Graphene oxide modified with aptamer-conjugated gold nanoparticles and heparin: a potent targeted anticoagulant

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Graphene oxide (GO) modified with 29-mer thrombin-binding-aptamer-conjugated gold nanoparticles (TBA\(_{29}\)-Au NPs/GO) can synergistically inhibit the thrombin-mediated cleavage of fibrinogen to fibrin. To further improve anticoagulation efficiency in human plasma, TBA\(_{29}\)-Au NPs/heparin/GO has been prepared from TBA\(_{29}\)-Au NPs/GO and heparin that can also bind thrombin. The dose-dependence of thrombin clotting time (TCT) delay caused by TBA\(_{29}\)-Au NPs/heparin/GO is 21.4, 17.0 and >100 times higher than that caused by the TBA\(_{29}\)-Au NPs, TBA\(_{29}\)-Au NPs/GO and commercially available drugs (heparin, argatroban, hirudin or warfarin), respectively.

Introduction
Blood coagulation involves a series of enzymatic reactions, largely localized on vascular and cellular surfaces, and results in the formation of a fibrin clot.\(^1\) Thrombin (activated factor II) is a multifunctional serine protease produced by the cleavage of prothrombin at two positions by the factor Xa-involved prothrombinase complex (factor Va, negatively charged phospholipid vesicles, and calcium).\(^2\) Thrombin plays many vital roles in the coagulation cascade, converting soluble fibrinogen into insoluble strands of fibrin, catalyzing many other coagulation-related reactions, including directly such as activating protein C and platelets, and providing feedback amplification of factors V, VIII, XI, and XII.\(^3\) Therefore, thrombin is a very attractive target for anticoagulation factors.

Many specific inhibitors have been used for regulating thrombin activity during surgical procedures and for treating patients with arterial or venous thromboembolisms.\(^4\) Thrombin (which has a molecular weight of 37 kDa) is a heterodimer consisting of two polypeptide chains, A and B. The catalytic domain in thrombin has a deep cleft around its catalytic site and two large electropositive surfaces, called exosite I (the fibrinogen–binding site) and exosite II (the heparin–binding site), on opposite sides of the active site cleft.\(^5\) The biological activity of thrombin is strongly affected by substrates, cofactors, and inhibitors that bind to exosites I and II.\(^6,7\) Many direct thrombin inhibitors, which block the enzymatic active site, are currently available for clinical use, but most of them cause serious side effects and suffer from narrow therapeutic windows due to their low specificity and indirect action.\(^8,9\) Thus, more efficient and specific anticoagulation agents are required for treating coagulation-related cardiovascular diseases. A 15-mer thrombin-binding-aptamer (TBA\(_{15}\)) has been shown as a potential anticoagulation drug because it can inhibit the activity of thrombin through its interaction with fibrinogen–binding exosite I.\(^10\) However, TBA\(_{15}\) at a high concentration is required to give an appropriate anticoagulant response because of its low binding affinity. Moreover, oligonucleotide aptamers are easily degraded by blood nucleases and have a short lifetime (~2 min) in blood, limiting their potential use.\(^11\) Conjugation of aptamers through phosphorothioate linkages and use of locked-nucleic acids has been shown to be efficient in improving the half-life and binding affinity of aptamers.\(^12\) However, toxicity of the expensive non-natural modified aptamers is a concern.

