Dendritic lipopeptides for high gene transfection efficiency with structure optimization by molecular dynamic simulation

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Statement of Purpose: Gene therapy as a novel cancer treatment has been attracting more and more attention. However, for the clinical application, delivery system is always considered as a bottleneck. It was found that the assembled branched structures provide unusually strong electrostatic attraction between DNA and siRNA molecules for higher surface potential, thereby improving the transfection capability through a tightly condensed form.[1] Obviously, different generation materials possess different surface potential, hydrophilic-hydrophobic properties and cytotoxicity. Thus, there should be balance among high compaction, well assembly and timely release and the suitable branching degree needs to be found. In this study, different generation of arginine-rich dendritic molecules mimicking cell-penetrating peptides of virus envelop have been synthesized.[2] We are now making effort to screen out the optimal structures with the highest transfection efficiency and illuminate the assembly, packing, disassociation mechanism through experiments coupling with molecular dynamic simulation. Moreover we investigated the influence of gene delivery and release by reduction sensitive disulfide bond.

Methods: We prepared three disulfide modified lipopeptides (R_1LS, R_2LS and R_3LS) with different generation arginine-rich dendritic periphery. The molecular structures were proved by nuclear magnetic resonance and mass spectra. Then we exploited dynamic light scattering and transmission electron microscope to detect the size, zeta potential and morphology of assemblies. The gene condensing and release were characterized by gel electrophoresis. The cytotoxicity of assemblies was determined by Cell Counting Kit-8 (CCK8) assay. *In vitro* DNA transfection efficiency was carried out on Hela and HepG2 cells. And the molecular dynamic simulation was used to optimize the structure of dendrimers. Moreover we prepared the contrast materials R_2L, which did not contain disulfide bond. We studied the cytotoxicity and *in vivo* gene transfection capacity of R_2LS and R_2L. The confocal microscopy was used to study the intracellular dissociation of R_2LS/DNA and R_2L/DNA complexes in Hela cells.

Results: These dendritic lipopeptides could self-assemble in aqueous solution with different morphology (fiber, sphere and spindle shapes), size (from 190-500 nm) and surface potential (10-32 mV). The gel electrophoresis assay showed that R_1LS could not condense DNA well at any given N/P ratios. R_2LS and R_3LS could completely retard DNA migration at the N/P ratios of 10 and 4, respectively. For gene release, R_2LS could completely release gene while R_3LS and R_2L showed no liberated band. In CCK-8 assay, R_1LS assemblies showed hardly any cytotoxicity even at high concentration (100 μg/mL) and the R_2LS was slightly cytotoxic at a wide range of concentration (10-100 μg/mL). However, R_2L and R_3LS assemblies obviously inhibited the cell viability with increasing concentration. In vitro, R_2LS showed high gene transfection efficiency versus liposome 2000 and PEI in 10 % FBS, which increased by 58.7 % for Hela cells. In vivo, R_2LS induced evidently high level of luciferase activity in the tumor with 6.2-fold higher protein expression than that of PEI. While R_2L and R_3LS could not release gene thus presented low gene transfetion efficiency. In single molecular simulation, R_2LS molecule showed better flexibility. In multiple molecular simulations, R_2LS system presented a tight integration while R_1LS and R_3LS system have large molecular interval.

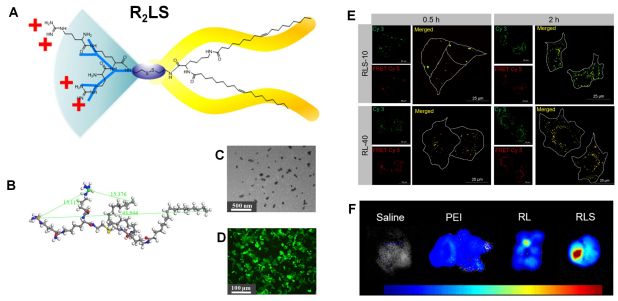

Figure 1. (A) The schematism of R_2LS molecule. (B) The optimized structure by molecular dynamic simulation. (C) The morphology of R_2LS assemblies by transmission electron microscope. (D) In vitro gene transfer effect. (E) The intracellular dissociation study. (F) In vivo gene transfer effect.

Conclusions: (i) The diversity in physicochemical properties of different generation assemblies induced different pDNA binding ability, cellular toxicity and transfection efficiency. (ii) High surface potential led to release gene difficultly although it was conducive to well condense gene. (iii) Disulfide bond has a crucial influence in gene release. (iv) High surface potential also led to high cytotoxicity. (v) R_2LS showed high transfection efficiency *in vitro and in vivo*. (vi) The simulation results indicated that R_2LS molecule has a better flexibility, which was favor to interact with cell membrane. And it could generate a tight integration in self-assembly, which led to a controllable release of cargos in reductive environment. **Overall, the 2nd generation dendrimers (R_2LS) possessed best performance in all respects.** Different generation dendritic assemblies have a significant influence on gene transfer effects. Thus we recommend to do structure optimization to obtain suitable branching degree due to different materials existing different optimum generation. Experiment coupling with computer simulation is a feasible means. Moreover, environmental responsiveness also plays a vital role.

References:

1. J. Yoo, J. Controlled Release. 2017; 246: 142-154.
2. X. Chen, J. Mater. Chem. B. 2017; 5: 1482-1497.