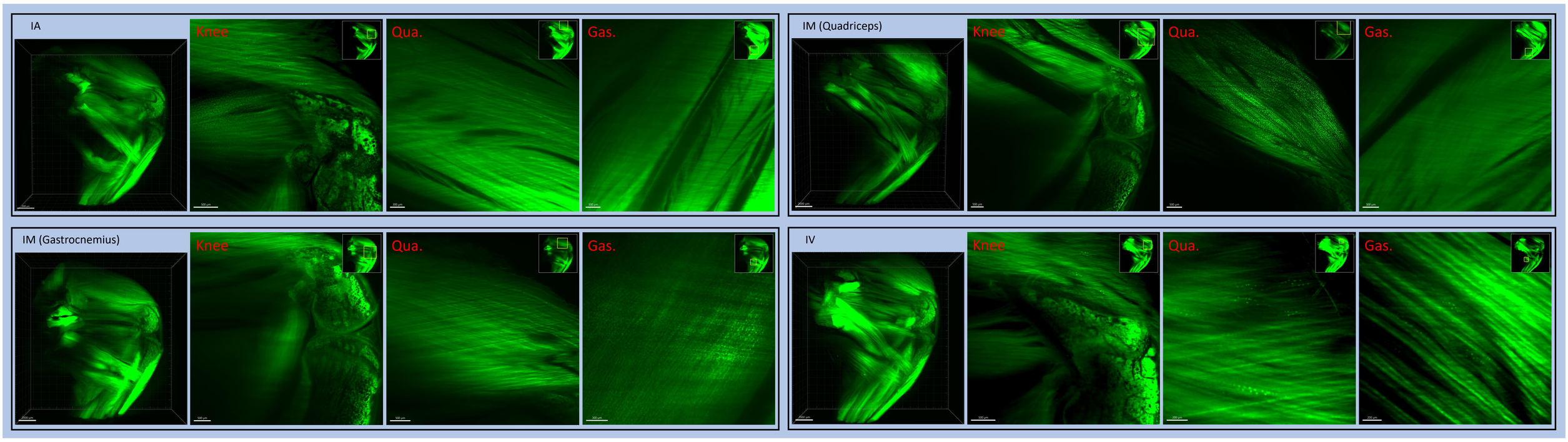

Figure 5. mGL signal in hindlimb after different routes of administration. IA: intraarticular injection, IM: intramuscular injection, IV: intravenous injection, Qua.: quadriceps area, Gas.: gastrocnemius area.

Conclusions

fDISCO was able to capture the histological information of fluorescent signal of mGL, thus being a suitable method to study the AAV-mediated expression of this reporter gene on a cellular level in 3D tissue samples.