R8-modified polysarcosine-b-PLL polypeptide to enhance circulation stability and gene delivery efficiency

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Introduction

Recently, polypeptide has been extensively researched in gene/drug delivery system for its similar structure to natural protein, which bring it with good biocampatibility and low cytotoxicity. Herein, we synthesized total-polypeptide copolymers azobenzene-modified polysarcosine-b-PLL(ASL) by NCA polymerization, which is a living polymerization method to produce polypeptides with high molecular weight and narrow MWDs[1]. PLL, a commonly used cationic polypeptide, was designed to bind DNA. Polysarcosine(PSAR) was used as PEG alternative for its outstanding hydrophilicity and excellent protein resistance ability[2,3]. Via host-guest interaction between azobenzene and cyclodextrin, a cell-penetrating peptide, arginine octamer(R8), was introduced to increase its cytomembrane penetrability[4].

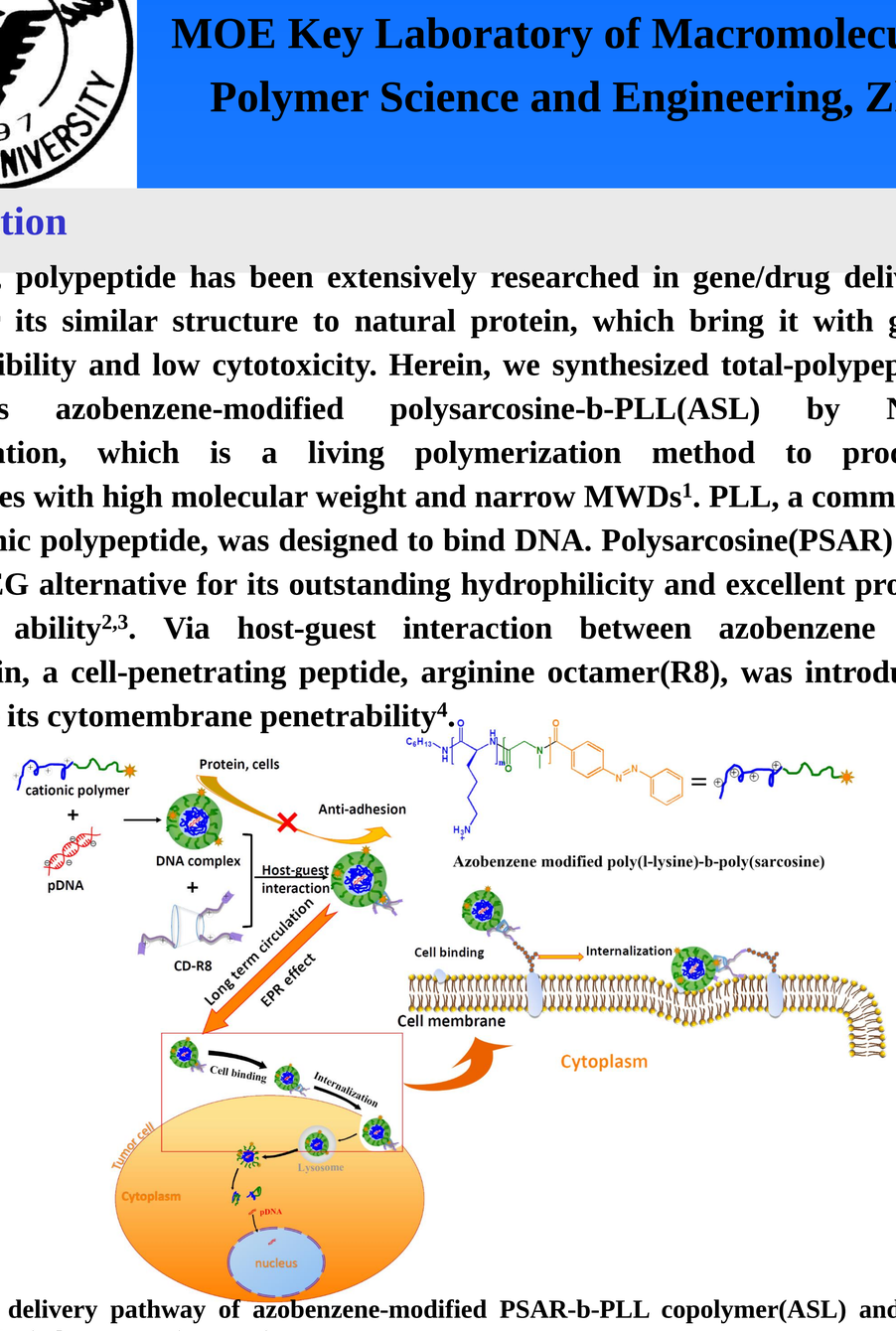


Fig. 1 Gene delivery pathway of azobenzene-modified PSAR-b-PLL copolymer(ASL) and R8-modified ASL via host-guest interaction

