

## Robust Hyaluronic Acid Nanoparticles with Bioreducible Core for Intracellular Drug Delivery

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**Statement of Purpose:** Nanocarriers with high stability and tumor targetability have been extensively investigated for improved therapeutic efficacy. Advantages of self-assembled polymeric nanoparticles in tumor diagnosis and therapy include its capability of solubilizing water-insoluble anticancer drugs and prolonging their circulation in the bloodstream which may increase their probability of reaching the target tumor sites after systemic administration *in vivo*. However, such particles suffer from poor *in vivo* stability in the bloodstream, resulting in severe side effects by premature drug release before reaching their target site. To improve the stability of the self-assembled nanoparticles, in this study, we have developed robust hyaluronic acid nanoparticles (CC-HANPs) through a simple core-crosslinking method. The physicochemical characteristics and stability of the nanoparticles were investigated in detail using transmission electron microscope (TEM), dynamic light scattering (DLS) and FT-IR spectroscopy.

**Methods:** All the chemicals used in the study were analytical grade and used as received.  $\alpha$ -Alkyne HA and PDSMA-N<sub>3</sub> were synthesized according to a previous publication with slight modification [1, 2]. HANPs were prepared by click chemistry using  $\alpha$ -alkyne HA and azide-functionalized pyridyl disulfide methacrylate (PDSMA-N<sub>3</sub>) [1, 3].

Doxorubicin (DOX)-loaded HANPs (DOX-HANPs) were fabricated by the dialysis method in the dark conditions. For crosslinking, an excess amount of dithiothreitol (DTT) was added to the solution containing DOX-HANPs and dialyzed for 9h against distilled water. Cross-linked DOX-HANPs (DOX-CC-HANPs) were obtained by lyophilization for 3days.

The kinetic stability of the nanoparticles was investigated by measuring the change in scattering intensities of the nanoparticle in the presence FBS or surfactant sodium dodecyl sulfate (SDS) as a function of time using a DLS

**Results:** The hydrodynamic size of the CC-HANPs (187nm) was found to be lower than that of HANPs (215 nm), indicating that the crosslinking results in the formation of compact nanoparticles. DOX-CC-HANPs were successfully prepared using the simple dialysis method with loading efficiency of ~ 80%. The physicochemical characteristics of CC-HANPs, HANPs, DOX-CC-HANPs and DOX-HANPs are summarized in Table 1.

Table 1. Physicochemical characteristics of nanoparticles.

Sample	Size (nm)	DOX feed amount (%)	Loading efficiency (%)	Loading content (%)
HANPs	215.74±6.98	-	-	-
CC-HANPs	187.67±2.65	-	-	-
DOX-HANPs	201.8±3.17	10	80.41±3.5	8.04±0.3
DOX-CC-HANPs	148.2±5.24	10	87.09±1.2	8.70±0.1

As shown Figure 1, the release rate of DOX from the DOX-CC-HANPs was slower than that from DOX-HANPs in the absence of glutathione (GSH). However, the release rate of DOX from CC-HANPs rapidly increased in 10 mM GSH, compared to HANPs. These results indicate that, at intracellular level of GSH, the disulfide linkage in the hydrophobic core is cleaved to accelerate the release of DOX.

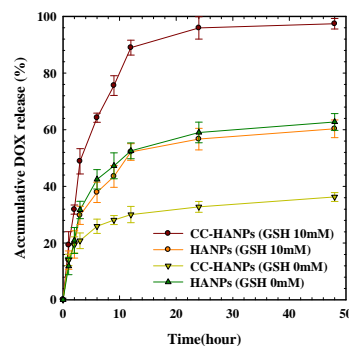


Figure 1. *In vitro* release profiles of DOX from cross-linked nanoparticles in the absence and presence of GSH.

**Conclusions:** In this study, we have successfully prepared CC-HANPs by the facile method using DTT without any toxic cross-linkers. CC-HANPs demonstrated enhanced stability even under harsh conditions, such as in the presence of SDS or FBS. The release of DOX from DOX-CC-HANPs was minimized in the physiological conditions, whereas rapid release of DOX was observed at the intracellular mimicking conditions. Overall, CC-HANPs might be a promising carrier for intracellular delivery of hydrophobic anticancer drugs.

### References:

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3. Kim SH, *Chem Commun*, 2013;49:10349-10351