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Synthetic high-density lipoprotein nanoparticles: good things in small packages

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Abstract

Medicine has been a great beneficiary of the nanotechnology revolution. Nanotechnology involves the synthesis of functional materials with at least one size dimension between 1 and 100 nanometers. Advances in the field have enabled the synthesis of bio-nanoparticles that can interface with physiological systems to modulate fundamental cellular processes. One example of a diverse acting nanoparticle-based therapeutic is synthetic high-density lipoprotein (HDL) nanoparticles (NP), which have great potential for treating diseases of the ocular surface. Our group has developed a spherical HDL NP using a gold nanoparticle core. HDL NPs: (i) closely mimic the physical and chemical features of natural HDLs; (ii) contain apoA-I; (iii) bind with high-affinity to SR-B1, which is the major receptor through which HDL modulates cell cholesterol metabolism and controls the selective uptake of HDL cargo into cells; (iv) are non-toxic to cells and tissues; and (v) can be chemically engineered to display nearly any surface or core composition desired. With respect to the ocular surface, topical application of HDL NPs accelerates re-epithelization of the cornea following wounding, attenuates inflammation resulting from chemical burns and/or other stresses, and effectively delivers microRNAs with biological activity to corneal cells and tissues. HDL NPs will be the foundation of a new class of topical eye drops with great translational potential and exemplify the impact that nanoparticles can have in medicine.

Keywords

chemical burn; cholesterol; cornea; eye drop; inflammation; lipoprotein; microRNA; nanotechnology; wound healing

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1. PAST

Nanotechnology and nanoparticles are part of our everyday lexicon; however, sixty years ago these terms were at best only hypotheses. The concept of nanotechnology is ascribed to the Noble Laureate, Richard Feynman, who, on New Year's Eve in 1959 postulated that extraordinarily small functional materials could be constructed through the controlled assembly of individual atoms. In an iconic speech entitled, *"There's Plenty of Room at the Bottom,"* Feynman discussed powerful opportunities and potential uses for such materials. For instance in biomedicine, he suggested a new approach to synthesizing drugs targeted to the site of disease that, upon arrival, would perform therapeutic tasks at the molecular level [1]. Fifteen years later, the term "nanotechnology" was first used and defined as the processing or separation, consolidation, and deformation of materials by one atom or one molecule [2]. Generally, the goals of scientists in this new field were to synthesize, characterize, assemble and ascribe function to nanoscale materials. A nanoparticle is customarily defined as a particle of matter that has at least one size dimension between 1 and 100 nanometers (nm) [3]. Because the radii of individual atoms are measured on the picometer (10^{-12} m) or, more commonly, ångström (10^{-10}m) scale, one can conceptualize that rationally assembling atoms results in nanoscale (10^{-9}m) materials.

Owing to the extraordinarily small size, the development and improvement of scientific tools was critical and, often, preceded or occurred simultaneously with important advances in nanotechnology. Interestingly, prior to the invention of new instruments, some of the novel properties of nanoparticles were appreciated as far back as the fourth century AD. Nanoparticles made of gold and silver, for instance, were used in the Lycurgus Cup and in stained glass windows because of their brilliant color and stability to photobleaching [4]. It was not until the mid-to-late 1980s, using scanning tunneling (STM) and atomic force (AFM) microscopes that silicon, xenon and carbon atoms were resolved in the form of stable spheres (see [5] and references therein). The impact of this work is exemplified by the fact that Richard Smalley, Robert F. Curl and Sir Harold W. Kroto were awarded the Nobel Prize in Chemistry in 1996 - the first for nanotechnology - for their, along with Jim Heath and Sean O'Brien, discovery of a carbon allotrope containing 60 atoms (C_{60}) called buckminsterfullerene [6, 7].