Results & Discussion

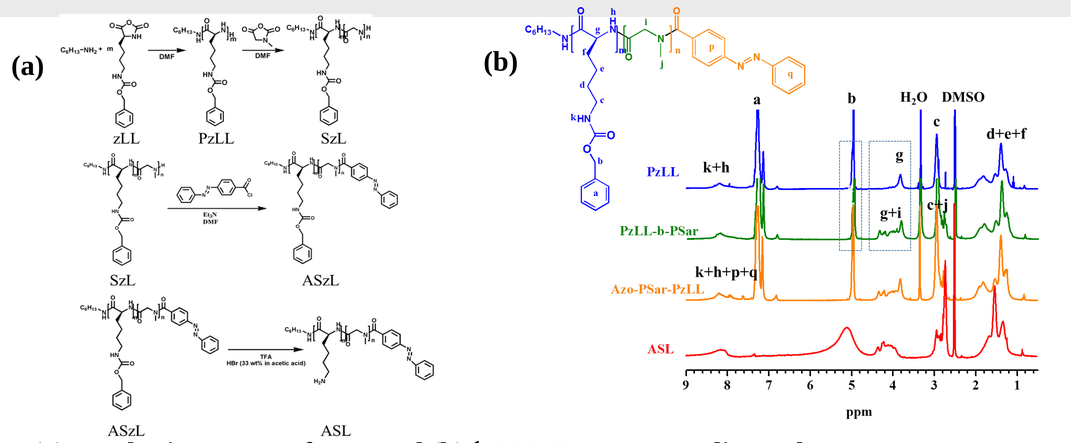
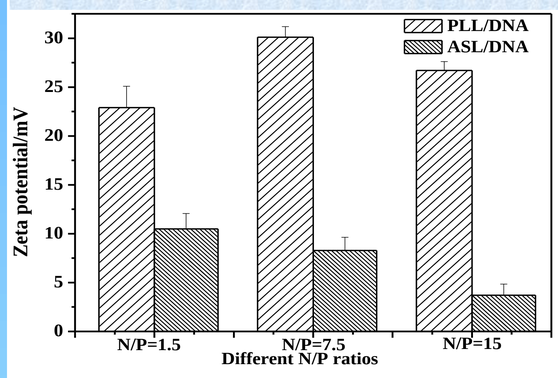


Fig. 2 (a) Synthesis process of ASL and (b) ^{1}H NMR corresponding polymers

➢ASL with 47 lysine units and 43 sarcosine units were successfully synthesized according to ^{1}H NMR and GPC.



➢The size of ASL/DNA was around 200 nm based on DLS. The shielding effects of poly(sarcosine) drew the potential to near zero.

Fig. 3 Z-ave diameters of gene complexes at various N/P ratios

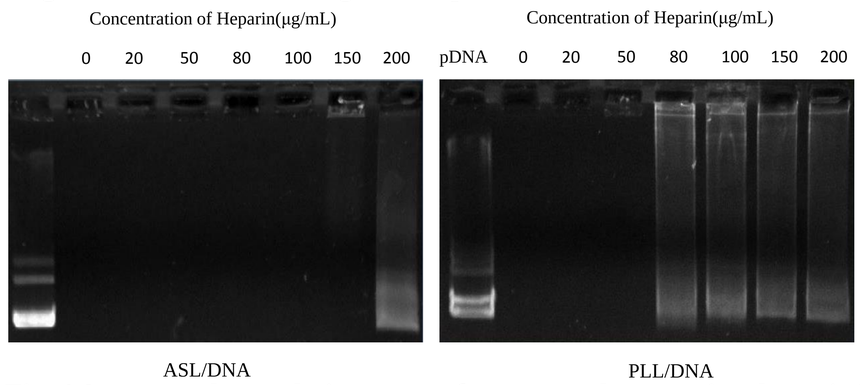


Fig. 4 Agarose gel retardation assay of gene complexes against heparin(N/P=7.5)

➢ASL/DNA showed excellent shielding effect against polyanion.

➢DLS data showed the ASL/DNA complex had superior physiological stability.

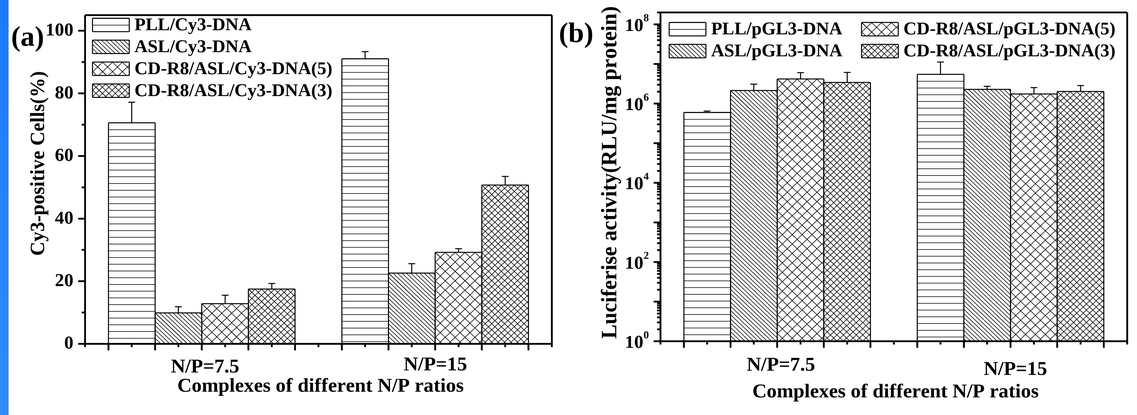


Fig. 5 (a) Cellular uptake assays and (b) in vitro transfection of gene complexes

