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578. Large Scale Preparation of Chitosan/ Nucleic Acid Nanoparticles Using an Automated Inline Mixing System

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Purpose: Polymer/nucleic acid (NA) nanoparticles (NPs) using the natural polysaccharide chitosan (CS) have been developed for efficient and safe delivery of plasmid DNA and siRNA (NAs), in vitro and in vivo. Further optimisation of this technology for clinical and commercial applications requires the implementation of large scale preparation methods. The rapid kinetics of NP formation (< 5 μs) has practical limitations on scalability, reproducibility and homogeneity of their production by manual mixing. In this study, an automated in-line mixing system was developed for large scale mixing of chitosan and NA solutions. This system was used to investigate the influence of mixing parameters on NP physicochemical properties.

Methods: A fully automated in-line mixing system consisting of two computer-controlled peristaltic pumps was developed to simultaneously drive large volumes of CS and NA solutions through a mixing connector, using equal volumetric ratios. NPs were prepared using a 10 kDa CS, and either plasmid DNA or siRNA. The influence of flow rate (Reynolds number, Re, ranging from 20 to 2000) and concentrations of CS (27 to 0.81 μg/ml of chitosan) and NAs (10 to 300 μg/ml) on the physicochemical properties of NPs was examined. Manually mixed NPs were prepared for comparison purposes. Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), and Environmental Scanning Electron Microscopy (ESEM) were used to assess the size, polydispersity (PDI), and morphology of NPs.

Results: The in-line mixing system allowed for the reproducible preparation of homogeneous NP batches of 1 L or more. In comparison, manual mixing resulted in broader particle size distribution and showed limited reproducibility. Mixing concentration had a very significant influence on NPs size. Indeed, a decrease in concentration of pDNA from 0.3 to 0.01 mg/mL resulted in a marked decrease of NPs hydrodynamic diameter and PDI (250 to 80 nm and 0.4 to 0.1, respectively). For an equivalent decrease in siRNA concentration, NP size and PDI decreased from 130 to 50 nm and from 0.21 to 0.18, respectively Aggregation occurred above a threshold NA concentration of 0.1 and 0.2 mg/mL for pDNA and siRNA, respectively. In contrast, the mixing flow rate or Re (in laminar flow regime) had no significant effect on NP size distribution. Below the aggregation concentration thresholds, ESEM observations of NPs prepared with either pDNA or siRNA revealed spherical or globular morphologies, independent of the mixing parameters.

Conclusion: The developed automated in-line mixing system is a promising method for the large scale and reproducible production of NPs. The significant influence of mixing concentration on chitosan NPs size revealed in this study will allow the preparation of homogeneous NPs of various sizes, which will be useful to provide NPs at the volumes and doses required in preclinical and clinical studies of these therapeutics.

Diabetes, Metabolic and Genetic Diseases II

579. Long-Term Follow-Up of Lentiviral Integration Profiles from Patients Undergoing Clinical Gene Therapy for X-Adrenoleukodystrophy

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HIV-1 based lentiviral vectors with self-inactivating (SIN) configuration are showing a reduced genotoxic risk in preclinical testing's compared to retroviral vectors harboring full LTRs. X-Adrenoleukodystrophy (X-ALD) has been the first monogenetic disease to be treated with gene therapy using HIV-1 based SIN vectors. In total, four patients have been enrolled in this clinical gene therapy trial. The first patient (P1) has been treated already more than six years ago, P2 5 years ago, P3 4.5 years and P4 two years ago. So far no severe adverse events have been reported from this and other clinical trials using HIV-1 based SIN vectors.

In the present study, we report an in depth integration site (IS) analysis of samples from 4 patients from this first clinical trial to treat a monogenetic cerebral disease using autologous hematopoietic stem cell transplantation with a HIV-1 based lentiviral SIN-vector. The correction of hematopoietic stem cells has not been accompanied by signs of clonal outgrowth or even premalignant disproportional of cellular contributions in the 4 treated patients. Linear amplification mediated PCR (LAM-PCR) performed on *ex vivo* transduced cells prior to reinfusion and on engrafted cells revealed a polyclonal hematopoietic reconstitution in all patients. High-throughput sequencing of LAM-PCR amplicons by 454 pyrosequecing and Illumina MiSeq sequencing identified a highly diverse clonal repertoire. In total, more than 33,000 unique IS have been retrieved from distinct time points and sorted cell fractions (P1: 6-74 months (M); P2: M6 – M64; P3: M2 – M54; P4: M1 – M24). Downstream bioinformatics analysis confirmed the characteristic insertion profile reported for lentiviral vectors. More than 70% of the detected IS are located within gene coding regions, which are known to be targeted preferentially by lentiviral vectors. Moreover, a favored integration on chromosomes 17 and 19 harboring gene dense regions has been observed. The affected genomic regions, close to KDM2A, PACS1 and HLA genes, are described for lentiviral clustering. The landscape of ALD vector integration was very similar in all four patients investigated. A successful ex vivo transduction of early hematopoietic progenitors is indicated by the detection of identical IS identified in myeloid and lymphoid lineages as well as in CD34+ cells. Lentiviral gene therapy shows to be safe and effective as the cerebral disease has been stabilized in 3 out of 4 patients.