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

Published in final edited form as:

Biomater Sci. 2016 December 20; 5(1): 38–40. doi:10.1039/c6bm00683c.

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

X. Luo[‡,a], **W. Wang**[‡,b,c], **J. R. Dorkin**[d], **O. Veiseh**[b,c,e], **P. H. Chang**[b,c], **I. Abutbul-Ionita**[f], **D. Danino**[f], **R. Langer**[b,c,e,g], **D. G. Anderson**[b,c,e,g], and **Y. Dong**[a,h,i,j]

[a]Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA

[b]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[c]Department of Anesthesiology, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA

[d]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[e]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[f]Department of Biotechnology and Food Engineering, Technion Institute of Technology and the Russell Berrie Nanotechnology Institute, Haifa 32000, Israel

[g]Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[h]Department of Biomedical Engineering, The Ohio State University, Columbus, OH 43210, USA

[i]The Center for Clinical and Translational Science, The Ohio State University, Columbus, OH 43210, USA

[j]The Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210, USA

Abstract

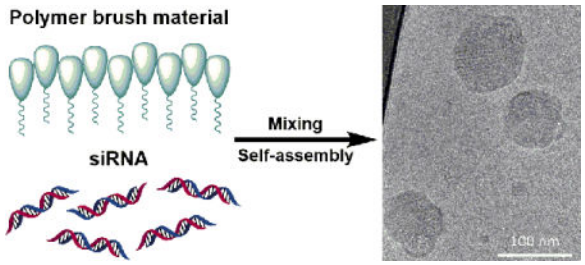
Delivery is the key challenge for siRNA based therapeutics. Here, we report the development of new poly(glycoamidoamine) brush nanomaterials for efficient siRNA delivery. GluN4C10 polymer brush nanoparticles, a lead material, demonstrated significantly improved delivery efficiency for siRNA against factor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

Graphical abstract

†Electronic supplementary information (ESI) available: Experimental details and ^{1}H NMR structure determination. See DOI: 10.1039/x0xx00000x

Correspondence to: D. G. Anderson; Y. Dong.

‡These authors contributed equally to this work.



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(glycoamidoamines) brush nanomaterials for efficient siRNA delivery both *in vitro* and *in vivo*.

Previously, we reported a class of poly(glycoamidoamines) brush materials and evaluated their efficiency for siRNA and mRNA delivery.[16] Analysis of structure-activity relationships indicated that 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(glycoamidoamines) brush materials. 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% and 73% (Fig. 2a–c). In order to evaluate 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 its stability and morphology. 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 showed similar stability at the same time course (Fig. S1). We also observed apparent cellular uptake of TarN3C10, TarN4C10 and GalN4C10 nanoparticles using Alexa 647-labelled siRNA (Fig. S2). Cryo-TEM image revealed the morphology of GluN4C10 nanoparticles with particle size consistent with the measurements from DLS

(Fig. 3b). Given the promising results of GluN4C10 nanoparticles *in vitro*, we evaluated delivery efficiency of GluN4C10 for siRNA against FVII *in vivo*. We then injected GluN4C10-FVII siRNA nanoparticles into mice through tail vain at three different doses: 0.3 mg/kg, 0.1 mg/kg, and 0.03 mg/kg. 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 the siRNA dose of 0.3 mg/kg, GluN4C10 showed effective and comparable FVII silencing activity (up to 95%) compared to TarN3C10. At a lower siRNA dose of 0.03 mg/kg, GluN4C10 displayed significantly higher FVII silencing than TarN3C10 (77% versus 30% at 0.03 mg/kg). Reflecting the results above, 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, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Cancer Institute Center of Cancer Nanotechnology Excellence at MIT-Harvard (U54-CA151884), the National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), as a Program of Excellence in Nanotechnology (PEN) Award, Contract #HHSN268201000045C, as well as by the NIH Grants R01-EB000244–27, 5-R01-CA132091–04, and R01-DE016516–03. O.V. was supported by Department of Defense Congressionally Directed Medical Research Program (DOD/CDMRP) postdoctoral fellowships (grant W81XWH-13-1-0215, respectively). Y.D. acknowledges supports from the Bayer Hemophilia Awards Program for the Early Career Investigator Award, the National PKU Alliance for Research Awards, the AAPS Foundation for New Investigator Grant, the National Institute of General Medical Sciences for the Maximizing Investigators' Research Award 1R35GM119679 as well as the start-up fund from the College of Pharmacy at The Ohio State University.