Recent studies have demonstrated that linearly assembled 15-mer thrombin-binding-aptamer (TBA\(_{15}\)) and TBA\(_{29}\) (using a poly-dA linker or a polyethylene glycol linker), synthetic dendritic TBAs, and nanostructured TBAs can inhibit thrombin activity efficiently through their multivalent interactions.\(^13-18\) More recently, we have found that TBA\(_{29}\)-conjugated gold nanoparticles (TBA\(_{29}\)-Au NPs) have a strong anticoagulant potency (82 times stronger than TBA\(_{29}\)) because they cause steric blocking and have high binding affinity for thrombin in a biological plasma mimic.\(^19\) In this study, we prepared TBA\(_{29}\)-Au NPs functionalized graphene oxide (GO) nanocomposite (TBA\(_{29}\)-Au NPs/GO), with the aim of further improving anticoagulant potency. TBA\(_{29}\) is easy folded to a G-quadruplex structure.\(^20\) The TBA\(_{29}\)-Au NPs functionalized on graphene oxide GO mainly through the π-π stacking interactions between the square planar guanine tetrad and GO.\(^21-24\) The multivalent TBA\(_{29}\) units on Au NPs result in the strong copperative π-π stacking interaction between TBA\(_{29}\)-Au NPs and GO. The hydrophobic and the hydrogen bonding interactions between TBA\(_{29}\) and GO could not be excluded.\(^25\) TBA\(_{29}\)-Au NPs/GO can bind to thrombin effectively to
inhibit the activity of thrombin to catalyze the cleavage of fibrinogen to form fibrin (Scheme 1a). The TBA$_{29}$–Au NPs/GO in comparison with TBA$_{29}$–Au NPs provided higher inhibitory potency toward thrombin, mainly because of the synergistic effects of TBA$_{29}$–Au NPs and GO. The anticoagulant efficiency of TBA$_{29}$–Au NPs/GO was 3.7 times stronger than that of TBA$_{29}$–Au NPs in a mimic physiological solution. We found that heparin–modified TBA$_{29}$–Au NPs/GO and commercially available rugs (heparin, argatroban, TBA$_{29}$–Au NPs/GO) had much stronger anticoagulation potency than that of TBA$_{29}$–Au NPs/GO and commercially available rugs (heparin, argatroban, and heparin, showing enhanced inhibitory efficiency toward thrombin).

Results and Discussion

Preparation and Characterization of TBA$_{29}$–Au NPs/GO Nanomaterials.

GO was synthesized using an improved Hummers’ method from graphite with a particle size of 7–11 μm. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) images show that the average size of the single-layer GO is about 400 nm, and the monolayer thickness is about 1.8 nm (Fig. 1a and 1c). From dynamic light scattering (DLS) measurements (Fig. S1), we estimated the hydrodynamic diameters of GO to be ~300 nm with narrow size distribution, indicating the GO were well dispersed in the solution. As-prepared GO (24 mg L$^{-1}$) and TBA$_{29}$–Au NPs (0.5 nM; 13 nm) in a sodium phosphate solution (5 mM; pH 7.0) were used to prepare TBA$_{29}$–Au NPs/GO. About 90% of the TBA$_{29}$–Au NPs was determined to be adsorbed onto the GO through comparing the absorbance values at 520 nm of the TBA$_{29}$–Au NPs in standard solution and in the supernatant after centrifugation (at a relative centrifugation force (RCF) of 1500 g for 10 min) of the TBA$_{29}$–Au NPs/GO mixture. Furthermore, the results of agarose gel separation of the mixtures of TBA$_{29}$–Au NPs (10 nM) and GO (0–600 mg/L) indicated >90% TBA$_{29}$–Au NPs were loaded on GO when the concentration of GO was higher than 120 mg L$^{-1}$ (Fig. S2, Supporting Information). A TEM image of TBA$_{29}$–Au NPs/GO (Fig. 1b) reveals that the TBA$_{29}$–Au NPs were assembled homogeneously on the surfaces of GOs. The AFM (Fig. 1d) and UV–vis absorption spectra of TBA$_{29}$–Au NPs/GO (Fig. S3, Supporting Information) further confirm that the Au NPs were dispersed (no aggregation) and well-distributed on the surface of each GO.

Effect of TBA$_{29}$–Au NPs/GO on the Inhibition to Thrombin Activity.

