Stability of gene delivery complexes for *in vitro* and *in vivo* transfection

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Introduction

Non-viral carriers are increasingly employed for delivery of therapeutic nucleic acids and are especially appealing for *in vivo* use, since viral vectors can induce undesirable responses [1]. Viral gene delivery to immunocompetent animals is less effective than immunocompromised animals in the case of bone regeneration [2], providing further need for non-viral gene delivery systems. Upon *in vivo* administration, either as free polymer/plasmid DNA complexes or complexes in a scaffold, gene delivery systems would need to be stable *in vivo* for hours/days for efficient transfection to be observed. This is especially true if host cells do not come in contact with the complexes immediately. Here, we investigate how transfection efficiency of complexes changes during prolonged incubation at body temperature, and whether this influences the effectiveness of *in vivo* gene delivery.

Materials & Methods

Complexes were made by mixing pDNA with polymer at a 2:10 weight ratio in saline at room temperature for 30 minutes. Polyethylenimine modified with linoleic acid (PEI-LA) or with polyethylene glycol (PEI-PEG) were synthesized as previously described [3,4]. For combination complexes, polymers were mixed in a 1:1 weight ratio prior to complex formation. Size of complexes was measured from the time of polymer addition up to 60 minutes and 24 hours after formation with dynamic light scattering. To examine changes in transfection efficiency, complexes were either used immediately or further incubated at 37 °C prior to exposure to cells. Complexes were either left in saline or modified with a Type A gelatin coating. *In vitro* transfections were performed with 293T cells grown in monolayer cultures, or seeded on absorbable gelatin sponges (Gelfoam) as a model for *in vivo* gene delivery. For monolayer transfections, complexes were added to media the day after cells were seeded. For sponge transfections, complexes were allowed to soak into the sponge and then cells were added to the loaded sponges. For *in vivo* transfections, sponges loaded with complexes (without cells) were implanted subcutaneously in rats. Sponges containing saline or pDNA alone (i.e. without a polymeric carrier) were included as controls. Sponges were harvested 1, 3, or 7 days after implantation, and no treatment saline used to normalize fluorescence. All animal protocols were approved by the University of Alberta Animal Welfare Committee. The pDNA was chosen based on ease of transgene detection for each type of transfection, and expressed one of Green Fluorescent Protein (GFP; monolayer), Bone Morphogenetic Protein-2 (BMP-2; *in vitro* sponge), or the red fluorescent protein dsRed (*in vivo* sponge). For uptake studies, pDNA was labeled with Cy3 with Cy-3 labeled pDNA alone as a control. The Kruskal-Wallis test (a non-parametric Analysis of Variance) followed by Dunn's multiple comparison test was used to determine the significance of *in vivo* results, since results did not follow a normal distribution.

Results

Complexes made from PEI-LA or a mixture of PEI-LA and unmodified PEI (PEI-LA/PEI) showed a steady increase from ~400 nm to ~1200 nm over the 60 minute period following complex formation. PEI-LA/PEI-PEG complexes showed no changes from the starting 200 nm size. Transfection with freshly prepared (0 hour) complexes led to high levels of GFP expression, as measured by flow cytometry, from all of PEI-LA, PEI-LA/PEI, and PEI-LA/PEI-PEG groups.

Incubation at 37 °C for 24 or 48 hours prior to cell exposure led to >90% drop in transfection efficiency for all three complex types, including the size stable PEI-LA/PEI-PEG complexes (See saline group in Figure 1A for typical transfection results).

A gelatin coating was found to prevent the decrease in transfection efficiency over time, but did not prevent the size increase in PEI-LA and PEI-LA/PEI complexes after a 24 hour incubation at 37 °C. Complexes coated in gelatin (1-0.01%) showed minimal or no decrease in transfection efficiency after a 24 hour incubation and still gave at least 50% of the original signal after a 48 hour incubation at 37 °C (Figure 1A). Transfection with gelatin-coated complexes also gave higher transfection compared to uncoated complexes on sponges (an *in vitro* model for *in vivo*), as determined by BMP-2 secretion over a 12-day period. The size-stable PEI-LA/PEI-PEG complexes gave much lower rates of BMP-2 secretion compared to PEI-LA and PEI-LA/PEI complexes, regardless of whether they were coated with gelatin. With PEI-LA and PEI-LA/PEI complexes, a gelatin coating increased BMP-2 secretion by 1.6-2.1 fold. To determine the mechanism behind the decrease in transfection, uptake of freshly prepared and incubated complexes was evaluated using flow cytometry. Cells transfected with freshly prepared complexes showed high percentage of cells (>90%) with pDNA. Saline-incubated complexes had low levels of uptake, with less than 10% containing pDNA. Gelatin-coated complexes, however, demonstrated minimal or no change in uptake after a 24 hour incubation at 37 °C. The gelatin-coated complexes led to better performance *in vivo* compared to uncoated complexes in saline, as measured by dsRed fluorescence. Sponges loaded with uncoated PEI-LA complexes led to significant fluorescence in scaffolds ($p<0.05$) only on day 3, whereas gelatin-coated complexes led to significant fluorescence in scaffolds ($p<0.01$) on Day 3 and 7 (Figure 1B).

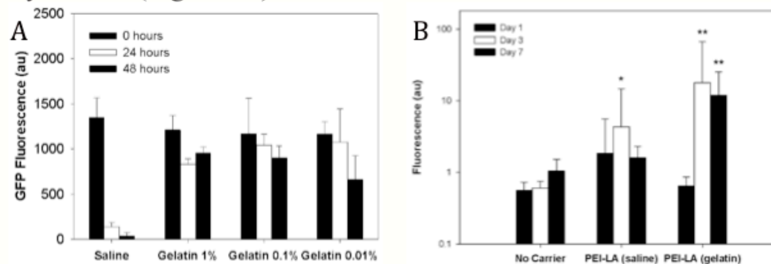

Figure 1: Transfection with PEI-LA complexes. 293T cells were transfected with complexes after 0-48 hour incubation at 37 °C (1A). Complexes loaded on sponges were implanted in rats for *in vivo* gene delivery (1B).

Discussion

The stability of polymer/pDNA complexes has often been determined using physicochemical characteristics such as size, but there have been no studies that have investigated how transfection efficiency changes during exposure to body temperature. In this study, we found that complexes rapidly lost their ability to transfect cells, and that size-stable complexes were not immune to this decrease. In agreement with *in vitro* data, the gelatin coating was also beneficial for *in vivo* transfections. Effectiveness of the gelatin-coatings in an animal model suggests that there is enough time between implantation and infiltration of the host cells for the complexes to lose transfection efficiency. This provides justification for more thorough investigations into how transfection efficiency changes over time during development of novel non-viral vectors.

References

[1] Kohn DS, Sadelain M, Glorioso JC. Nat Rev Cancer 2003;3(7):477-88.
[2] Kang Q, Sun M, Cheng H, Peng Y, Montag A, Deyrup A, et al. Gene Ther 2004;11(17):1312–20.
[3] Neamnark A, Suwantong O, KC R, Hsu C, Supaphol P, et al. Mol Pharm 2009;6(6):1798-1815.
[4] Zhang S, Kucharski C, Doschak MR, Sebald W, Uludag H. Biomaterials 2010;31(5):952-63.