➢ Endocytosis decreased for the shielding of PSAR. The introduction of R8 via host-guest interaction highly enhanced cellular uptake for the transmembrane effect of R8. Transfection efficiency of ASL/DNA was similar to that of PLL/DNA.

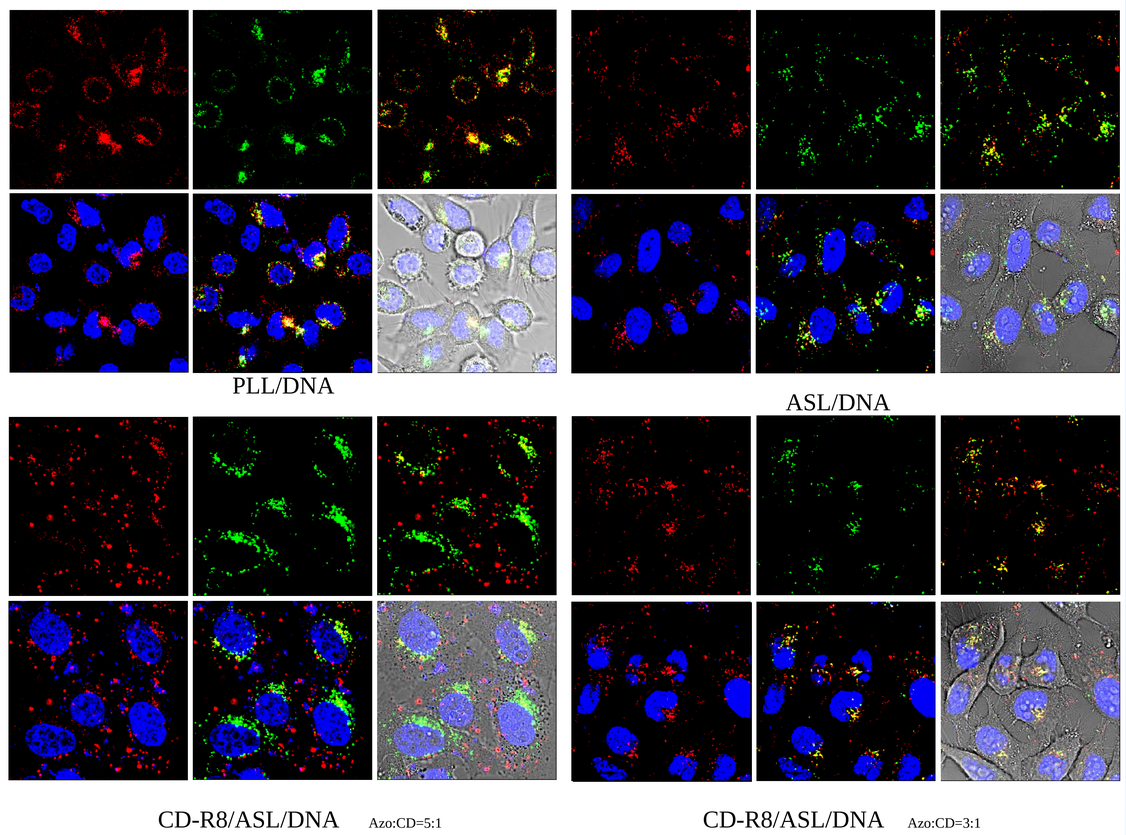


Fig. 6 CLSM images of cells exposed to different polymers complexed with cy3-DNA (Red) for 4.5 h incubation and incubated for another 12 h. The nuclei were stained with DAPI(blue), and endosome were dyed green by LysoTracker® Green DND.

➢It was difficult for PLL/DNA and ASL/DNA complexes to escape from endosome. Due to cell penetrating effect, CD-R8/ASL/DNA complex entered cytoplasm, with a few penetrating into nucleus.

Conclusion

In summary, poly(sarcosine) was, for the first time, applied in gene delivery system as PEG alternative and showed outstanding shielding effects, which stabilized the polymer complexes in physiological saline and polyanion solution. Furthermore, the introduction of R8 could not only enhance the cellular uptake, but also promote the internalization of gene into cytoplasm. Also, the short PLL chain may release DNA easily in complicated intracellular circumstance and lead to comparable transgene expression to optimal PLL carriers.

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References

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