Using Nucleic Acid Aptamers to Develop Artificial Antibodies for Drug Delivery

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Statement of Purpose: Because of high affinity and specificity, antibodies have been widely used in many areas of life sciences.[1] However, antibodies have many shortcomings that may limit their applications. First, they are produced in organisms. The performance of the same antibody varies from batch to batch. They are also sensitive to environmental conditions. During the manufacturing, modification, shipping, and storage, they can easily lose their binding functionalities. More importantly, antibodies are often highly immunogenic. Studies have shown that monoclonal antibodies that have been largely 'humanized' by substituting much of their nonhuman component with human sequence can elicit immune responses. The immunogenicity can not only induce side effects, but also reduce binding efficiency and prevent the repeated *in vivo* administration. Therefore, there is a clear need to develop nanobiomaterials that can mimic the functionalities of antibodies but with superior properties. The objective of this research is to synthesize and characterize artificial antibodies with nucleic acid aptamers and dendrimers.

Methods: The model aptamer used in this study was a DNA aptamer selected against human T lymphocytic leukemia CCRF-CEM cells. The truncated format of this DNA aptamer (sgc8c) was reacted with the poly(amidoamine) (PAMAM) dendrimer of generation 5. The chemical conjugation procedure was shown as follows. PAMAM were activated with NHS and DCC in anhydrous dimethyl sulfoxide (DMSO) at room temperature overnight. Fluorescein cadaverine (FC) was then added to the activated PAMAM solution. The reaction mixture was transferred to 2-[N-morpholino] ethane sulfonic acid (MES) solution containing sgc8c aptamer. The conjugation product was separated by polyacrylamide gel electrophoresis and purified by 10k molecular weight cut-off filter unit. The size of nanomaterial was measured by Zetasizer Nano S dynamic light-scattering detector. The images of polyacrylamide gel were captured with Bio-Rad Gel Doc XR Imager System. CCRF-CEM cells were cultured and maintained in RPMI medium 1640 (Hyclone, Logan, UT) supplemented with 10% FBS and 100 units/ml penicillin–streptomycin. $5x10^5$ CCRF-CEM cells were washed twice with 700 µL washing buffer (Dulbecco's PBS with $CaCl_2$ and $MgCl_2$ was supplemented with 4.5 g/L glucose and 5 mM $MgCl_2$) and re-suspended in 200 µL binding buffer (washing buffer with 1 mg/mL BSA and 20% FBS) containing nanomaterial or its controls. After incubation for 1 hr, the cells were washed once with 1 mL washing buffer and then immediately subjected to flow cytometry analysis or microscopy imaging. The flow cytometry was performed using BD FACSCalibur™ flow cytometer. The microscopy images were captured with Zeiss inverted microscope and also equally processed with Adobe PhotoShop software to increase the legibility.

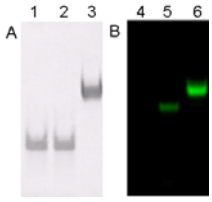

Figure 1. Electrophoresis of reaction products in 6% polyacrylamide gel. (A) Gel was stained with ethidium bromide and imaged by Bio-Rad Gel Doc System: 1, sgc8c; 2, sgc8c mixed with inactive PAMAM; 3, FC-PAMAM-sgc8c. (B) Gel was imaged by Kodak Image Station 2000MM: 4, PAMAM; 5, FC-PAMAM; 6, FC-PAMAM-sgc8c.

Results:

The image of ethidium bromide-stained gel shows that almost sgc8c aptamers were conjugated with PAMAM (**Figure 1A**). To further demonstrate the band in lane 3 in **Figure 1A** was FC-PAMAM-sgc8c not PAMAM-sgc8c or FC-PAMAM, we used gel electrophoresis to characterize the mobility of FC-PAMAM-sgc8c and FC-PAMAM by imaging the conjugated FC. **Figure 1B** confirmed that the band was the product. Therefore, the data show that the nucleic acid aptamer could be conjugated with dendrimer to form nanomaterial and carry the molecules of interest. We also characterized the size of FC-PAMAM-sgc8c in the aqueous solution. The D_h of sgc8c is 1.34±0.08 nm. It is much smaller than PAMAM whose D_h is 6.75±0.45 nm. The D_h of FC-PAMAM-Sgc8c is 8.06±0.16 nm that is almost equal to the summation of the sizes of sgc8c and PAMAM. In comparison, FC-PAMAM-Sgc8c is smaller than antibody that is ~14 nm in D_h. The flow cytometry experiment showed that FC-PAMAM-sgc8c could bind to CCRF-CEM cells and cause the shift of signal to the right. The data of flow cytometry was further confirmed by the microscopy images. Cells labeled with FC-PAMAM-sgc8c had the strongest fluorescence intensity in comparison to controls.

Conclusions: We have demonstrated for the first time that nucleic acid aptamer and dendrimer could be conjugated to form artificial antibodies with the capability of binding to target cells. Artificial antibodies hold great potential for various biomedical applications such as drug delivery.

References:

1. Kozlowski S, Swann P. Current and future issues in the manufacturing and development of monoclonal antibodies. Adv Drug Deliv Rev. 2006;58:707-722.