By 2000, nanotechnology was impacting a wide range of disciplines including physics, chemistry, biology, computer science and engineering, though medicine has been the area of the most significant advances in nanotechnology [5]. Within the medical field, nanotechnology has impacted a number of areas; however, with differences in the pace of progress. Advances in miniaturizing the dimensions of existing technologies, a common approach across nanotechnology disciplines, led to significant advances in the performance and throughput capabilities of nucleic acid sequencing, drug screening and many other examples in basic and applied molecular diagnostics [8-11]. Furthermore, the area of nanodiagnostics benefited by new approaches combining biological molecules (e.g. nucleic acids and proteins) with nanoparticles (e.g. colloidal gold nanoparticles and semiconductor quantum dots) such that the conjugates specifically bound to their molecular counterpart and the nanoparticles, in turn, imparted signal transduction opportunities for realizing next generation molecular diagnostic assays [12, 13]. Finally, the brilliant physical properties of

nanoparticles have been used to develop probes for passive and targeted *in vitro* and *in vivo* imaging applications, including clinical imaging modalities such as magnetic resonance imaging (MRI) [14, 15]. Overall, the impact of nanomaterials and nanotechnology has, and continues to, contribute greatly to molecular detection and medical diagnostics.

The introduction and application of nanotechnology for developing medicines to treat human disease, even though this process is not new, requires increased resources and takes time. Much of the early work to develop new nanotherapies was focused on cancer. To some degree, this was not by chance as the National Cancer Institute (NCI) set aside funds for designated academic Centers of Cancer Nanotechnology Excellence (CCNE). The CCNE program lasted for three funding cycles, 5 years/ cycle, and started in 2005. The ending of the CCNE program in 2020 resulted in retrospectives and commentary regarding its success [16-18]. Certainly, over this time a number of nanomedicines gained approval and an increasing number are currently in human clinical trials [19]. Drawing on lessons learned from the last 20 years, there is an optimism that novel nanotherapies with increasing sophistication will take more prominent positions in pharmaceutical developmental pipelines for an expanding number of clinical indications in, and outside of, the cancer field [20] which is a topic of significant current national interest [21].

Overall, nanomaterials and nanoparticles can be used to synthesize higher order supramolecular materials that seamlessly interface with biological systems to modulate fundamental biologic processes such as cell proliferation, migration, inflammation, autophagy and metabolism [5, 22]. Our multidisciplinary group is particularly interested in developing new topical and molecularly targeted therapies for ophthalmology, which is a clinical area that has heretofore received little attention. We have demonstrated and proposed a mono-therapeutic nanoparticle platform that exemplifies diversity of action in the form of synthetic high-density lipoprotein (HDL) nanoparticles (NP) [23], which will be the focus of this review.

2. PRESENT

2.1 HDL NPs.

High-density lipoproteins (HDLs) are natural nanoparticles, ranging in size from 7-13 nm in diameter, that circulate in the bloodstream and move into and out of human tissues. HDLs are responsible for targeted lipid, mainly cholesterol and cholesterol ester, transport [24-26]. Apolipoprotein A-1 (apoA-I) is the most abundant and important protein of HDL and is responsible for HDL size, shape, and molecular targeting. In addition to apoA-I, the HDL particle is typically modeled as being comprised of cholesterol, cholesterol esters, and phospholipids. Mature, cholesterol-ester-rich, spherical HDL particles target their high-affinity ligand, scavenger receptor type B1 (SR-B1) [27]. Upon binding the receptor, diffusion of free cholesterol between the cell and the surface of the HDL particle is initiated, and the delivery of cholesterol esters from the core of the HDL particle to the cell occurs. The synthesis of immature versions of HDLs consisting of mixtures of apoA-I and phospholipids is commonplace [28]; however, because of the chemical complexity of cholesterol-rich spherical HDLs, and the biological/ enzymatic steps required for biosynthesis, little success had been achieved in developing reliable and scalable methods of

