A t-PA/gold nanoparticle conjugate based on bio-affinity ligation

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Statement of Purpose: A major issue in the therapeutic use of tissue plasminogen activator(t-PA) is its very short half-life in the circulation(2–6 min) due to the effects of inhibitors, enzymes and antibodies in blood.[1] In previous study, t-PA was conjugated to synthetic materials to improve the circulation time mainly by covalent bonding or electrostatic interactions, which are "invasive" and generally accompanied by loss of protein activity and immunogenic responses.[2,3] In present work, a t-PA/gold nanoparticle (t-PA/AuNP) conjugate was prepared via bio-affinity ligation based on specific interactions between t-PA and ε-lysine (a ligand that has affinity to a specific domain in t-PA) immobilized on AuNP surface through polyvinyl pyrrolidone (PVP) as a spacer. The resulting conjugate can not only retained almost full enzymatic activity, but also protect t-PA from inhibition by PAI-1 to some extent as compared with free t-PA *in vitro*. Moreover, the conjugate showed prolonged circulation time in *vivo*.

Methods: ε-lysine-containing RAFT agent was synthesized and subsequently used for preparing well-defined ε-lysine-terminated Polyvinyl pyrrolidone (PVP-Lys) via RAFT polymerization. The PVP-Lys capped AuNPs (AuNPs-PVP-Lys) were prepared via a ligand exchange procedure. The t-PA and AuNPs-PVP-Lys were co-incubated at 37 ºC in PBS at pH 7.4 for 1 h to prepare t-PA/AuNPs-PVP-Lys conjugate. The interactions between t-PA and AuNPs-PVPLys were investigated by DLS, SDS-PAGE and UV-Vis spectroscopy. Enzymatic activity of the conjugate (both *in vitro* and *in vivo*) was determined using chromogenic reaction method used previously.[4]

Results: According to DLS measurement (Figure 1A), the increase in hydrodynamic diameter of the particle upon addition of t-PA indicate t-PA was conjugated to gold nanoparticles. SDS-PAGE and UV-Vis spectroscopy further demonstrated the specificity of the interaction between the AuNPs-PVP-Lys and t-PA (Figure 1B, Figure 1C).

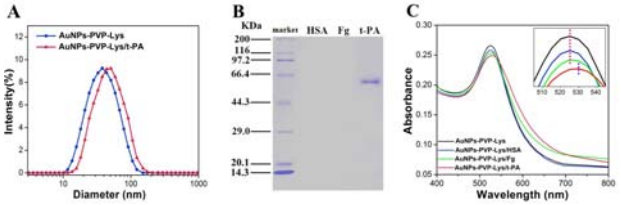

Figure 1. (A) Hydrodynamic diameter distributions of AuNPs-PVP-Lys and AuNPs-PVP-Lys/t-PA determined by DLS; (B) SDS-PAGE analysis of proteins adsorbed on AuNPs-PVP-Lys (lanes 2-4) from single protein solutions of t-PA, albumin and fibrinogen; and (C) UV-visible spectra of AuNPs-PVP-Lys in buffer and in the presence of HSA, Fg and t-PA, respectively.

Enzymatic activity assay indicated that the conjugate retained almost full enzyme activity with respect to free t-PA (Figure 2A). Figure 2B showed that conjugate was inactivated by PAI-1 more slowly than free t-PA, indicating that the t-PA in the conjugate was protected from PAI-1 to some extent. Free t-PA showed a mean half-life of ~7 min while the conjugate increased the half-life to ~20 min according to *in vivo* study (Figure 2C). Moreover, free t-PA was almost completely cleared 1 h after administration, while for the AuNPs-PVP-Lys/t-PA conjugates ~20% of the initial dose remained.

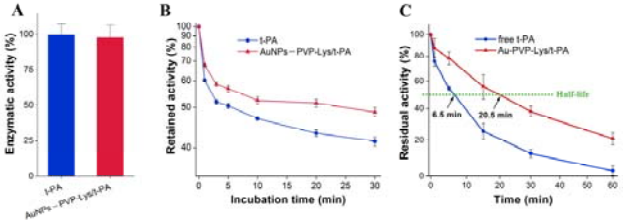

Figure 2. (A) Enzymatic activity of free t-PA and conjugated t-PA determined using chromogenic substrate S-2288. (B) *In vitro* enzymatic activity of free t-PA and conjugated t-PA vs. time in the presence of PAI-1. (C) Plasma amidolytic activity-time curve of t-PA after intravenous administration of free t-PA or AuNPs-PVP-Lys/t-PA conjugate to rats (mean±S.D., n=3).

Conclusions: In conclusion, a t-PA/AuNPs-PVP-Lys conjugate was prepared via bio-affinity ligation based on the affinity interactions between a lysine-binding domain in t-PA and ε-lysine exposed on the surface of AuNPs-PVP-Lys under physiological conditions. Compared with free t-PA, the conjugate retained almost full enzyme activity and clot-dissolving efficiency, and the t-PA was protected from inhibition by PAI-1 to some extent. Moreover, the conjugate showed prolonged circulation time *in vivo*.

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