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Poly(glycoamidoamine) brush nanomaterials for systemic siRNA delivery *in vivo*†

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Delivery is the key challenge for siRNA based therapeutics. Here, we report the development of new poly(glycoamidoamine) brush nanomaterials for efficient siRNA delivery. GluN4C10 polymer brush nanoparticles, a lead material, demonstrated significantly improved delivery efficiency for siRNA against factor VII (FVII) in mice compared to poly(glycoamidoamine) brush nanomaterials reported previously.

Small interfering RNA (siRNA) has been extensively applied for biological and therapeutic purposes in the past two decades.[1–6] Clinical results demonstrated the potential of siRNA for treating a wide variety of diseases.[5,7,8] Although tremendous efforts have been made to improve the delivery of siRNA, systemic and effective delivery of siRNA remains a challenging issue for its broad therapeutic applications.[6,9–15] Here, we report the design, synthesis, and characterization of new poly(glycoamidoamine) brush nanomaterials for efficient siRNA delivery both *in vitro* and *in vivo*.

Previously, we reported a class of poly(glycoamidoamine) brush materials and evaluated their efficiency for siRNA and mRNA delivery.[16] Analysis of structure–activity relationships indicated that an increased number of amines in the monomer and short alkyl tails facilitated RNA delivery.[16] Based upon these design criteria, we synthesized three new materials (Fig. 1).[16] Three modified poly(glycoamidoamine) polymers consisting of tartarate (Tar), galactarate (Gal), or glucarate (Glu) sugars were first obtained using the method reported by Reineke.[17–22] 1,2-Epoxydecane then underwent ring-opening reactions with these polymers to afford the designed poly(glycoamidoamine) brush materials. The structures of the polymer brush materials were confirmed by ^{1}H NMR.

Polymer brush materials were subsequently formulated with DSPC, cholesterol (Chol), DMG-PEG$_{2000}$, and siRNA against Fluc into polymer–siRNA nanoparticles. Then, we characterized these nanoparticles:[16,23] particle size ranged from 114 nm to 159 nm; surface charge was neutral or slightly positive; and siRNA encapsulation efficiency was between 53%

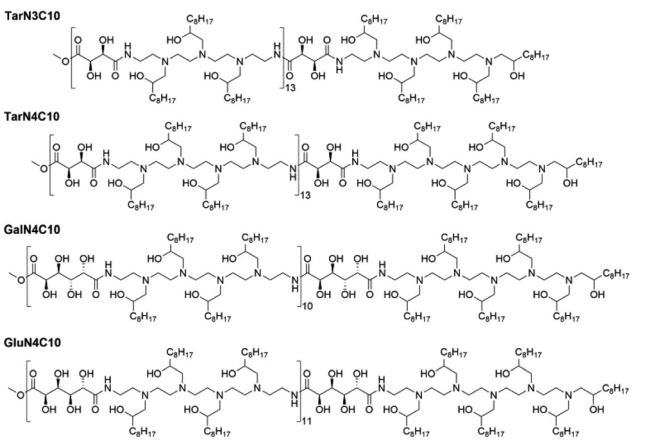

Fig. 1 Structures of polymer brush materials. The nomenclature is a combination of all three building blocks: the sugar units, the number of amines in the monomer, and the number of carbons in the epoxides.

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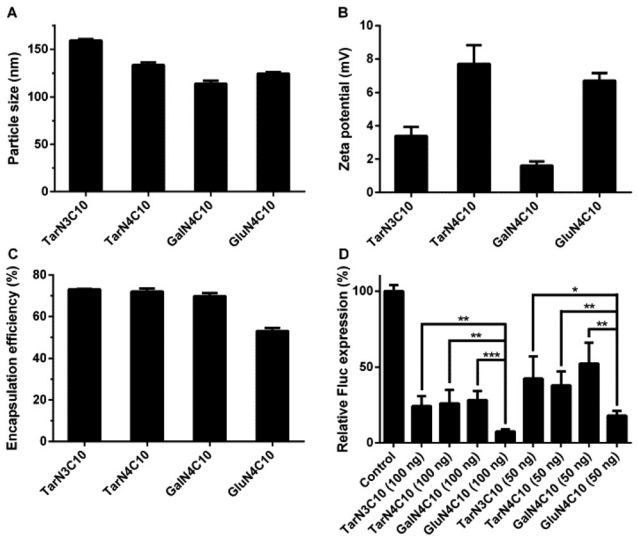

Fig. 2 Polymer brush nanoparticle characterization and siRNA delivery *in vitro*. (a–c) Characterization of polymer–siRNA nanoparticles: particle size, particle surface charge, and siRNA encapsulation efficiency. (d) Fluc silencing of polymer–siRNA nanoparticles. Formulation GluN4C10 showed significantly higher gene silencing activity compared to other formulations. (Quadruplicates; two-tailed *t*-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.)

and 73% (Fig. 2a–c). In order to evaluate the siRNA delivery efficiency of these formulations *in vitro*, dual-HeLa cells expressing both firefly and *Renilla* luciferase were treated with polymer brush nanoparticles.[24,25] As shown in Fig. 2d, the formulation GluN4C10 silenced Fluc expression 93% at a siRNA dose of 100 ng and 82% at a siRNA dose of 50 ng, which was significantly more effective compared to other formulations including TarN3C10, a lead material reported previously.[16] Consequently, GluN4C10 was selected for further studies.

