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Beyond chemotherapeutics: Cisplatin as a temporary buckle to fabricate drug-loaded nanogel

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Abstract

Cisplatin templated nanogel (NG) with targeting capability was synthesized, inspired by cisplatin as a chemotherapeutic drug. After crosslinking the natural polysaccharide hyaluronan, a ligand for CD44, cisplatin could be removed by simple dialysis in salt solution while the withheld drug remains entrapped.

Graphical Abstract

Cisplatin, a widely used first line chemotherapeutic drug for various cancers, has attracted intense research attentions since it was introduced to the market over 40 years ago¹. After its cellular uptake either by passive diffusion or copper transporters, cisplatin attacks DNA, blocks replication and induces cell apoptosis^{2, 3} (Scheme 1a). Although cisplatin effectively shrinks the tumor, its nonspecific accumulation in both tumor and normal tissues has also caused severe side effects such as nephrotoxicity, myelosuppression, and neurotoxicity⁴. Numerous strategies are thus developed to improve the performance of cisplatin either by

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modifying the structure or introducing it into a drug delivery system including polymeric nanoparticles^{1, 5}. Similar to its DNA binding mechanism, cisplatin conjugates to polymers through the coordination between platinum and the functional groups, such as carboxyl groups. This coordination is a reversible process, the bound cisplatin, in the presence of high chloride ions, could be freed from the carrier polymer to react with DNA^{6, 7}, and kills cells^{8–15}. Based on this unique property, various carriers such as synthetic polymers^{8–11}, PEG-p-Glu¹² and hyaluronan (HA)^{13–15} have been proposed to improve the therapeutic effect of cisplatin. Intrigued by this reversible reactivity, it was hypothesized that cisplatin, beyond chemotherapeutics, could be an ideal temporary crosslinker for nanoparticle formation. It could facilitate the assembling process, and then be removed by a simple salt exchange process.

Nanogel (NG), nanoparticles composed of crosslinked hydrophilic polymer networks, have demonstrated great potentials in loading drugs or dyes with different solubilities for therapeutic and imaging applications^{16, 17}. Current strategies to encapsulate payloads into polymers are mainly self-assembly, crosslinking, emulsion and nanoprecipitation¹⁸, which may require complex preparations, tedious chemical purifications, and/or harsh conditions, that result in compromised drug efficiency or an increase in cost. To avoid these issues, we sought to investigate the applicability of cisplatin as a removable crosslinker to mediate the formation of drug-loaded NG.

HA (Fig. S1, ESI[†]), a natural polysaccharide from the extracellular matrix, was chosen as the building polymer for its high density of available carboxyl groups for cisplatin coordination, biodegradability and low immunogenicity for safety, and CD44 targeting capability for delivery^{15, 19}. Imitating the disulfide bonds assisted protein folding; cisplatin was used as a crosslinker to bring HA into a globular state.

In the presence of a cationic-aromatic drug, the cisplatin and HA created local environment would promote the drug's π – π stacking and facilitate the drug entrapment into the NG (Scheme 1b). Like the polyion complex micelles^{20, 21} and drugs-tuned self-assembly^{22–24} for drug delivery, thus the acquisition of NG here would only involve the non-covalent interactions. To demonstrate the concept, rhodamine B (Rb, Fig. S1, ESI[†]) was employed as a model drug, because of its strong absorption, fluorescence, and mitochondrial labeling capability could ease the subsequent analysis and characterization including quantification, aggregation assessment, and intracellular tracking of NG.

A simple mixing of HA with cisplatin and Rb at 90 °C for 3 hours, followed by a dialysis in pure water, led to the creation of a non-viscous solution (HA/Cis/Rb) with a clear color change and a hydrodynamic size of around 168.4 nm (Fig. 1b and Fig. S2a, ESI[†]). The absorption spectra of HA/Cis/Rb demonstrated a clear broadened red shift in comparison to the free Rb (Fig. 1c). This implied that the aggregation of Rb in the NG was possibly due to π – π stacking effects^{25, 26}, which was further supported by the fluorescent quenching of Rb (Fig. S3, ESI[†]). For comparison, HA was mixed with only Rb (HA@Rb), or only cisplatin (HA/Cis), heated at 90 °C for 3 hours, and then dialyzed in water following the same procedure. The collected solution of HA@Rb barely demonstrated Rb color and had a size over 1612.8 nm and PDI around 0.833, indicating that Rb could not be held by HA without a

crosslinker (Fig. S2, ESI[†]). In contrast, the HA/Cis solution contained broadly distributed large particles, size = 726.9 nm and PDI = 0.629 (Fig. S2, ESI[†]). Certainly, no characteristic absorption of Rb was observed in HA/Cis preparation (Fig. S2b, ESI[†]). The above information suggested that the cisplatin triggered the self-assembly of NG and the interactions between Rb and HA further tightened the assembly. The platinum coordination crosslinked HA and enhanced the entrapment of Rb; while filling the gel with Rb, local interactions such as the π - π stacking of Rb and the electrostatic interactions between the negatively charged HA and the positively charged Rb resulted in a compact and homogeneous NG.