producing spherical forms of HDL [29]. Major breakthroughs in the synthetic HDL bioengineering field came with the development by the Thaxton Lab of a spherical HDL biomimetic synthesized using a gold nanoparticle (NP) core as a size and shape-restrictive template for apoA-I and lipid assembly, whereupon the conjugates tightly bind and regulate cell cholesterol [30-32] (Fig. 1). A similar synthetic approach was used by others to synthesize HDL-like nanoparticle imaging agents [33]. As therapeutics, HDL NPs: (i) are endowed with a surface chemistry that closely mimics the physical and chemical features of natural HDLs; (ii) contain apoA-I; (iii) bind with high-affinity to SR-B1; (iv) are not toxic to healthy cells in vitro or in vivo; (v) and can be chemically engineered to display nearly any surface or core composition desired. [30]. Another core property of native HDLs is their ability to transport cholesterol and other small molecules and nucleic acids and deliver these payloads to cells expressing SR-B1 [34-38]. Because of their targeted nature, the inherent functions of native HDLs, the ability to carry precious cargo for delivery and their biocompatibility, HDLs are an intriguing natural nanomaterial to synthetically harness.

2.2 HDL and the Cornea.

It is recognized that HDL protects against cardiovascular disease by regulating cholesterol efflux from tissues and reducing inflammation [39, 40]. Other positive effects of HDL are their antioxidant, antifibrotic, vasoprotective, and antithrombotic properties [39, 40]. There have also been reports that HDL can maintain the integrity of the endothelium [41]. Overall, data support that HDLs play a powerful role in maintaining epi- and endo-thelial cell homeostasis and can potently reduce inflammation. To our knowledge, materials with inherent targeting ability and tunable structure-function properties, like synthetic HDL NPs, have not been considered as a therapeutic agent by the ophthalmology community. With this in mind, we have reported on the effects of HDL NPs on corneal epithelial regeneration and inflammation [23].

2.3 HDL NPs enter cells and tissues of the cornea.

SR-B1 is the major receptor that controls the selective uptake of HDL cargo into cells [42]. Not surprisingly, single cell RNA sequencing showed that SR-B1 mRNA was detected in all cell populations of the mouse cornea [43]. Consistently, SR-B1 protein was observed on primary human corneal epithelial cells (HCECs) as well as human and mouse corneal and limbal epithelia [23]. SR-B1 was also present on the stromal keratocytes. In a series of in vitro and in vivo experiments, HDL NPs were demonstrated to enter HCECs and intact mouse corneas [23]. Specifically, following the addition of HDL NPs to HCECs, non-membrane-bound gold particles were readily observed, free in the cytoplasm; few if any particles were detected in the nucleus or in the proximity of the cell membrane. This suggested a non-endocytic mechanism of HDL NP transport into HCECs via SR-B1, similar to what has been described in other cells [42, 44, 45]. More importantly, twenty-four hours after a fluorescent HDL NP conjugate in PBS was topically applied to intact mouse corneas, fluorescent signal was readily detected in the superficial and basal corneal epithelial cells as well as in the stromal keratocytes [23].

2.4 HDL NPs accelerate re-epithelialization in vivo.

Using a diet-induced obesity (DIO) mouse model, which has an impaired wound healing response [46, 47], HDL NPs applied topically to the corneal surface after a debridement wound sealed wounds significantly faster than controls (Fig. 2; [23]). Such a positive effect on wound closure is due, in part, by HDL NPs up-regulation of the Akt signaling pathway [23], which is involved in re-epithelialization [48]. Furthermore, Akt signaling regulates actin remodeling and cell migration [49]. Thus, it is not surprising that a marked increase in F-actin was observed at the leading edge of the HDL NP-treated migrating cells compared with the control NP-treated cells [23]. This suggests that HDL NPs are positive regulators of F-actin polymerization during the initial migratory phase of cell migration [50]. Finally, phosphorylation of EphA2 at S897 via Akt can signal an increase in cell migration through the reorganization of actin filaments at the leading edge of a migrating sheet [51]. Treatment of cells with HDL NPs resulted in a dramatic increase in p-EphA2-S897 expression compared with control NPs, providing compelling evidence that HDL NPs positively affect cell migration via targeting EphA2 [23]. In the future, it is necessary to understand how Akt/pS897-EphA2 signaling impacts corneal epithelial wound healing when treated with synthetic HDL NPs.

