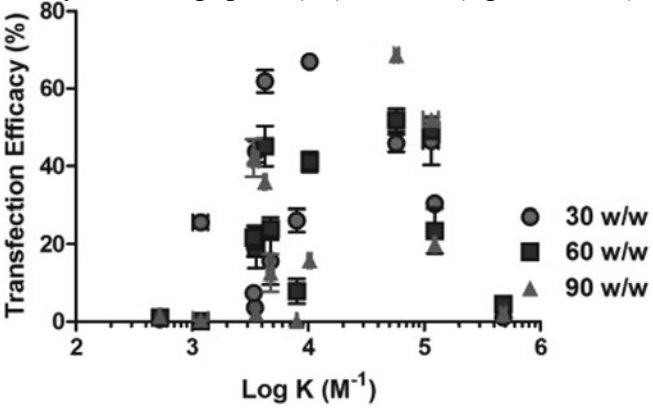
| | |
|---|---|
| **Titre:** Title: | Lyophilized chitosan nanoparticles for pDNA and siRNA delivery: Physico-chemical properties, transfection efficiency, and cytotoxicity |
| **Auteurs:** Authors: | Daniel Veilleux, Marc Lavertu, Kristof Biniecki, & Michael D. Buschmann |
| **Date:** | 2014 |
| **Type:** | Communication de conférence / Conference or Workshop Item |
| **Référence:** Citation: | Veilleux, D., Lavertu, M., Biniecki, K., & Buschmann, M. D. (mai 2014). Lyophilized chitosan nanoparticles for pDNA and siRNA delivery: Physico-chemical properties, transfection efficiency, and cytotoxicity [Affiche]. American Society of Gene & Cell Therapy 17th Annual Meeting, Washington, DC. Publié dans Molecular Therapy, 22(S1). https://doi.org/10.1016/s1525-0016(16)35365-5 |

JACS 2008). Time-correlated single photon counting allowed the determination of the amount of free and bound EB in solution based on the ratio of the areas under the decay associated spectra, which were used in the Hill equation to determine K (Bishop, JACS 2013). Polyplex diameter and x were quantified using NanoSight and dynamic light scattering. Transfection and cytotoxicity were assessed using flow cytometry and CellTiter 96®. Results As molecular weight decreased and as the number of carbons increased in the backbone and sidechain, K decreased. There was not a significant trend between K and either particle size, x, or cytotoxicity. There was a biphasic response when comparing transfection against K in both cell lines with optimal Ks ranging from (1-6) x 104 M-1 (Figure 1; GB319).




Conclusions A K in the range of (1-6) x 104 M-1 was necessary but not sufficient for optimal transfection. The binding affinity can be modified to be in the optimal range via GPC fractionation or through the addition of carbons in the backbone or sidechain. Investigating subtle structural differences revealed new quantitative and mechanistic relationships for gene delivery.

## 352. Lyophilized Chitosan Nanoparticles for pDNA and siRNA Delivery: Physico-Chemical Properties, Transfection Efficiency, and Cytotoxicity

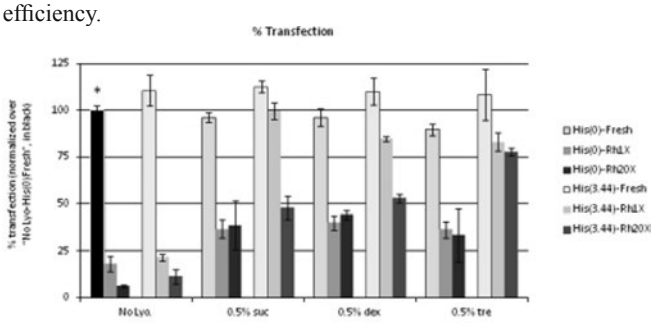
Daniel Veilleux,[1] Marc Lavertu,[1] Kristof Biniecki,[2] Michael D. Buschmann.[1]

*[1]Biomedical and Chemical Engineering, Ecole Polytechnique de Montreal, Montreal, QC, Canada; [2]ANRis Pharmaceuticals Inc, Kirkland, QC, Canada.*

**Purpose:** Polymer/nucleic acid nanoparticles (NP) using the natural polysaccharide chitosan (CS) have been optimised for efficient and safe delivery of plasmid DNA and siRNA, *in vitro* and *in vivo*. Further development of this technology for clinical and commercial applications requires stability and storage through lyophilisation. Specific compositions and methods were identified in this study to retain structural properties and biological activity of lyophylized chitosan nucleic acid nanoparticles and to permit concentration by rehydration in reduced volumes to achieve high doses.