References

1. Davis ME. Mol Pharm. 2009; 6:659–668. [PubMed: 19267452]
2. Kim DH, Behlke MA, Rose SD, Chang MS, Choi S, Rossi JJ. Nat Biotechnol. 2005; 23:222–226. [PubMed: 15619617]
3. Davis ME, Zuckerman JE, Choi CH, Seligson D, Tolcher A, Alabi CA, Yen Y, Heidel JD, Ribas A. Nature. 2010; 464:1067–1070. [PubMed: 20305636]

4. Juliano R, Bauman J, Kang H, Ming X. Mol Pharm. 2009; 6:686–695. [PubMed: 19397332]
5. Kanasty R, Dorkin JR, Vegas A, Anderson D. Nat Mater. 2013; 12:967–977. [PubMed: 24150415]
6. Whitehead KA, Langer R, Anderson DG. Nat Rev Drug Discovery. 2009; 8:129–138. [PubMed: 19180106]
7. Burnett JC, Rossi JJ, Tiemann K. Biotechnol J. 2011; 6:1130–1146. [PubMed: 21744502]
8. Barros SA, Gollob JA. Adv Drug Deliv Rev. 2012; 64:1730–1737. [PubMed: 22732527]
9. Tan SJ, Kiatwuthinon P, Roh YH, Kahn JS, Luo D. Small. 2011; 7:841–856. [PubMed: 21374801]
10. Yin L, Zheng N, Cheng J. Methods Mol Biol. 2016; 1364:37–47. [PubMed: 26472440]
11. Wang Y, Miao L, Satterlee A, Huang L. Adv Drug Deliv Rev. 2015; 87:68–80. [PubMed: 25733311]
12. Islam MA, Reesor EKG, Xu Y, Zope HR, Zetter BR, Shi J. Biomaterials Science. 2015; 3:1519–1533. [PubMed: 26280625]
13. Liao W, Li W, Zhang T, Kirberger M, Liu J, Wang P, Chen W, Wang Y. Biomaterials Science. 2016; 4:1051–1061. [PubMed: 27221980]
14. Krishnamurthy S, Vaiyapuri R, Zhang L, Chan JM. Biomaterials Science. 2015; 3:923–936. [PubMed: 26221931]
15. Keles E, Song Y, Du D, Dong WJ, Lin Y. Biomaterials Science. 2016; 4:1291–1309. [PubMed: 27480033]
16. Dong Y, Dorkin JR, Wang W, Chang PH, Webber MJ, Tang BC, Yang J, Abutbul-Ionita I, Danino D, DeRosa F, Heartlein M, Langer R, Anderson DG. Nano Lett. 2016; doi: 10.1021/acs.nanolett.5b02428
17. Tranter M, Liu Y, He S, Gulick J, Ren X, Robbins J, Jones WK, Reineke TM. Mol Ther. 2012; 20:601–608. [PubMed: 22186793]
18. Ingle NP, Malone B, Reineke TM. Trends Biotechnol. 2011; 29:443–453. [PubMed: 21705101]
19. Liu Y, Reineke TM. Biomacromolecules. 2010; 11:316–325. [PubMed: 20058913]
20. McLendon PM, Fichter KM, Reineke TM. Mol Pharm. 2010; 7:738–750. [PubMed: 20349982]
21. Liu Y, Wenning L, Lynch M, Reineke TM. ACS Symp Ser. 2006; 923:217–227.
22. Liu Y, Wenning L, Lynch M, Reineke TM. J Am Chem Soc. 2004; 126:7422–7423. [PubMed: 15198572]
23. Li B, Luo X, Deng B, Wang J, McComb DW, Shi Y, Gaensler KM, Tan X, Dunn AL, Kerlin BA, Dong Y. Nano Lett. 2015; 15:8099–8107. [PubMed: 26529392]
24. Love Kevin T, Mahon Kerry P, Levins Christopher G, Whitehead Kathryn A, Querbes W, Dorkin JR, Qin J, Cantley W, Qin Liu L, Racie T, Frank-Kamenetsky M, Yip Ka N, Alvarez R, Sah Dinah WY, de Fougerolles A, Fitzgerald K, Koteliansky V, Akinc A, Langer R, Anderson Daniel G. Proc Natl Acad Sci U S A. 2010; 107:1864–1869. [PubMed: 20080679]
25. Dong Y, Love KT, Dorkin JR, Sirirungruang S, Zhang Y, Chen D, Bogorad RL, Yin H, Chen Y, Vegas AJ, Alabi CA, Sahay G, Olejnik KT, Wang W, Schroeder A, Lytton-Jean AK, Siegwart DJ, Akinc A, Barnes C, Barros SA, Carioto M, Fitzgerald K, Hettinger J, Kumar V, Novobrantseva TI, Qin J, Querbes W, Koteliansky V, Langer R, Anderson DG. Proc Natl Acad Sci U S A. 2014; 111:3955–3960. [PubMed: 24516150]