Two well-known TBAs, a 15-mer (TBA$_{15}$) and a 29-mer (TBA$_{29}$) were used to prepare TBA-modified AuNPs (TBA–AuNPs) previously and employed to detect and control the activity of thrombin. We demonstrated that TBA$_{29}$–P$_7$T$_{15}$–Au NPs (Au NPs conjugated with TBA$_{29}$ with a linker of 15 thymine (T) and 9 stem pairs (P) at the termini of the TBA$_{29}$) interact strongly with thrombin ($K_c$<0.01 nM) due to its high local concentration of TBA ligands on the particles’ surfaces. In addition, we also demonstrated that 15 thymine units in the linker and 8 stem pairs at the termini of the TBA are important to provide stability and flexibility for strong binding with thrombin. Here, the descriptor “TBA$_{29}$–Au NPs” was simply used to represent Au NPs conjugated with approximately 70 TBA units presenting T$_{15}$ and P$_9$. We tested the inhibitory potencies of TBA$_{29}$, GO, TBA$_{29}$–Au NPs, and TBA$_{29}$–Au NPs/GO by performing typical clotting tests using fibrinogen (1.14 µM), bovine serum albumin (BSA; 100 µM) and thrombin (15 nM) in physiological conditions. The sequences of TBA$_{29}$ and other DNA fragments used in this study are listed in Table S1 (Supporting Information). The real–time coagulation kinetics of thrombin–induced fibrin formation in mimic physiological solution (25 mM tris(hydroxymethyl)aminomethane (Tris)–HCl at pH 7.4, 150 mM NaCl, 5.0 mM KCl, 1.0 mM MgCl$_2$, 1-3
and 1.0 mM CaCl₂ containing BSA (100 µM) was investigated by measuring the scattered–light intensity caused by fibrin as a function of time (Fig. S4, Supporting Information). The amount of each oligonucleotide present was kept constant (35 nM). Because of the formation of fibrin–gel, the turbidity and light scattering of the reaction mixtures increased with increasing thrombin activity.²⁹

Through comparing the inhibition potencies of these inhibitors toward thrombin (based on the initial reaction rates, i.e., within 800 s; a high rate reveals a weak inhibitory potency), we found that the potencies decreased in the order TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO > TBA₁⁵⁻⁻¹⁵⁻ᵒ⁻Au NPs > GO > TBA₂⁹⁻⁻²⁹⁻⁻Au NPs (Fig. S5, Supporting Information). As controls, the 25-mer random–sequence DNA with T₁₅⁻⁻linker (rDNA, 35 nM), rDNA–modified Au NPs (rDNA–Au NPs), rDNA–Au NPs/GO, and BSA–modified Au NPs (BSA–Au NPs) all provide negligible inhibition toward thrombin (data not shown). We also found that TBA₁⁵⁻⁻¹⁵⁻⁻Au NPs than TBA₂⁻⁻⁴⁻⁴⁻Au NPs and TBA₁⁵⁻⁻¹⁵⁻⁻Au NPs/GO than TBA₂⁹⁻⁻²⁹⁻⁻Au NPs/GO provided much lower inhibitory abilities, mainly because TBA₁⁵⁻⁻¹⁵⁻⁻Au NPs than TBA₂⁹⁻⁻²⁹⁻⁻Au NPs has a weaker binding affinity for thrombin.²⁹ Notably, the TBA₂⁹⁻⁻²⁹⁻⁻Au NPs/GO inhibition (128 cps/sec) was much stronger than that (476 cps/sec) of the TBA₂⁻⁻⁴⁻⁴⁻Au NPs, mainly because of ultrahigh binding ligands present on the surface of GO (Fig. S5, Supporting Information) and/or synergistic effects of TBA₂⁻⁻⁴⁻⁴⁻Au NPs and GO. Compared with free TBA₂⁻⁻⁴⁻⁴⁻ (K₅₋₋ = 0.5 nM),²⁹ TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO had a much higher binding affinity for thrombin (K₅₋₋ = 7.0 × 10⁻¹² M; Fig. S6, Supporting Information). The binding affinity of TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO for thrombin was comparable to our previous bivalent TBA₁⁵⁻⁻¹⁵⁻⁻/TBA₂⁻⁻⁴⁻⁴⁻Au NPs (K₅₋₋ = 8.86 × 10⁻¹²M).²⁸ The ultrahigh TBA density on the surface of the Au NPs and TBA₂⁻⁻⁴⁻⁴⁻Au NPs on the surface of GO provided high local concentrations of TBA ligands, enhancing the binding affinity for thrombin.²⁹,³¹ In addition, steric blocking and electrostatic interactions between thrombin and GO (zeta potential ~ −45 mV; specific surface area ~2600 m²/g) could not be excluded for such a strong inhibition. Thrombin activity was strongly inhibited by unmodified 60 mg L⁻¹ GO (Fig. S7, Supporting Information) in the absence of background protein (BSA, 100 µM). We believe these synergistic effects (interactions of thrombin with TBA₂⁻⁻⁴⁻⁴⁻ and with GO, and steric effects) are the main contributors to the ultrahigh inhibitory function of TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO. Compared to GO covalently modified with oligonucleotides,³⁰ the preparation of TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO is relative very simple. Highly dense aptamer molecules could be easily loaded on the GO through multivalent base–graphene pi stacking. When the aptamers were covalently bonded to GO, the aptamer molecules most likely existed as flattened structures, leading to weak affinity toward its targeted molecules. However, the TBA₂⁻⁻⁴⁻⁴⁻ on the surfaces of the Au NPs/GO provided high flexibility and an appropriate orientation, allowing stronger interaction with thrombin.