Learning from the reversible coordination of cisplatin, the prepared solution of HA, cisplatin and Rb was subjected to dialysis for 3 days in PBS with a high concentration of chloride ions (pH 7.4, 140 mM Cl⁻), instead of pure water, to obtain HA/Rb. To verify the cisplatin's removal, a modified o-phenylenediamine (OPDA) assay^{27, 28} was used to quantify cisplatin in NGs based on the characteristic of absorbance in the adduct of cisplatin and OPDA (see the ESI[†] and Fig. S4 for details). After reacting HA/Cis/Rb with OPDA, the solution demonstrated two absorption peaks, which belong to Rb (~ 544 nm) and the cisplatin-OPDA adduct (705 nm), indicating the co-existence of Rb and cisplatin in the prepared NG. Conversely, HA/Rb only gave a clear Rb peak, and the cisplatin-OPDA absorption was almost eliminated (Fig. 1d). Compared with 0.455 mg/ml of Rb and 0.478 mg/ml of cisplatin in HA/Cis/Rb, there was 0.423 mg/ml of Rb and only 0.027 mg/ml of cisplatin left in HA/Rb (Fig. S5, ESI[†]). The relative constant amount of Rb in both preparations revealed a tight packing of Rb in NG. A near 98.2% of fluorescence quenching and a significant red shift in absorption further evidenced the strong compactness of Rb (Fig. 1c and Fig. S3, ESI[†]). After the removal of cisplatin, HA/Rb swelled slightly with a hydrodynamic size of 210.4 nm compared with 168.4 nm for HA/Cis/Rb (Fig. 1b). The size of the dried HA/Rb and HA/Cis/Rb determined by the transmission electron microscopy (TEM) was only 57.9 nm and 50.5 nm, respectively (Fig. S6, ESI[†]). The morphology of HA/Rb appeared more irregular compared with HA/Cis/Rb after the removal of cisplatin (Fig. 1a). It was noted that the zeta potential of HA/Rb decreased in comparison to HA/Cis/Rb (Fig. S7, ESI[†]), re-emphasizing that cisplatin was removed, as more occupied carboxyl groups were released. The slight loss of Rb in HA/Rb may also partly contribute to the zeta potential drop since electrostatic interaction between Rb and HA screened the negative charges of HA. Nevertheless, the contribution of Rb loss to zeta potential change here was believed to be minor given that the decrease of Rb was limited.

To further confirm the removal of cytotoxic cisplatin in HA/Rb, a cell viability assay on human fibrosarcoma HT1080 cell line was conducted. HA/Cis/Rb and HA/Rb were introduced into the cell cultures, at the same HA concentration, for 3 days. HA/Rb showed no cytotoxicity up to 167 μ g/ml HA (Fig. 1e), while HA/Cis/Rb demonstrated severe cytotoxicity as free cisplatin in a concentration dependent manner. The HA/Rb treated cells turned fluorescent and grew healthily, as did the untreated control cells, while the total cell number of the HA/Cis/Rb treated cells was significantly reduced (Fig. S8, ESI[†]).

Rb is well known for its specific binding to mitochondria²⁹, 30-minutes of pulse incubation with Rb showed a bright signal within the mitochondria; however, the intracellular Rb was

quickly expelled from the cells in less than 6 hours (Fig. 2a). When HT1080 was incubated with HA/Rb, the Rb signal was found to co-localize with the lysosomes rather than mitochondria because NG were internalized through endocytosis,³⁰ and then the encapsulated Rb was released within the lysosomes. When the incubation was elongated from 1 to 6 hours, the Rb signal of the HA/Rb treated cells slightly increased possibly due to the further release and dequenching of Rb. Interestingly, a weak Rb signal appeared in the mitochondria after 6 hours, indicating that some released Rb molecules escaped from the lysosome and migrated to the mitochondria. The intracellular release was simulated by incubation of HA/Rb in the presence of hyaluronidase (HAase) (Fig. S9, ESI[†]), an enzyme responsible for degradation of HA. With the assistance of HAase, the Rb fluorescence was increased more rapidly compared with the control (only PBS), suggesting the degradation of HA led to more Rb release and recovery of quenched fluorescence. The TEM observation further verified the fragmentation of HA/Rb NG after the incubation with HAase for 2 days (Fig. S9b–c, ESI[†]). This observation suggested that NG itself was degradable and the drug in NG can be slowly released and access its subcellular target, a key merit for an effective drug delivery system.