We then characterized GluN4C10 nanoparticles for their stability and morphology. The particle size was measured weekly by dynamic light scattering (DLS). The results indicated that this formulation was stable at 4 °C for at least 4 weeks (Fig. 3a). TarN3C10, TarN4C10, and GalN4C10 nanoparticles

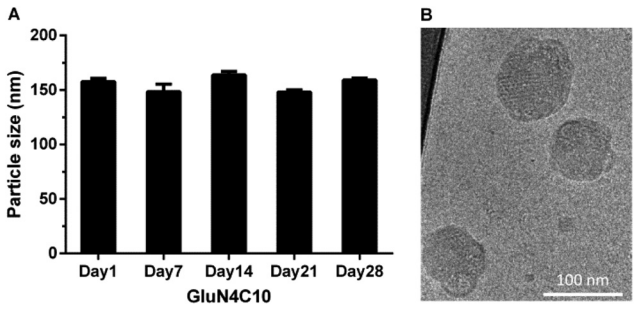

Fig. 3 Stability test and Cryo-TEM of GluN4C10 polymer brush nanoparticles. (A) Particle size of GluN4C10 remained constant at 4 °C for four weeks. Data represent group mean ± SD ($n = 3$). (B) A representative Cryo-TEM image of GluN4C10 nanoparticles.

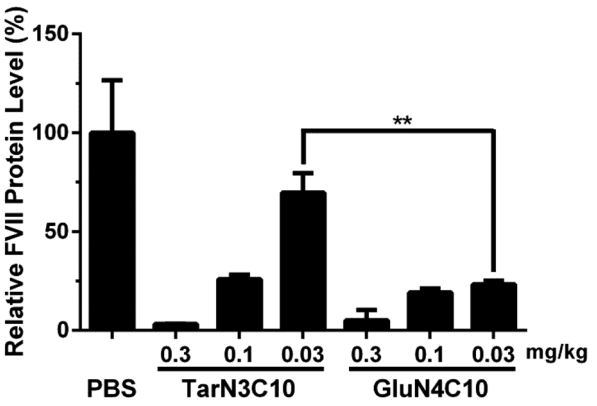

Fig. 4 siRNA delivery efficiency of GluN4C10 polymer brush nanoparticles *in vivo*.

showed a similar stability at the same time course (Fig. S1†). We also observed apparent cellular uptake of the GluN4C10, TarN4C10 and GalN4C10 nanoparticles using Alexa 647-labelled siRNA (Fig. S2†). The Cryo-TEM image revealed the morphology of the GluN4C10 nanoparticles with particle size consistent with the measurements from DLS (Fig. 3b). Given the promising results of the GluN4C10 nanoparticles *in vitro*, we evaluated the delivery efficiency of GluN4C10 for siRNA against FVII *in vivo*. We then injected the GluN4C10-FVII siRNA nanoparticles into mice through their tail vain at three different doses: 0.3 mg kg^{-1}, 0.1 mg kg^{-1}, and 0.03 mg kg^{-1}. TarN3C10 nanoparticles served as a positive control. As shown in Fig. 4, both TarN3C10 and GluN4C10 polymer brush nanoparticles displayed dose-dependent silencing of FVII. At a siRNA dose of 0.3 mg kg^{-1}, GluN4C10 showed effective and comparable FVII silencing activity (up to 95%) compared to TarN3C10. At a lower siRNA dose of 0.03 mg kg^{-1}, GluN4C10 displayed a significantly higher FVII silencing than TarN3C10 (77% *versus* 30% at 0.03 mg kg^{-1}). Reflecting the results above, the GluN4C10 polymer brush nanoparticles were capable of efficiently delivering siRNA molecules *in vivo*.

Conclusions

In summary, we designed and synthesized three new polymer brush materials based on the design criteria established previously. The formulation GluN4C10 nanoparticles demonstrated efficient siRNA delivery both *in vitro* and *in vivo*. We speculate that sugar units may play a more critical role in this series, and thereby improve delivery efficiency. Most importantly, GluN4C10 were capable of silencing 77% of FVII expression at a dose of 0.03 mg kg^{-1}, significantly more potent than TarN3C10. Therefore, GluN4C10 polymer brush nanomaterials are promising siRNA delivery vehicles and merit further development for therapeutic applications.

All procedures used in animal studies conducted at MIT were in compliance with Massachusetts laws or guidelines, were approved by the Institutional Animal Care and Use

Committee (IACUC) and were also consistent with local, state, and federal regulations as applicable.

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