



Non-viral Gene Therapy Approaches for Osteoarthritis

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INTRODUCTION

Disability associated with osteoarthritis (OA) is a significant medical complication that is compounded by poor treatment options. Gene therapy may have applications for treating OA by offering the ability to produce local and sustainable levels of therapeutic proteins required to achieve a positive clinical outcome or to provide a mechanism whereby deleterious gene products can be inhibited (RNAi). Use of non-viral delivery vectors for gene based therapeutics may have benefits over viral based vectors by potentially allowing for multiple administrations and offering reduced safety concerns¹. Additionally, polymeric non-viral delivery systems are amenable to chemical modifications that allow for functionalized delivery systems to promote DNA protection, cellular uptake and cell targeting. Here we present work evaluating use of non-viral polymeric delivery systems for intra-articular (IA) plasmid delivery.

MATERIALS AND METHODS

Female Sprague-Dawley rats were used for studies evaluating the ability of various polymeric-based delivery systems to promote plasmid uptake following injection into the knee. Rats were injected (under anesthesia) IA into the right and left knees with 100 µg formulated plasmid in a total volume of 100 µl. The plasmid (pLuc) encoded for the luciferase reporter gene driven by a CMV promoter. Luciferase expression levels were analyzed using a commercially available assay (Promega, Madison, WI).

Evaluation of optimized polymer delivery system (NCPB-1) was performed in a rat model of OA². In this model OA was surgically induced by performing a medial meniscectomy along with transection of the ligaments. Following a 4 week recovery period 250 µg reporter gene plasmid (pSeAP or pGFP tagged with c-Myc) formulated with NCPB-1 was injected IA two times (once/week). At the termination of the study (one day after second injection) the animals were euthanized, treated knees were harvested and prepared for immunohistological analysis using standard procedures in order to evaluate transgene expression distribution. Serum was collected via retro-orbital puncture for determinations of systemic SeAP expression levels (Tropix, Bedford, MA).

For DNase protection assay, DNA formulation was prepared at final DNA concentrations of 0.1 mg/ml in presence various amounts of crown polymer in saline. A total of 1 µg DNA was incubated with 1 international unit of DNase I in 1X digestion buffer for 10 minutes at 37°C. Samples were loaded on 1% agarose gel and electrophoresed at 100 V for one hour. Female ICR mice were used for IA injections. Plasmid formulated at 1.0mg/ml was injected IA (15 µl/knee) or IM (25 µl/tibialis). The human IL-4 plasmid (pORF-hIL-4) was purchased from Invivogen (San Diego, CA).

RESULTS

Initial studies were performed to evaluate transfection efficiencies of various classes of polymeric delivery systems following IA delivery into the rat knee (Figure 1). From these studies the non-condensing neutral charged polymer 4 and excipient 2 yielded the highest expression levels and were selected for further evaluation and optimization studies (data not shown). It was subsequently determined that a combination of these (named NCPB-1) led to the highest transfection efficiencies.

Evaluating the transfection efficiency of plasmid formulated with various polymers and excipients for intra-articular delivery

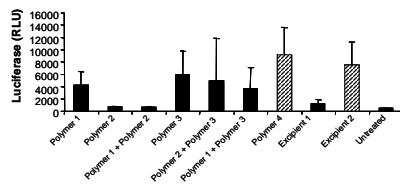


Figure 1. Reporter gene expression levels following IA injection of plasmid formulated with various polymeric delivery systems. At 24 hours after injection joint capsules were harvested and homogenates prepared for luciferase analysis. Values are mean ± SD, n=5

REFERENCES

1. Partridge KA, et al: Tissue Engineering 10:295-307, 2004.
2. Williams JM, et al: J Anat 134:103-9, 1982.

In a rat model of OA, following intra-articular injection of NCPB-1 formulated plasmid, it was seen that cells within the synovial membrane showed the highest amount of staining for c-Myc tagged GFP protein (Figure 2). Positive "patches" of c-Myc expression were localized to skeletal muscle fibers associated with the knee architecture (Figure 3). Similar staining patterns were noted in intact knees injected with formulated plasmid.

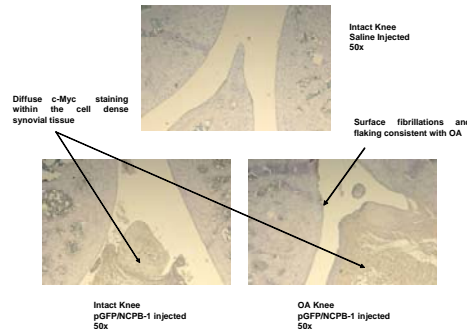


Figure 2. c-Myc staining of rat knee joints. Comparison of saline injected knees to intact and OA knees injected two times with pGFP formulated with NCPB-1 (250 µg each injection). Tissues were harvested 24 hours after the second injection.