**Thrombin Clotting Time.**

We showed that TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO had an ultrahigh capacity for inhibiting the activity of thrombin in a mimic biological solution. We further tested its capacity in human plasma samples by performing TCT tests. TCT tests are commonly performed on patients suspected of suffering from coagulopathy and are used to screen for factors I, IIa, and XIII.²⁹ We first compared the inhibitory potencies of TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO, TBA₂⁻⁻⁴⁻⁴⁻Au NPs, and commercial anticoagulant drugs, including three direct thrombin inhibitors (heparin, argatroban, and hirudin) and one indirect thrombin inhibitor (warfarin) by performing TCT assays (Fig. 2 and Fig. S8, Supporting Information). The dose–dependence of the TCT delay indicated that the anticoagulation potency of TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO in plasma was stronger than that of the four commercial drugs, but only slightly stronger than that of the TBA₂⁻⁻⁴⁻⁴⁻Au NPs. The TCT using TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO ([TBA₂⁻⁻⁴⁻⁴⁻] = 8.75 nM) was 87 ± 2 s, which is longer than the TCT in the absence of the inhibitor (20 ± 3 s), whereas the TCTs were 69 ± 3 s, 23 ± 3 s, 21 ± 2 s, 22 ± 4 s, and 21 ± 5 s for TBA₂⁻⁻⁴⁻⁴⁻Au NPs ([TBA₂⁻⁻⁴⁻⁴⁻] = 8.75 nM), heparin (8.75 nM), argatroban (8.75 nM), hirudin (8.75 nM), and warfarin (8.75 nM), respectively. We suggest that the nonspecific binding of the plasma proteins to GO weakened the binding strength between TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO and thrombin.

Heparin–modified TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO (TBA₂⁻⁻⁴⁻⁴⁻Au NPs/heparin/GO) was prepared (see the experimental section for detailed preparation of TBA₂⁻⁻⁴⁻⁴⁻Au NPs/heparin/GO) to attempt to decrease nonspecific binding and improve the binding affinity. The heparin molecules conjugated with GO probably through multivalent hydrogen bonding and hydrophobic interactions.³⁴,³⁵ Determination by light scattering experiments revealed about 95% of the heparin was adsorbed onto the GO, through comparing the concentration of heparin in the supernatant after centrifugation of the TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO. The TCT using TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO (TBA₂⁻⁻⁴⁻⁴⁻Au NPs/heparin/GO) was significantly prolonged compared to the TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO without heparin. The anticoagulation potency of TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO in the presence of heparin was stronger than that of the four commercial drugs, and only slightly stronger than that of the TBA₂⁻⁻⁴⁻⁴⁻Au NPs. The TCT using TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO ([TBA₂⁻⁻⁴⁻⁴⁻] = 8.75 nM) was 20 ± 3 s, whereas the TCTs were 69 ± 3 s, 23 ± 3 s, 21 ± 2 s, 22 ± 4 s, and 21 ± 5 s for TBA₂⁻⁻⁴⁻⁴⁻Au NPs ([TBA₂⁻⁻⁴⁻⁴⁻] = 8.75 nM), heparin (8.75 nM), argatroban (8.75 nM), hirudin (8.75 nM), and warfarin (8.75 nM), respectively. We suggest that the nonspecific binding of the plasma proteins to GO weakened the binding strength between TBA₂⁻⁻⁴⁻⁴⁻Au NPs/GO and thrombin.
NPs/heparin/GO mixture to that of control solutions. The heparin did not cause the aggregation of TBA$_{329}$–Au NPs/go after heparin adsorbed on GO (Fig. 1e and Fig. S3). We noted the TBA$_{329}$–Au NPs was not desorbed from the GO after heparin adsorbed on GO from the comparison of absorbance values at 520 nm of the TBA$_{329}$–Au NPs in standard solution and in the supernatant after centrifugation (at a RCF of 1500 g for 10 min) of the TBA$_{329}$–Au NPs/heparin/GO mixture. The inactivation of thrombin and activated factor X (factor Xa) induced by heparin (a sulfated polysaccharide) is through an antithrombin–dependent mechanism.

