Hyaluronic Acid with Tunable Amphiphilicity for Therapeutic Delivery

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INTRODUCTION: Self-assembled amphiphilic synthetic polymers have been of interest for drug delivery and regenerative medicine applications for decades. Such information is much less available for amphiphilic biopolymers, in part due to challenges associated with their reproducible chemical modifications and purifications, in part due to drastically different solubilities of hydrophobic and hydrophilic components. We hypothesize that when cyto-compatible hydrophobic motifs are conjugated with biopolymer hyaluronic acid (HA) that is prevalent in native tissues using high-fidelity bioconjugation chemistry with tunable stoichiometry, amphiphilic HA can be leveraged into versatile microstructures and self-assemble into nanoparticles (NPs). To test this hypothesis, we covalently conjugated dibenzocyclooctyne (DBCO)-modified cholesterol onto azide-functionalzied HA via catalyst-free strain-promoted azide−alkyne cycloaddition (SPAAC) click chemistry to prepare cholesteryl HA (Chol HA) (Fig. 1a) NPs and explored their potentials for therapeutics delivery.

METHODS: The size distribution of Chol HA NPs as a function of MW was determined by dynamic light scattering (DLS). Small-angle X-ray scattering (SAXS), cryo-TEM and molecular dynamics simulation (Schrödinger Desmond engine) were utilized to investigate the microstructure of the self-assembled NPs. Cellular internalization of Nile red-loaded NPs was visualized with confocal laser scanning microscope (CLSM). Osteogenesis of rat bone marrow-derived stromal cells (rBMSCs) induced by pre-loaded NPs was examined after 2-3 weeks of culture as a function of NP cargos and frequency of NPs supplementation as visualized and quantified by alizarin red and alkaline phosphatase staining. Cargos are either pre-loaded in NPs or dispersed in the medium, treatments including loaded dexamethasone + vitamin C (NP(DEX+VitC)), loaded DEX + un-loaded VitC-2-phophate (NP(DEX) + VitC-2-P), or loaded VitC + un-loaded water-soluble DEX in beta-CD (NP(VitC)+DEX/CD). All data and error bars shown were from three repeats and statistical significance of the differences were analyzed with One-way ANOVA and LSD test ($p < 0.05$).

RESULTS: As cholesterol conjugation content increased, the turbidity of Chol HA in PBS kept increasing while the viscosities increased initially and peaked at 5.5 mol% of cholesterol after which viscosities significantly dropped. DLS showed that Chol HA conjugates of 10 to 100 kD formed negatively charged spherical NPs with hydrodynamic diameters from 200 to 500 nm in the aqueous buffer (Fig. 1b). SAXS and cryo-TEM unveiled the onion-like multilamellar structures of the NPs with a size distribution of 25-50 nm, much smaller than the hydrodynamic dimension determined by DLS that is consistent with a thick hydrated shell of HA and associated counterions (Fig. 1c). Rapid cellular internalization of Nile red (0.4 w/w%)-loaded Chol HA (50 kD) NPs by rBMSCs occurred within 5 min according to the time-lapse CLSM (Fig. 1d). Chol HA NPs also readily encapsulate DEX (0.12% w/w%) and protected VitC from degradation as shown by ELISA and UV spectroscopy. Osteogenesis of rBMSCs was more significantly enhanced when the culture was supplemented by NPs carrying both osteoinductive molecules DEX and VitC than by NPs carrying either factor alone, whether the supplementation was repeated every 2-3 days (Fig. 2a) or one-time only (Fig. 2b). Chol HA NPs did not significantly impact the proliferation of rBMSCs over 7 days with fresh supplementation of NPs every two days.

DISCUSSION: Chol HA underwent phase transitions from liquid to physical gel to particle suspension as the cholesterol content increased in aqueous solution. Above 5.5 mol% of cholesterol, the formation of discrete NPs was favored, with their microstructures dominated with a multilamellar core and a hydrated HA surface layer. Experimental and computational results corroborated the multilayered NPs consisting of alternately packed hydrophobic layers with overlapping cholesterol moieties and hydrophilic HA layers. Rapid cellular internalization and cytocompatibility of Chol HA NPs were likely facilitated by the high affinity between cholesterol and lipid membranes as well as HA and membrane surface CD44. Robust osteogenesis of rBMSCs induced by DEX/VitC-loaded Chol HA NPs was also demonstrated, paving the way for potential translational applications of the NPs such as bone regeneration *in vivo*.

SIGNIFICANCE/CLINICAL RELEVANCE: Diverse regenerative medicine applications from guided tissue regeneration to drug delivery or the combination of both often require discrete material designs that inevitably increase compositional and translational complexity. The prospect of addressing these challenges by tailoring straightforward formulations of Chol HA is exciting. Biocompatible and cell-permeable Chol HA NPs capable of both carrying hydrophobic dexamethasone and protecting hydrophilic vitamin C can be explored for bone tissue regeneration and beyond.

IMAGES AND TABLES:

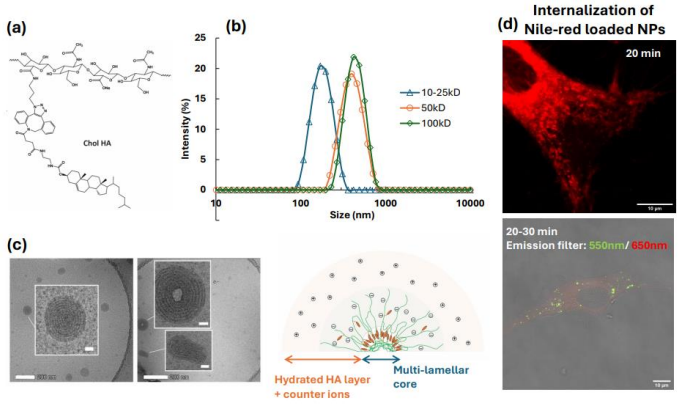

Figure 1. Chemical structure of Chol HA (a); Size distribution of Chol HA NPs of varying MW by DLS (b) and the morphology by cryo TEM (c); The internalization of Nile red-loaded NPs and the accumulation of NPs in endosomes of rBMSCs within 30 min (d).

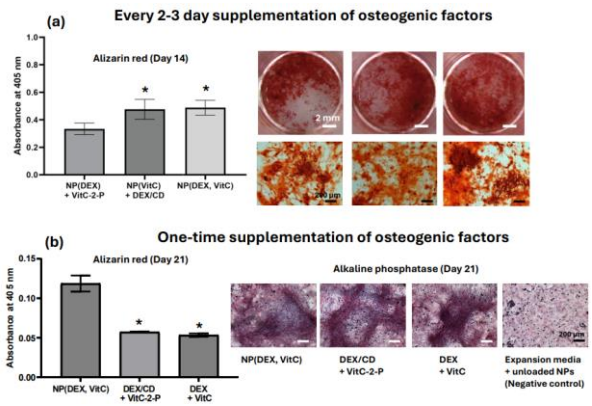

Figure 2. Alizarin red quantification and staining of osteogenesis of rBMSCs induced by pre-loaded NPs with 2-3 day supplementation (a); Alizarin red quantification and alkaline phosphatase staining of rBMSCs with one-time supplementation of pre-loaded NPs.