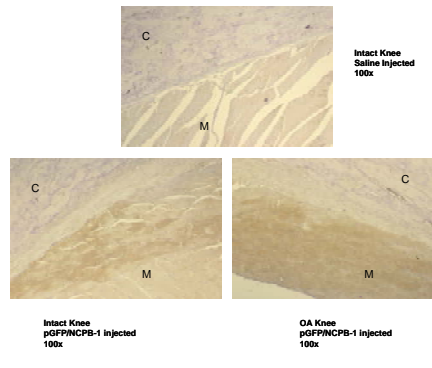


Figure 3. Positively stained muscle fibers that are adjacent to the knee joint in pGFPNCPB-1 treated intact and OA knees. Muscle (M), Collagenous fibers/tendon (C).

Expression levels were quantified using the human secreted alkaline phosphatase (SeAP) reporter gene (Figure 4). Local SeAP protein levels (in synovial aspirates) of OA and intact animals were very similar one day following injection of formulated plasmid. Systemic SeAP expression levels were maintained over at least 7 days and could be increased by a 2nd intra-articular injection. Systemic SeAP expression levels tended to be higher in OA animals than in intact animals but this was not statistically significant (p = 0.19).

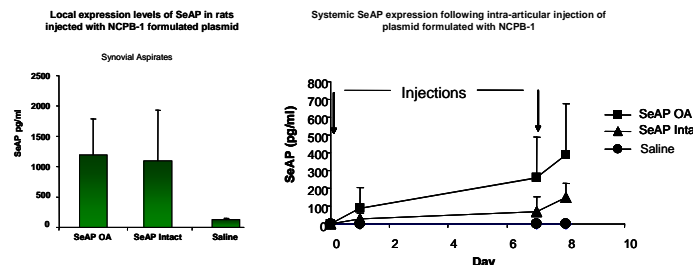
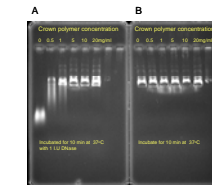


Figure 4. Local and systemic SeAP expression levels in rats. Formulated plasmid was injected IA into rats 2x (days 1 and 7). One day following second injection synovial exudates were extracted for SeAP analysis (A). Serum was collected and analyzed throughout the duration of the experiment (B).

A second generation "NCPB-1 like" non-viral delivery system (crown polymer) has been synthesized that can protect DNA from DNase degradation and produce significantly greater transgene expression levels compared to NCPB-1 following IM and IA injection in mice (Figure 4). Use of crown polymer to deliver a plasmid encoding for human IL-4 a potentially therapeutic relevant cytokine produced significant levels of IL-4 protein within the joint and systemically (Figure 5).

Protection of formulated plasmid against DNase challenge.



Serum SeAP expression levels in mice 1 day following injection of formulated plasmid

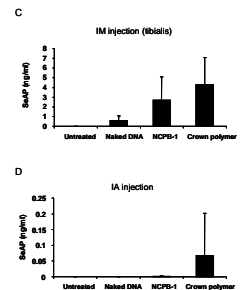


Figure 4. In-vitro DNase protection and in-vivo activity of crown polymer. Plasmid (pLuc) was formulated with various amounts of crown polymer and (A) incubated at 37°C for 10 minutes with DNase (1 IU) or (B) incubated at 37°C for 10 minutes without DNase. Systemic SeAP expression in mice following IM (C) or IA (D) injection of plasmid formulated with crown polymer in comparison to NCPB-1 and naked DNA. Values are mean ± SD, n=5.

Intra-articular delivery of formulated plasmid encoding for human IL-4

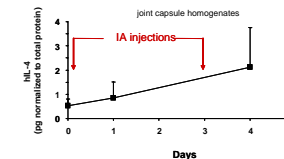
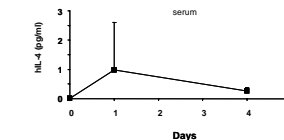


Figure 5. In-vivo expression of hIL-4 following IA delivery in mice. Plasmid was formulated with crown polymer and injected 2x over three days. One day after each injection tissues were harvested from animals for analysis by ELISA. Values are mean ± SD, n=5.



CONCLUSIONS

- Optimized polymeric systems for IA gene delivery were developed through a series of screening experiments. Transgene expression profiles suggest that levels were most prominent in synovial tissue and skeletal muscle tissue associated with the joint.
- Long-term local and systemic transgene expression was achieved from IA injections with the ability for repeated injections (a likely requirement for treatment of chronic degeneration associated with OA).
- Additional polymer modifications aimed at increasing DNA protection from DNases resulted in improved transgene expression levels following both IM and IA injection.
- Administration of plasmid encoding for the anti-inflammatory cytokine IL-4 produced significant local and systemic protein levels and suggests that polymeric delivery systems may be useful for gene therapy applications in the treatment of diseases of the joint.

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