The inactivation of thrombin by antithrombin III can increase by nearly three orders of magnitude in the presence of heparin. The binding of thrombin to the highly negatively charged heparin appears to be a predominantly electrostatic interaction at a site proximal to the pentasaccharide of the heparin, with an intrinsic $K_d$ of 5–10 $\mu$M. The dose–dependence of the TCT delay and TCT delay to 600 s (TCT$_{600}$) (Fig. 2) shows that the anticoagulation ability of TBA$_{329}$–Au NPs/heparin/GO was not only much higher than that of the four commercial drugs but also much higher than that of TBA$_{329}$–Au NPs/GO, TBA$_{329}$–Au NPs, and heparin-modified GO (heparin/GO). The TCTs of TBA$_{329}$–Au NPs/heparin/GO ($[\text{TBA}]_0 = 8.75$ nM), heparin/GO ($[\text{heparin}]_0 = 8.75$ nM), TBA$_{329}$–Au NPs/GO ($[\text{TBA}]_0 = 8.75$ nM), and TBA$_{329}$–Au NPs ($[\text{TBA}]_0 = 8.75$ nM) were approximately 75.0, 15.0, 4.4, and 3.5 times longer ($t/t_0$) is the TCT in the absence of the inhibitor, and $t_0$ is the TCT in the presence of the inhibitor), respectively, than that of the TCTs in the absence of any inhibitor (Fig. S9, Supporting Information). The TCT delay caused by TBA$_{329}$–Au NPs/heparin/GO was 21.4 times higher than that caused by the TBA$_{329}$–Au NPs and 17.0 times higher than that caused by the TBA$_{329}$–Au NPs/GO. The results clearly show the advantages of using TBA$_{329}$–Au NPs/heparin/GO over TBA$_{329}$–Au NPs/GO and TBA$_{329}$–Au NPs with respect to blood coagulation time. The ultrahigh anticoagulant potency of TBA$_{329}$–Au NPs/heparin/GO was mainly caused by heparin-passivated GO, decreasing the nonspecific binding between plasma background proteins and GO. In addition, multivalent binding of thrombin with TBA$_{329}$ and heparin molecules also account for enhanced potency. Steric blocking of fibrogenin provided by heparin and TBA$_{329}$–Au NPs on the GO surfaces cannot be excluded for the obtained stronger inhibitions.

We have further evaluated the anticoagulant capability of TBA$_{329}$–Au NPs/heparin/GO by the measurement of prothrombin time (PT) and activated partial thromboplastin time (aPTT) in plasma samples. aPTT measurement is a screening test for coagulant factors II, V, VIII, IX, X, XI, and XII of the extrinsic and common pathways, while PT measurement is the test for factors II, V, VII, and X of the intrinsic and common pathways. Fig. 3 shows the comparison of elongation of PT and aPTT by TBA$_{329}$–Au NPs/heparin/GO and four commercial drugs (heparin, argatroban, hirudin, and warfarin). Prolonging the PT and aPTT ($t/t_0$ and $t$ are the PT or aPTT in the absence and presence of inhibitor, respectively) in plasma samples by TBA$_{329}$–Au NPs/heparin/GO, heparin, argatroban, hirudin, and warfarin led to values of 5.9/3.9, 1.5/1.2, 1.0/1.1, 1.1/1.0, and 1.0/1.0 times, respectively. The results further clearly demonstrate the advantages of our TBA$_{329}$–Au NPs/heparin/GO over commercial drugs with respect to blood coagulation time.

Stability of TBA$_{329}$–Au NPs/heparin/GO.

First, we study the long-term stability of TBA$_{329}$–Au NPs/heparin/GO in physiological buffer by DLS measurements. The

![Fig. 3](image_url)
been demonstrated that GO nanosheets exhibited long blood circulation time and low uptake in reticuloendothelial system even if its sizes larger than 100-nm when compared with other carbon nanomaterials. The extraordinary stability of TBA$_{29}$–Au NPs/heparin/GO suggests that our newly developed anticoagulant may have a long plasma half-life. The small sized GO may be employed for loading of TBA$_{29}$–Au NPs and heparin if the TBA$_{29}$–Au NPs/heparin/GO are easy to uptake in reticuloendothelial system in animal studies in future.

