LYSINE CO-HISTIDINE HYPERBRANCHED POLYMERS FOR SIRNA DELIVERY

Nour Allahham[1], Asma Buanz[1], Cameron Alexander[2], Steve Brocchini[1], Gareth R. Williams[1]

[1] Department of Pharmaceutics, UCL School of Pharmacy, WC1N 1AX, UK; [2] School of Pharmacy, University of Nottingham, NG7 2RD, UK

Background:

Small interfering RNA (siRNA) has made clinical progress because of its promise for gene silencing. siRNA works via the RNA interference (RNAi) mechanism, where it can potentially degrade any mRNA of interest and inhibit its translation into a protein. Developing safe and more efficient formulation strategies for siRNA remains a complex challenge. Previous work suggested that lysine-co-histidine hyperbranched polymers (pKH) are effective carriers for plasmid DNA. We investigated the one-step synthesis of pKH polymers for possible siRNA complexation.

Methods:

pKH polymers were prepared in a one-pot thermal polycondensation. Lysine (K) and histidine (H) with molar ratios of 4:1 and 4:2 were dissolved in a small amount of water in the presence of KOH and stirred at 170ºC for 8 or 16 h under a stream of N_2 to evaporate water to allow melt polymerisation as the reaction proceeded. The structure of pKH hyperbranched polymers was confirmed with NMR and Mn was obtained by GPC. For complexation, we used a short double strand DNA oligo (21bp) as a siRNA surrogate for proof of concept. Complexation was confirmed with agarose gel electrophoresis.

Results:

NMR analysis confirmed the formation of hyperbranched pKH polymers, however the starting stoichiometry of lysine and histidine was not maintained during the reaction, resulting in different/inconsistent lysine and histidine ratios. Variations in the molecular weight were also observed between batches, suggesting heterogenicity and that reproducibility was process dependent. When complexing pKH polymers with DNA oligos, different pKH batches displayed varying complexation efficiencies.

Conclusions:

The heterogenicity of hyperbranched pKH polymers results in inconsistent complexation efficiency with short DNA oligos. Process dependent factors related to water removal and heat transfer during pKH preparation are difficult to control and may be the cause of batch-to-batch variability. We are currently examining the solution-phase synthesis of pKH to increase the reproducibility of its complexation efficiency.