Since HA is a specific ligand for CD44, the targeting potential of HA/Rb was checked using MDA-MB-231 and MCF-7 cell lines, which have a high and low CD44 expression (Fig. S10, ESI[†]), respectively. As expected, more Rb signal was observed in MDA-MB-231 cells with higher CD44 expressions. This uptake could be inhibited by the competition assay with extra free HA (Fig. 2b), indicating the HA in NG still maintain its targeting capability. Similar to that in HT1080 cells, the Rb signal in MDA-MB-231 cells were observed after 24 hours as more Rb molecules were released within cells.

The aforementioned studies with the model drug Rb have confirmed that cisplatin can be used as a temporary crosslinker to facilitate NG formation. At the end of its duty, the used cisplatin can then be dialyzed away as proposed. Importantly, the resulting NG, although has no buckle, retains its contents and size.

Advancing from the model formulation, the next rational step is to prepare NG with different drugs. The FDA-approved hydrophobic dasatinib (Dasa, Fig. S1, ESI[†])³¹, a tyrosine kinase inhibitor used for leukemia, and hydrophilic doxorubicin (Dox, Fig. S1, ESI[†])³², a chemotherapeutic drug for various cancers, were incorporated into NG using the developed protocol assisted by cisplatin. For a better comparison, the corresponding NGs without the removal of cisplatin were included in the study (Fig. S11–15, ESI[†]). Following the established protocol, the HA/Dasa and HA/Dox were formed with a TEM size around 58.2 nm and 36.3nm, while their hydrodynamic size was around 249.6 nm and 182.1 nm, respectively (Fig. S11b, d and Fig. S12c, d, ESI[†]). The size of the NG is drug type and concentration dependent. The hydrophobicity of the drugs play a role in compactness, and the initial concentration of the drugs could dictate the particle formation. Systematic tuning is required for each drug to obtain the preferred NG. The drug content in the NGs and drug loading efficiency were summarized in Table S1 and S2, ESI[†]. Generally the drug quantities remain constant within the NGs, while the majority of the cisplatin was removed by reversed coordination. The heating used during the preparation of NG didn't affect the integrity as well as cytotoxic potential of the drug as demonstrated by LC-MS and MTS assay,

respectively (Fig. S13, ESI†). In consistence with Rb-loaded NGs, both Dasa and Dox NGs demonstrated a clear red shift in absorption, size swelling, zeta potential drop and fluorescent quenching (Fig. 3a, d and Fig. S12, 14 and 15, ESI†), thereby strengthening the generality of our strategy. The Dasa or Dox in the NG demonstrated a slow release behavior in PBS/BSA (Fig. 3b, e), which was reasonable as these NG already survived from the PBS dialysis during which the loosely bound drug has been removed. However, the release of the drug in cells was expected to be faster since natural biopolymer HA could be degraded in cells. HA/Dasa and HA/Dox demonstrated a greater cytotoxicity towards MDA-MB-231 cells with a higher CD44 expression (Fig. 3c, f), indicating the NG's potential in targeted drug delivery.

In summary, cisplatin has been exploited as a removable crosslinker to trigger the assembly of dye or drug-loaded NG inspired by its action mechanism in cancer therapy. This method did not require multiple stepped chemical reactions, or strong solvents and complicate formulation processes; thus it represents a 'green chemistry' for the synthesis of NG to load drug of a different solubility. The acquired NG features a highly flexible formulation (with or without cisplatin) and drug candidate (drug with aromatic and amine groups), which enables it as a delivery platform for imaging, therapy or even theranostics. Given that all materials used could be an FDA-approved component and the preparation can be scaled up with ease, the NGs hold great potential to be translated towards clinical settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

(a) TEM image of HA/Cis/Rb and HA/Rb. The cartoon shows the components of NGs. (b) Hydrodynamic size of NG. (c) Absorption spectra of NGs. Free Rb was included as control to demonstrate the red shift. (d) Absorption spectra of the OPDA reaction product of NG. Free HA, Rb and cisplatin were set as controls. Absorption around 544 nm and 705 nm are correlated to the Rb and cisplatin content. (e) Cytotoxicity of NGs on HT-1080 cells for 72 h. Free cisplatin and Rb with the same concentration with that in HA/Cis/Rb were included for comparison. The concentration of NG was expressed in HA content.

Fig. 2.

(a) Colocalization Rb (red) of NG with lysosome (blue) and mitochondria (green). Cells were pulsed with Rb and HA/Rb for 30 min and followed by imaging after 1h and 6h. The rectangle area was further exaggerated. After 6h, appearance of tubular structure of Rb signal (colocalization with mitochondria) was observed for HA/Rb treated cells. (b) Specific uptake of NG in MDA-MB-231 and MCF-7 cells

Fig. 3.
(a,d) Absorption spectra of HA/Dasa and HA/Dox. (b,e) Drug release of HA/Dasa and HA/Dox in PBS/BSA. BSA was used to simulate the effects of proteins in biological medium. (c, f) Cytotoxicity of HA/Dasa and HA/Dox in vitro (72h).