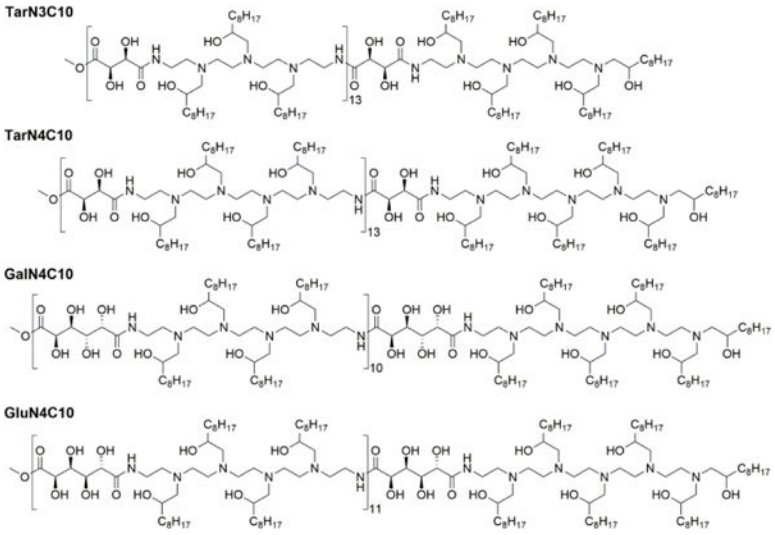


Fig. 1.

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.

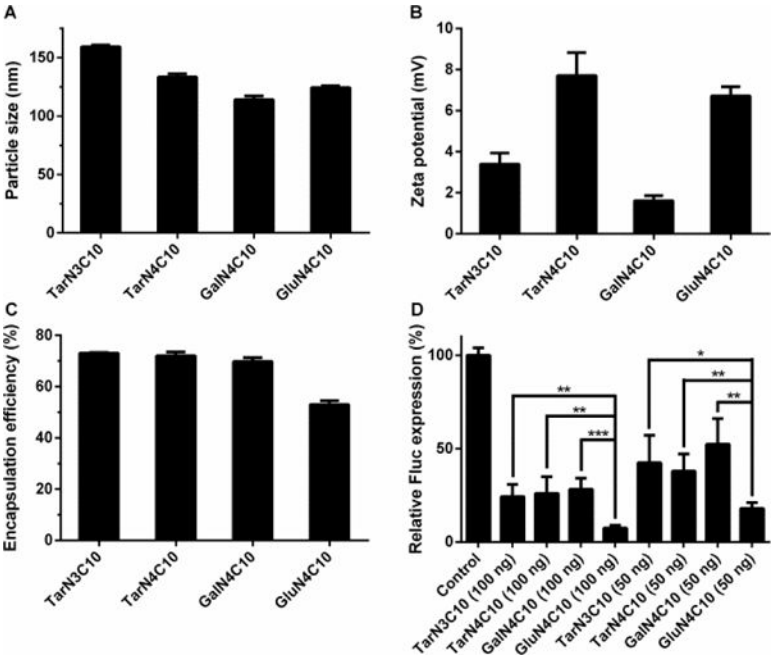


Fig. 2.

Polymer brush nanoparticles 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; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

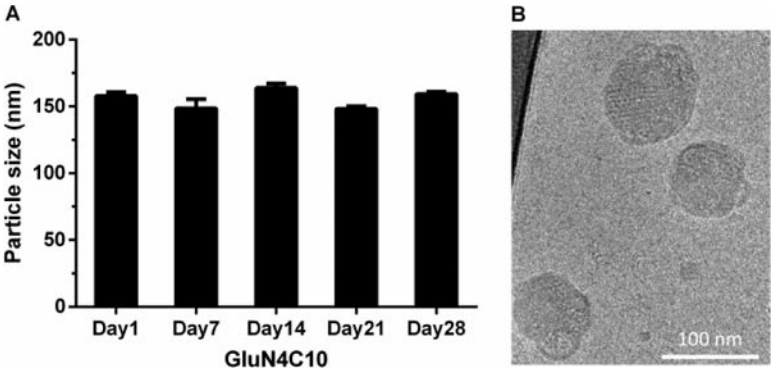


Fig. 3.

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.

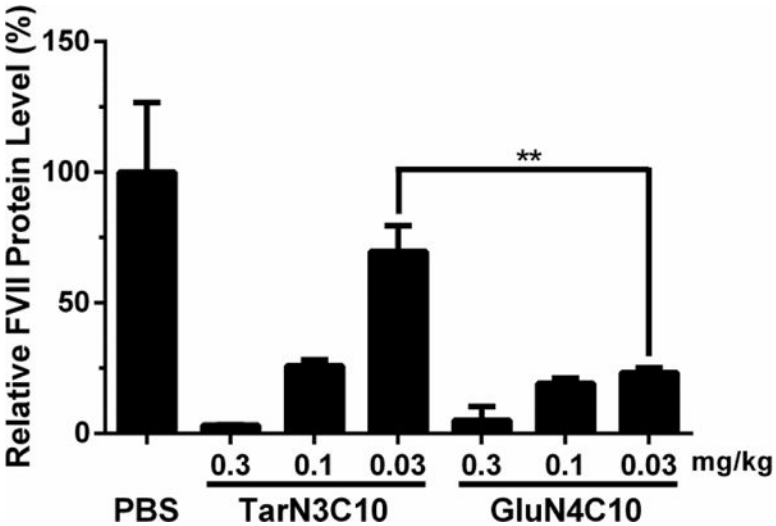


Fig. 4.

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