**Methods:** CS/DNA NP were prepared using a 10kDa CS and the plasmid eGFPLuc, either formulated with or without 0.5% (w/V) lyoprotectant (sucrose, dextran 5kDa, or trehalose) and/or buffer (3.44 mM histidine at pH 6.5). NP formulations were then subjected or not to FD and rehydration at various concentrations. For each formulation, NP size and polydispersity index (PDI) were assessed by Dynamic Light Scattering (DLS); zeta potential, by Laser Doppler Velocimetry; and morphology, by Scanning Electron Microscopy (SEM). Transfection efficiency of formulations was quantified *in vitro* using a HEK293 cells, measuring the expression of reporter genes eGFP and Luciferase by flow cytometry and luminometry respectively. Cytotoxicity of formulations was measured *in vitro* using the lactate dehydrogenase (LDH) assay, the alamar blue assay, and the annexin V/propidium iodide apoptosis assay.

**Results**: Data showed that both lyoprotectant and buffer were required to prevent complex aggregation following FD and rehydration: hydrodynamic diameters ranged from 125 to 235nm, with PDI values from 0.15 to 0.25 and zeta potentials from +19 to +25mV. Optimal formulations could be concentrated up to 20-fold upon rehydration in lower volumes (Rh20X), while achieving near physiological pH and tonicity. Complexes obtained with these formulations were mainly spherical. Following FD and rehydrated to initial volume (Rh1X), these formulations had similar transfection efficiencies as fresh complexes without excipient, whereas after Rh20X, formulations with trehalose and histidine had the best efficiency.




Transfection results were normalized over control "No Lyo-His(0)-Fresh" (*), which had a 53% transfection efficiency in HEK293 cells. Excipients had little effect on transfection, with fresh compositions having transfection efficiencies above 90% of control. Both lyoprotectant and buffer were required to maintain transfection levels of at least 50% of control after FD; trehalose being the most effective lyoprotectant with a transfection efficiency of 70% of control after Rh20X.

Luciferase expression levels of formulations with histidine and sucrose or trehalose were not affected by FD and rehydration. All formulations had negligible cytotoxicity. Similar outcomes for CS siRNA systems for gene silencing were also found.

**Conclusion:** These novel biocompatible DNA and RNA delivery systems permit broad applications, increased doses, and improved storage properties. Characterization of these systems during accelerated and long term stability studies is ongoing.

## 353. Approaches for Improved Polymeric Gene Delivery To Umbilical Cord Blood-Derived Mesenchymal Stromal Cells

Laura Rose,[1] Cezary Kucharski,[1] Dulan Jayasooriya,[1] Leah A. Marquez-Curtis,[2] Hilal Gul-Uludag,[1] Anna Janowska-Wieczorek,[2] Hasan Uludag.[1]

*[1]Chemical Engineering, University of Alberta, Edmonton, Canada; [2]Canadian Blood Services, Edmonton, Canada.*

**Introduction:** Mesenchymal stromal cells harvested from umbilical cord blood (CB) offer vast potential for cell-based therapies, but their modification with plasmid DNA (pDNA) remains inefficient. Here, we investigated various approaches for improving transfection using small molecular weight (2 kDa) polyethylenimine modified with linoleic acid (PEI-LA) [1] as a low toxicity alternative to lipofection. Approaches pursued included centrifugation of polymer/pDNA complexes after addition to cells, modifying complexes with hyaluronic acid (HA) to target CD44 receptors, and spiking complexes with non-silencing RNAs. **Materials & Methods:** CB-derived cells [2] were transfected in 24-well plates with pDNA expressing GFP. PEI-LA or Lipofectamime™ 2000 was mixed with pDNA at controlled polymer/pDNA weight ratios for 30 minutes at room temperature before addition to cell cultures. After the complexes' addition, plates were centrifuged for 5 minutes at 210 g. Media was replaced after 4 hours. For coatings, gelatin or HA was added to complexes after formation. As a control, gelatin or HA was mixed with pDNA prior to PEI-LA. Finally, either control non-silencing small interfering RNA (siRNA) or micro RNA (miRNA) was mixed with