2.5 HDL NPs as anti-inflammatory agents.

HDL inhibits inflammatory and oxidative damage that are processes involved in atherogenesis [52], in part, by preventing monocyte recruitment into the arterial wall [53], and by decreasing production and inactivation of neutrophil NADPH [54]. It is now recognized that synthetic HDLs can reduce acute inflammation and oxidative stress in rabbit carotid arteries (in vivo) and in primary human coronary artery endothelial cells [55]. Furthermore, the type of phospholipid used in the reconstituted HDL has significant influence on anti-inflammatory and anti-atherosclerosis properties [56]. Thus, it is apparent that various recombinant ApoA-I proteins and ApoA-I mimetic peptides used for the preparation of synthetic HDLs exhibit properties similar to those of endogenous HDL suggesting potential as anti-inflammatory agents (see [57] and references therein). Our group has demonstrated that synthetic HDL NPs made using inorganic [58] and organic core [59] scaffolds endowed with apoA-I and certain phospholipids potently reduce the in vitro (human cell lines) and ex vivo (primary human cells) inflammatory response induced by exposure to lipopolysaccharide (LPS, Gram-negative bacterial endotoxin). The use of HDL NPs as anti-inflammatory agents in an ocular setting is scant [23].

2.5.1 HDL NPs are effective in treating alkali burn-induced corneal inflammation.—

In the alkaline burn model to induce an inflammatory response in mouse corneas, the corneal epithelium, stromal, and inflammatory cells are involved in the injury, repair, and wound healing processes, which are accompanied by the production of numerous cytokines [60, 61]. Following such a wounding protocol, mice were treated daily for 4 days with a topical solution of HDL NPs, control NPs in PBS, or PBS [23]. By day 7 post-wounding, PBS- and control NP-treated corneas remained opaque whereas the HDL NP-treated mice showed a 40-50% ($p < 0.05$) improvement in corneal opacity and surface integrity (Figs. 3, a, b). Control NP treated corneas displayed a range of thickened and disorganized corneal epithelia as well as a wide spectrum of stromal alterations, ranging

from a stroma filled with inflammatory cells (Fig. 3 c) to randomly oriented collagen bundles resulting in a disorganized appearance (Fig. 3 c). In contrast the HDL NP-treated corneas displayed a well-organized stratified epithelium (Fig. 3 d) and a stroma with collagen bundles highly organized in a plywood-like fashion that were relatively devoid of inflammatory cells (Fig. 3 d). In some HDL NP treatment groups, stromal keratocytes were prominent (Fig. 3 d).

Immune cell recruitment after corneal injury is mediated by proinflammatory cytokines released from epithelial cells and keratocytes at the injured site. IL-1, lL-6 and TNF are important [60, 62] and aid in attracting neutrophils, which are the first cells infiltrating the cornea after injury [63, 64]. Shortly after neutrophils enter the cornea, macrophages extravasate from the limbal vessels, infiltrate the stroma from superficial to deeper layers and migrate towards the center of the cornea. Macrophages aid in corneal wound closure by secreting TGF;β to promote the differentiation of fibroblasts to myofibroblasts. In addition to removal of debris and apoptotic cells, macrophages are essential mediators of angiogenesis after severe and prolonged corneal injury [65]. Several chemokines and their receptors have been identified in the inflamed cornea. CXCL1, CXCL8 and MCP-1/CCL2 mRNA levels were found to be elevated in human inflamed corneas. Additionally, CCR7 and its ligand CCL21 were upregulated in inflamed corneas, mediating MHC II+ cell recruitment [66-68]. Evaluation of the alkali burn-induced cytokine expression pattern after treatment with HDL NPs revealed that day 1 post-injury, *Il1a, Il1b, Il6* and *Ccl2* were most highly expressed (Fig. 4), which is consistent with an initial stage of inflammation. By day 3, HDL NP treatment significantly reduced the expression levels of *Il1a, Il1b, Il6, Inos, Mmp9* and *Ccl2* when compared with control NPs (Fig. 4) [23]. Chemokines such as CCL2 play important roles in the recruitment of macrophages to the site of injury during an inflammatory event [69, 70], as well as the inflammatory mediator iNOS, which is associated with activated macrophages [71]. Elevated levels of Gelatinase or MMP-9 are associated with numerous diseases of the cornea and can facilitate corneal ulceration [72]. All genes evaluated, returned to pre-treatment levels by day 7 (Fig. 4). Collectively, these findings strongly indicate that topical application of HDL NPs to the corneal surface following a chemical burn can aid in attenuating the inflammatory response.