Biocompatibility of TBA$_{29}$–Au NPs/heparin/GO.

Recent reports suggest aptamer–modified Au NPs has good biocompatibility for mammalian cells. Although GO could cause some rupture to cell membrane, most studies report mammalian cell viabilities decrease lesser than 20% after exposure to GO concentrations and lower than 10 mg L$^{-1}$ during 24 h or longer. The cytotoxicity of TBA$_{29}$–Au NPs/heparin/GO towards mammalian cells was evaluated using an Alamar Blue assay. After 24 h of incubation of human embryonic kidney cells (293T cell line) and breast adenocarcinoma cells (MDA–MB–231 and MCF–7 cell lines) with the TBA$_{29}$–Au NPs/heparin/GO ([GO] = 0–24 mg L$^{-1}$), we found that the TBA$_{29}$–Au NPs/heparin/GO had little influence on cells viability (Fig S11, Supporting Information). Therefore, we suggest the TBA$_{29}$–Au NPs/heparin/GO have good biocompatibility toward mammalian cells, considering the TBA$_{29}$–Au NPs/heparin/GO at the concentration of 24 mg L$^{-1}$ (GO) show ultrahigh inhibitory ability to thrombin in plasma (Fig. 2). Similar to TBA$_{29}$–Au NPs/heparin/GO, TBA$_{29}$–Au NPs/GO had little influence on cells viability when the concentration was lower than 24 mg L$^{-1}$ (data not shown). The TBA$_{29}$–Au NPs/heparin/GO and TBA$_{29}$–Au NPs/GO show very low cytotoxicity in selected human cell lines probably due to the GO surfaces passivated by high biocompatible aptamer molecules.

We further studied the in vitro hemolysis of TBA$_{29}$–Au NPs/heparin/GO to verify the satisfactory biocompatibility of TBA$_{29}$–Au NPs/heparin/GO. The hemolysis assay was performed in defibrinated blood (human) to test the rupture of red blood cells (RBCs) to withstand swelling in contact with TBA$_{29}$–Au NPs/heparin/GO solution. The hemolysis of RBCs were not observed after incubation with TBA$_{29}$–Au NPs/heparin/GO solution at 37 °C for 4 h (Fig. S12, Supporting Information), revealing that the TBA$_{29}$–Au NPs/heparin/GO could serve as a potentially safe anticoagulant–nanomaterials. The TBA$_{29}$–Au NPs/heparin/GO exhibit insignificant cytotoxicity to the mammalian cells and hemolysis insinuate the emergence of this nanocomposite as a future putative anticoagulation drug. However, acute and chronic studies using animal models in the future should be conducted to ensure the potential of TBA$_{29}$–Au NPs/heparin/GO as a viable drug.

Conclusions

We synthesized a nanocomposite TBA$_{29}$–Au NPs/heparin/GO which acts as a highly effective anticoagulant through controlling the thrombin activity towards fibrinogen. Such a high anticoagulant activity could be due to i) the high selectivity of TBA$_{29}$ towards thrombin helps heparin to easily access the target and the anticoagulant activity of heparin leads to the inhibition of thrombin activity, and ii) the steric hindrance caused by the TBA$_{29}$–Au NPs to thrombin, minimizing the access of fibrinogen to the active sites of thrombin. More importantly, the easily prepared and highly active TBA$_{29}$–Au NPs/heparin/GO is an efficient anticoagulant in human plasma, showing its potential for treating coagulation–related cardiovascular diseases. Furthermore, the in vitro cytotoxicity and hemolysis experiment results show that TBA$_{29}$–Au NPs/heparin/GO has low cytotoxicity and hemolysis effect, revealing that the TBA$_{29}$–Au NPs/heparin/GO could serve as a potentially safe anticoagulant. In addition, this study shows that GO modified with nanomaterials may be useful for regulating molecular function, controlling protein and DNA binding, and manipulating enzyme activities.

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Notes and references


47. P. J. Huang and J. Liu, Small, 2012, 8, 977.


Synthesis of a nanocomposite of aptamer-conjugated gold nanoparticles and heparin co-immobilized graphene oxide acts as a highly effective anticoagulant through controlling the thrombin activity towards fibrinogen.