Hyaluronic Acid-Gold Nanoparticle-Tocilizumab Complex for the Treatment of Rheumatoid Arthritis

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Statement of Purpose: Rheumatoid arthritis (RA) is a chronic inflammatory disease caused by the inflammation of synovial membrane, leading in turn to articular cartilage destruction [1]. In this work, hyaluronic acid (HA)-gold nanoparticle (AuNP)-Tocilizumab (TCZ) complex was synthesized for the treatment of RA. TCZ is a humanized monoclonal antibody against the interleukin-6 (IL-6) receptor and used as an immunosuppressive drug by interfering IL-6 in the pathogenesis of RA. AuNP, which has an anti-angiogenic effect, is not only beneficial for the treatment of RA but also working as a contrast agent and a drug carrier. HA is known to have cartilage-protective and lubricant effects. Taken together, these components were exploited to develop a new therapeutic system of HA-AuNP-TCZ complex for RA.

Methods: Thiol end-functionalized HA was synthesized by reductive amination of HA with cystamine and then reduction with DTT. The HA-SH was immobilized onto the surface of AuNP via Au-thiol chemistry [2]. Meanwhile, TCZ was oxidized by sodium periodate to introduce aldehyde groups on the Fc portion of a glycosylated TCZ and conjugated to amine-PEG-thiol linker using sodium cyanoborohydride as a reducing agent [3]. Finally, TCZ-PEG-SH was also attached to HA-AuNP via Au-thiol chemistry. The resulting HA-AuNP-TCZ complex was characterized by UV-Vis spectra, transmission electron microscopy (TEM), and dynamic light scattering (DLS).

Results: Figure 1a shows a schematic representation of HA-AuNP-TCZ complex. After preparation of TCZ-PEG-SH (Figure 1b), HA-AuNP-TCZ complex was prepared by the conjugation of both HA-SH and TCZ-PEG-SH to AuNP via Au-thiol chemistry.

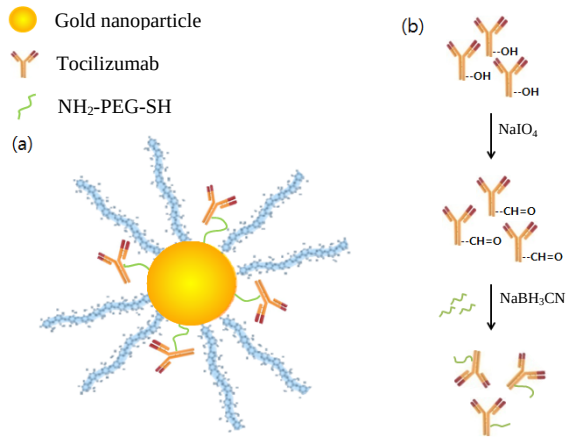

Figure 1. (a) Schematics of HA-AuNP-TCZ complex and (b) the protocol for the preparation of TCZ-PEG-SH.

The formation of HA-AuNP-TCZ complex was assessed by UV-vis spectra (Figure 2a). The direct interactions of HA-SH and TCZ-PEG-SH to AuNP were confirmed from the red shift in the surface plasmon resonance (SPR) peaks. The SPR peak of free AuNP appeared around 520 nm and the stepwise binding of HA-SH and TCZ to AuNP shifted the SPR peak to 523 nm and 524 nm, respectively. The monodisperse formation of the HA-AuNP-TCZ complex was also confirmed by TEM (Figure 2b). The size of AuNP was *ca.* 20 nm. Furthermore, the hydrodynamic size of the complex was analyzed by DLS. The size of the AuNP was *ca.* 23.04 nm with a narrow PDI of 0.18 and that of HA-AuNP was *ca.* 39.89 nm with a PDI of 0.23. The diameter of HA-AuNP-TCZ complex was *ca.* 44.52 nm with a PDI of 0.25. The content of TCZ bound to a single AuNP was determined by measuring the absorbance of FITC-labeled TCZ, which revealed that *ca.* 25.82 of TCZs were bound to a single AuNP. The therapeutic effect of HA-AuNP-TCZ complex is currently investigated in RA animal models.

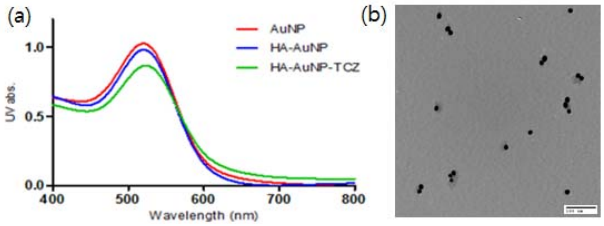

Figure 2. (a) UV-Vis spectra and (b) TEM image of HA-AuNP-TCZ complex.

Conclusions: HA-AuNP-TCZ complex was successfully synthesized for the treatment of RA. The formation of HA-AuNP-TCZ complex was clearly confirmed by UV-vis spectra, TEM, and DLS analyses. Currently, the therapeutic effect of the complex on RA is investigated, which will corroborate the feasibility of HA-AuNP-TCZ complex for further development. The novel platform of HA-AuNP-antibody complex can be exploited for various therapeutic applications.

References:

[1] Kim KS et al. Acta Biomaterialia, 2011;7:666-674.
[2] Lee MY. ACS Nano 2012; 6:9522-9531.
[3] Kumar S et al. Nature Protocols, 2008;3:341-320.