2.6 HDL NPs are an effective platform for the delivery of microRNAs to cells.

miRNAs are small (~22 nucleotides in length), “noncoding” or “non-messenger” RNAs that are part of the RNAi silencing machinery [for reviews see [73-76]]. Consequently, miRNAs influence the regulation of a myriad of biological processes in both normal and disease circumstances. Hence, miRNAs hold great promise as potential therapeutic agents. The effective formulation and delivery of therapeutic miRNAs to the cytoplasm of target cells in a biologically active form, has proven to be a major hurdle toward realizing this goal. A conceptual breakthrough to this problem occurred with the demonstration that natural HDLs, isolated from human serum contained miRNAs and that these HDL-bound miRNAs had improved stability compared with naked miRNAs [38]. Furthermore, as mentioned above, native HDLs deliver bound miRNAs to cells that express the high-affinity receptor of HDLs, SR-B1, whereupon the miRNA regulates the expression of the target gene [77]. Capitalizing on these observations, the Thaxton laboratory has demonstrated that the HDL NP platform

can be extensively tailored to efficiently deliver any desired individual (i.e. similar to miRNAs) or complementary pairs of RNA strands (e.g. siRNA duplex pairs) on separate HDL NPs or on the same HDL NP for potent target gene regulation [37]. In vitro and in vivo data reveal that the HDL NP platform can be utilized to efficiently target prostate and ovarian cancer [78, 79].

2.6.1 HDL NPs complexed to miR-205 accelerate corneal epithelial cell migration.—

In the context of corneal epithelial biology, microRNA-205 (miR-205) has well-established regulatory roles [80-83]. Specifically, miR-205 negatively regulates the lipid phosphatase SHIP2 in epithelial cells resulting in the activation of Akt signaling [83]. Consequently, by suppressing SHIP2, miR-205 promotes epithelial migration via cofilin activation [82]. Because of such easily monitored outcomes, HDL NPs were complexed with miR-205 (HDL NP-miR-205) and this compound was interrogated as a positive effector of corneal epithelial migration [23]. When HCECs were exposed to miR-205-HDL NPs for 48 hours: (i) the complex was readily taken up; (ii) remained stable; (iii) RNA increased; and, (iv) no signs of toxicity were observed [23]. As anticipated, a decrease in SHIP2 and an increase in p-Akt levels were observed compared to control NPs. To determine further the biological activity of the miR-205-HDL NP compound, a series of scratch wound assays using a limbal-derived corneal epithelial cell line (hTCEpi) [84, 85] revealed that miR-205-HDL NP treatment sealed wounds after 6 hrs compared to an 18 hr closure time for the scrambled-miR-HDL NP treatment [23]. Taken together, these findings firmly established that HDL NPs are an effective platform for delivery of biologically active miRNAs to corneal epithelial cells.

2.6.2 miR-146a-HDL NPs reduce NF-kB activity.—

As a second proof of concept for the efficacy of HDL NPs to serve as a platform for delivery of miRNAs into cells, miR-146a was evaluated. miR-146a is expressed in response to pro-inflammatory stimuli and can inhibit NF-κB transcriptional activity [86-88]. When miR-146a-HDL NPs were exposed to J774-dual macrophages, which express the secreted alkaline phosphatase (SEAP) gene downstream of the NF-κB consensus transcriptional response element, the signal of LPS-induced SEAP was significantly reduced compared with controls [23]. This is consistent with the miR-205 data indicating that miR-146a complexed with HDL NPs can penetrate cells and retains biological activity.

