Lysine co-histidine hyperbranched polymers for siRNA delivery

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Background:

siRNA has gained an increasing interest because of its great promise in the field of gene silencing. siRNA works via RNAi mechanism (Fig 1), where it can potentially degrade any mRNA of interest and inhibit its translation into a protein. Developing a safe and more efficient delivery platform for siRNA remains a complex challenge to date.

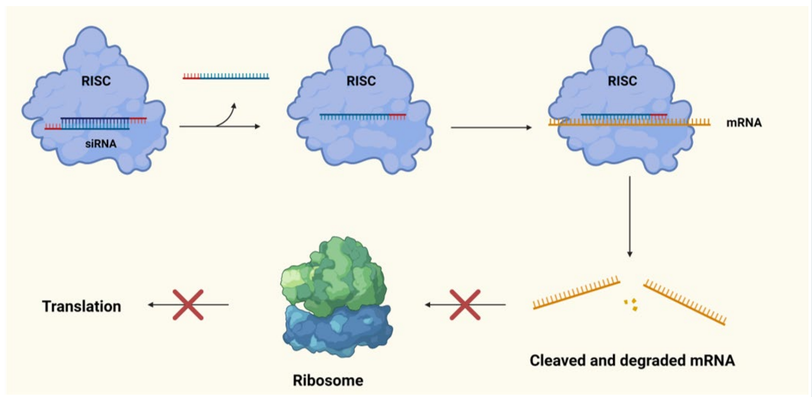

Fig 1. *RNA interference (RNAi) mechanism. siRNA: small interfering RNA. RISC: RNA-induced silencing complex.*

Objectives:

To develop a biodegradable cationic polylysine co-histidine hyperbranched polymer (pKH) (Fig 2) that can encapsulate anionic siRNA and deliver it safely and efficiently.

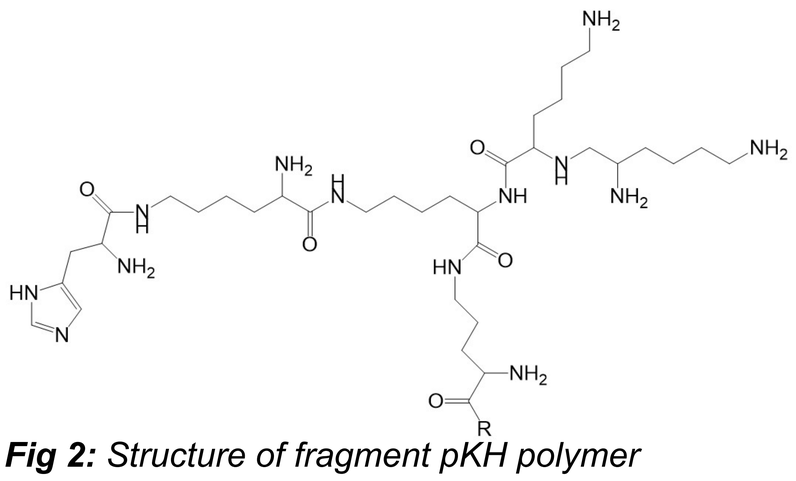

Fig 2: *Structure of fragment pKH polymer*

Methods

pKH polymers were synthesized by thermal polycondensation[1]. Lysine (K) and histidine (H) with molar ratios 4:1 and 4:2 were dissolved in water in the presence of KOH and stirred at 170ºC for 8 or 16h under a stream of N_2. The structure of pKH was confirmed with NMR and Mn was obtained by GPC. For complexation we used double strand DNA oligo (21bp) as a proof of concept.

Results & Discussion:

pKH synthesis and characterisation:

Peak integrals show that the starting stoichiometry of lysine and histidine was not maintained during the reaction.

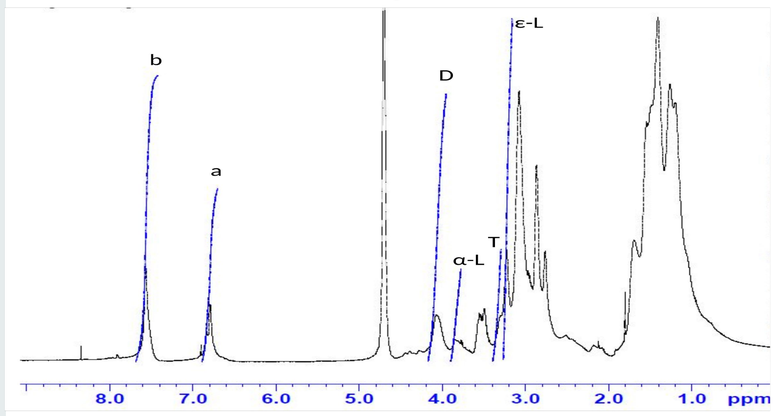

Fig 3*. [1]H NMR spectrum of pKH (500mHz in D_2O) δ 7.56 and 6.79 (CH in imidazole ring, b and a) 4.07 (COCH(R)NH dendritic, D) 3.83 (COCH(R)NH α-linear, α-L), 3.31 (COCH(R)NH terminal, T) 3.27 (COCH(R)NH ε-linear, ε-L).*

Variations in molecular weight (Fig 4) are observed between batches

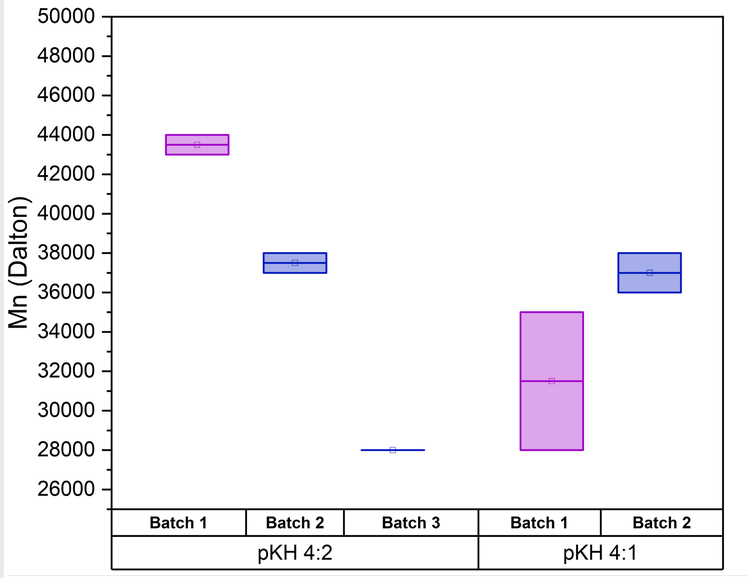

Fig 4 *Molecular weight (Mn) for different batches of pKH. Blue and purple bars represent batches synthesised at 8 and 16h respectively.*

Polyplex formation & complexation efficiency:

Polyplexes were formed at different amine:phosphate (N:P) ratios. Fig 5, shows polyplexes formed with pKH 4:2 at N:P ratio 10:1

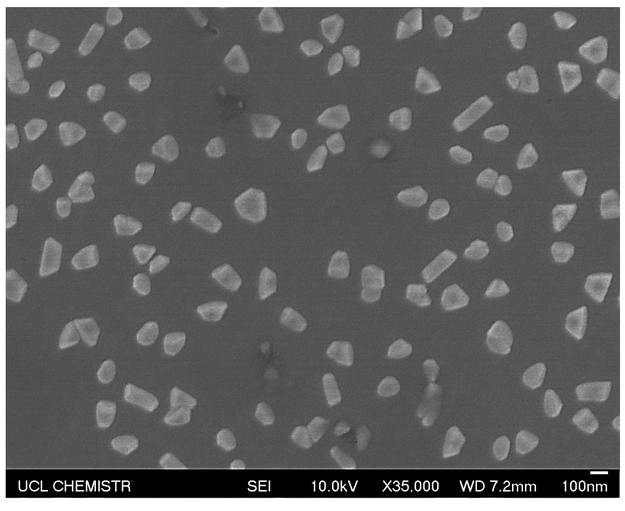

Fig 5 *SEM image of polyplex of pKH 4:2 with double strand DNA oligo at N:P ratio 10:1*

Different batches of pKH showed different complexation efficiencies at the same amine:phosphate (N:P) ratio (Fig 6). Differences in fluorescence intensities indicate differences in complexation efficiency

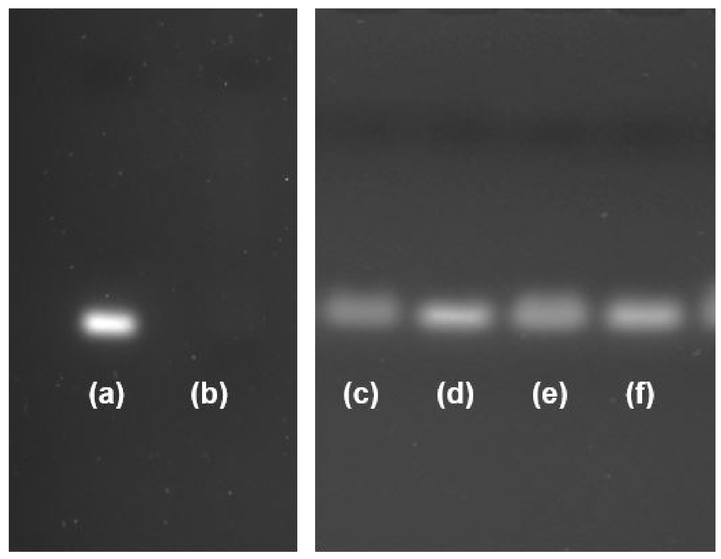

Fig 6. *Agarose gel electrophoresis of pKH complexation with oligos N:P 25:1. (a) free oligos. (b, c, e) pKH 4:2 (d, f) pKH 4:1.*

Conclusions and future work:

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.

References:

Alazzo, A., Lovato, T., Collins, H., Taresco, V., Stolnik, S., Soliman, M., Spriggs, K., and Alexander, C. (2018) Journal of Interdisciplinary Nanomedicine, 3:38-54

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