AAV (Adeno-associated virus)-attached allogenic nerve graft to promote the nerve regeneration in peripheral nerve defects

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INTRODUCTION: Peripheral nerve defects exceeding 3 cm cannot be effectively repaired by direct neurorrhaphy, and nerve grafting is required to restore function. Autologous nerve grafts are regarded as the gold standard because they provide viable Schwann cells and sustained release of neurotrophic factors to support axonal regeneration. However, their use is limited by donor-site morbidity, inevitable sensory loss, and surgical complications. Allogenic nerve grafts eliminate the need for donor nerve harvest but lack Schwann cells, resulting in inferior regenerative outcomes. Localized gene therapy using adeno-associated virus (AAV) has recently emerged as a promising strategy to address this limitation. AAV enables long-term, site-specific expression of therapeutic molecules, allowing continuous supply of neurotrophic factors that are critical for nerve regeneration. Therefore, this study was designed to investigate whether coating allogenic nerve grafts with AAV can provide sustained local delivery of regenerative factors after transplantation, and to evaluate its potential to enhance axonal regeneration and functional recovery as a novel therapeutic approach for peripheral nerve repair.

METHODS: To evaluate the binding efficiency of AAV to allogenic nerve grafts, rat sciatic nerves were cut into ~3 mm segments and incubated with AAV1, AAV5, or AAV7 (1×10^9 GC/mL) at room temperature for 1 h. After incubation, the grafts were washed, and the amount of virus bound to the tissue was quantified by qPCR. For *in vitro* transduction analysis, each AAV-coated nerve graft was co-cultured with primary Schwann cells for 3 days, after which GFP expression was examined using fluorescence microscopy. For *in vivo* evaluation, a 6-mm segment of the sciatic nerve was excised in Sprague–Dawley rats and replaced with an AAV5-coated allogenic nerve graft. Two weeks post-transplantation, nerves were harvested, cryosectioned, and analyzed by immunofluorescence microscopy to detect GFP expression within the donor graft as well as in the proximal and distal segments of the recipient nerve.

RESULTS SECTION: In this study, qPCR analysis showed that AAV5 had the highest binding efficiency (51.9%) to allogeneic rat sciatic nerve grafts, markedly surpassing AAV1 (0.27%) and AAV7 (1.51%) (Fig. A). Fluorescence microscopy revealed that co-culturing AAV-coated nerve grafts with primary Schwann cells for three days resulted in markedly stronger GFP expression with AAV5 compared to AAV1 or AAV7 (Fig. B). *In vivo*, transplantation of an AAV5-coated allogenic nerve graft into a 6-mm sciatic nerve defect in Sprague–Dawley rats, immunofluorescence revealed GFP signals in the proximal and distal segments of the recipient nerve, with a weaker signal within the graft. These results suggest that, after transplantation, much of the AAV5 in the graft was lost, and that only a portion of the AAV5 was transduced to host Schwann cells (Fig. C).

DISCUSSION: Our study demonstrates that AAV5 serves as an effective vector for efficient and localized gene delivery to Schwann cells both *in vitro*. In particular, this study suggests that the selection of an appropriate AAV serotype is critical for achieving targeted gene delivery to specific tissues or cell types. Our findings indicate that localized AAV5 delivery would help overcome the limitations of allogenic nerve grafts by providing sustained, site-specific expression of regenerative factors during nerve regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates that AAV5-coated allogenic nerve grafts enable efficient, localized gene delivery in a peripheral nerve defect model. This approach may represent a clinically viable strategy to enhance axonal regeneration and functional recovery in patients requiring nerve grafting, while potentially mitigating the limitations of conventional allogenic grafts.

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IMAGES AND TABLETS: