Electronic Supplementary Information

Structural variations in hyperbranched polymers prepared via thermal polycondensation of lysine and histidine and their effects on DNA delivery

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Table of Contents

1 Synthesis and characterization of hyperbranched polymers .......................................................... 2

Figure s1: FT-IR spectra of hyperbranched polymers. ................................................................. 2
Figure s2: H-NMR spectrum of hb-polyk. .................................................................................. 3
Figure s3: 13C-NMR spectrum of hb-polyk. .............................................................................. 4
Figure s4: H-NMR spectrum of hb-polykh ............................................................................... 5
Figure s5: 13C-NMR spectrum of hb-polykh. ........................................................................... 6
Figure s6: H-NMR spectrum of polymers in group-A stacked together to show the growth in the imidazole peaks with increase in molar ratio of histidine. .............................................................................. 7
Figure s7: H-NMR spectrum of polymers in group-B stacked together to show the growth in the imidazole peaks with increase in molar ratio of histidine. ................................................................. 7
Figure s8: HSQC spectrum of hb-polyk .................................................................................. 9
Figure s9: HSQC spectrum of hb-polykh .............................................................................. 10
Figure s10: Chemical shifts of imidazole amines in hyperbranched polymers at different pH values .......... 11

2 Polypelexes preparation and characterization .............................................................................. 12

Figure s11: (a) The histograms of AFM images, analysed by image-j, show the size distribution of polypelexes. (b) Hydrodynamic radii of polypelexes prepared in PBS, pH 7.4 at different N/P ratios using polymers of group-a and group-b. .............................................................................. 12
Figure s12: (a) Zeta potential measurements, (b) ethidium bromide displacement assay and (c) agarose gel electrophoresis of hyperbranched polymers/dna polypelexes prepared in PBS, pH 7.4 at different N/P ratios. ................................................................. 13

3 Biological evaluation of polypelexes ....................................................................................... 14

Figure s13: Metabolic activity of a549 cells treated with (a) the hyperbranched polymers and (b) their polypelexes. ........................................................................................................ 14
Figure s14: Luciferase activity of polypelexes prepared in PBS, pH 7.4 with polymers of high molecular weight polypelexes. .................................................................................. 15
Figure s15: Heparin competition assay ........................................................................................ 16
Figure s16: LDH assay .................................................................................................................. 16
Synthesis and characterization of hyperbranched polymers

Figure S1: FT-IR of hyperbranched polymers

Vibrational absorptions at (3283, 3063, 2925, 2862, 1644, 1529, 1439, 1369, 1309, 1253 cm\(^{-1}\)) and strong peaks at 1640 cm\(^{-1}\) and 1529 cm\(^{-1}\) (amide bands I and II) confirm the formation of the peptide bonds.
Figure S2: $^1$H-NMR spectrum of hb-polyK, shows the structural units of the polymers, (400 MHz, D$_2$O) δ = 4.13 (b, 1H, COCH(R)NαH, dendritic unit), 3.85 (br, 1H, COCH(R)NαH, α-linear unit), 3.33 (br, 1H, COCH(R)NαH, terminal unit), 3.23 (br, 1H, COCH(R)NαH, ε-linear unit), 3.11 (br, 2H, -CH$_2$-NεH), 2.68 (br, 2H, -CH$_2$-NεH$_2$), 1.8–1.2 (br, 6H, -CH$_2$-).
Figure S3: $^{13}$C-NMR spectrum of $hb$-polyK, (100 MHz, D$_2$O) $\delta$ = 177.3 (-C(O)-NH), 54.6 (-COCH(R)NαH), 38.9 (-CH$_2$-NεH$_2$), 34.0 (-CH$_2$-CH-NαH$_2$), 28.2 (-CH$_2$-CH$_2$-NεH), 22.3 (-CH$_2$-CH$_2$-CH$_2$-NεH$_2$).
Figure S4: $^1$H-NMR spectrum of $hb$-polyKH, (400 MHz, D$_2$O) δ = 7.59 (br, 1H, CH of imidazole ring), 6.81 (br, 1H, CH of imidazole ring), 4.11 (br, 1H, COCH(R)NαH, dendritic unit), 3.85 (br, 1H, COCH(R)NαH, α-linear unit), 3.31 (br, 1H, COCH(R)NαH, terminal unit), 3.22 (br, 1H, COCH(R)NαH, ε-linear unit), 3.10 (br, 2H, -CH$_2$-NeH), 2.77 (br, 2H, -CH$_2$-NeH), 1.8−1.2 (br, 6H, -CH$_2$−).
Figure S5: $^{13}$C-NMR spectrum of $hb$-polyKH, (100 MHz, D$_2$O) δ = 177.3 (C(O)-NH), 135.8 (C- OF IMIDAZOLE RING), 54.6 (COCH(R)NaH), 38.9 (-CH$_2$-NεH$_2$), 34.0 (-CH$_2$-CH$_2$-NaH$_2$), 28.28 (-CH$_2$-CH$_2$-NεH), 22.38 (-CH$_2$-CH$_2$-CH$_2$-NεH$_2$).
Figure S6: $^1$H-NMR spectra of polymers in Group-A. The integrals corresponding to the protons of the imidazole ring (7.59 ppm and 6.81 ppm) increase with the increase in molar ratio of histidine.
Figure S7: $^1$H-NMR spectra of polymers in Group-B. The integrals corresponding to the protons of the imidazole ring (7.59 ppm and 6.81 ppm) increases with the increase in molar ratio of histidine.
Figure S8: HSQC spectrum of \( hb \)-polyK.

The spectrum shows the resonances of the various \( \alpha\)-CH protons linked to those for the \( \alpha\)-C of \(^{13}\)C-NMR spectrum, which confirms that there are several environments for these protons dependent on the different branch units.
Figure S9: HSQC spectrum of $hb$-polyKH, confirming the incorporation of histidine in the polymers
Figure S10: Chemical shifts of imidazole protons of hyperbranched polymers at different pH values, which were used to calculate the pKa of imidazole amine.
2 Polyplexes preparation and characterization.

A

hb-polyK-13kDa (N/P ratio of 5)  hb-polyKH2-18kDa (N/P ratio of 5)

B

Figure S11: (A) the histograms of AFM images, analysed by Image-J, show the size distribution of polyplexes. (B) Hydrodynamic radii of polyplexes prepared in PBS, pH 7.4 at different N/P ratios using polymers of Group-A and Group-B.
Figure S12: (A) Zeta potential measurements, (B) ethidium bromide displacement assay and (C) agarose gel electrophoresis of hyperbranched polymers/DNA polyplexes prepared in PBS, pH 7.4 at different N/P ratios.
Figure S13: Metabolic activity of A549 cells treated with (A) the hyperbranched polymers and (B) their polyplexes.

(A) A549
Figure S14: Luciferase activity of polyplexes prepared in PBS, pH 7.4 with polymers of high molecular weight.
Figure S15: Heparin competition assay, the polyplexes of hb-polyK-13kDa, hb-polyKH₂-18kDa, hb-polyK-33kDa and hb-polyKH₂-34kDa at N/P ratio of 10 were incubated with increased amount of heparin sulfate for 30 min and observed by gel electrophoresis.

Figure S16: LDH assay of hb-polyK-13kDa and hb-polyKH₂-18kDa polyplexes of N/P ratio of 10 in A549 and H1299 cell lines, which reflect the stable nature of A549 membrane in comparison with H1299 where the difference between hb-polyK-13kDa and hb-polyKH₂-18kDa in their ability to interact and permeabilise the membranes can be seen clearly in H1299 but not in A549.