3. FUTURE

3.1 Organic core scaffolds

One of the major benefits of the synthetic HDL NPs is that the 5nm gold core can be functionalized with phospholipids and apoA-I and targeted to specific cells to modulate cellular lipid metabolism [37, 89]. Another feature of these synthetic HDL NPs is their stability after cell binding [59, 90]. However, this property has a potential downside in ocular tissues since gold particles binding to and interacting with cells that do not rapidly turnover (e.g., keratocytes, endothelial cells) might theoretically impair the passage of light to the lens. Recently we have successfully replaced the inorganic gold nanoparticle with an organic, transparent Lipid-conjugated core (oc) scaffold (Fig. 5) [59]. These ocHDL NPs

have the size, shape, surface chemistry composition, protein structure, cholesterol transport properties and targeting properties similar to mature human HDLs [59]. Functionally, they possess cholesterol transport and anti-inflammatory capabilities similar to native HDLs [59]. Delivery of these highly innovative and optically transparent ocHDL NPs in various ocular cells and tissues should pose no problems with light transmission. We envision that such ocular core scaffolds will form the foundation of a unique class of nanoparticle-based products particularly well-suited for the ocular community.

3.2 Modification of existing synthetic HDL NPs: potential for novel therapeutics.

As mentioned previously, modifying the qualitative and quantitative nature of the phospholipids that comprise the synthetic HDL NPs can have profound effects on the biological nature of these particles [30, 58]. Specifically, HDL NPs endowed with phospholipids that can be metabolized to polyunsaturated fatty acids are particularly appealing. In fact, native HDL binding to macrophages has been reported to stimulate production of pro-resolving lipids [91]. Therefore, adding phospholipids to synthetic HDL NPs that are pro-resolving through metabolism, including certain ω6 and ω3 fatty acids [92-94] as well as cardiolipin, will be potentially more potent activators of wound healing and the resolution of inflammation. Having demonstrated the targeted delivery of microRNA cargo using the HDL NP platform [23], a logical next phase is the generation of targeted HDL NP therapies that incorporate small molecules such as Vitamin D3 (Vit D3).

3.3 Vitamin D3-HDL NP: a potential enhanced anti-inflammatory eye drop.

Vit D3 is well-recognized as an immunomodulator through direct inhibition of NF-κB activation, suppression of TNF-α and iNOS expression, as well as activation of autophagy [95-99]. Vit D3 is converted intracellularly to the active form in macrophages, a critical cell population activated following stress that exacerbates local cellular inflammation. The presence of the Vit D receptor (VDR) was detected in the human corneal epithelium, as well as the corneal endothelium [100]. Additionally, the presence of vitamin D hydroxylases (CYP27B1, CYP27A1, CYP2R1, and CYP24A1) are present in corneal epithelial and endothelial cell lines [101, 102], indicative that these cells have the ability to initiate and regulate Vit D3 metabolism. With respect to corneal inflammation, topical administration of Vit D3 to sutured mouse corneas (a model for inflammation) inhibited Langerhans cell migration and maturation, while delaying neovascularization in the central cornea (see [103] and references therein). Vit D3 protected corneal graft rejection by inhibiting the proinflammatory cytokines IL-1α and TNF-α, in rats [104]. In vitro studies in corneal epithelial cells demonstrated immunomodulatory activity of Vit D3 via attenuation of proinflammatory mediators while increasing antimicrobial peptides and anti-pseudomonas activity [105]. We propose that Vit D3 has potential for treatment of a variety of corneal inflammatory diseases because it targets macrophages and suppresses inflammation without the side effects commonly associated with long-term steroid usage. In addition, Vit D3 can activate autophagy [99], a critical stress response process to differentiate macrophages towards an anti-inflammatory repair phenotype. We have demonstrated that autophagy has an important role in maintaining limbal epithelial stem cell homeostasis [43, 106].

Given the anti-inflammatory properties of HDL NPs as well as Vit D3, we envision development of a “super” HDL NP-Vit D3 eye drop in order to reduce inflammation in the anterior segment. The HDL NP platform affords significant synthetic control and latitude in order to rationalize and develop targeted materials with desired characteristics. Specifically, HDL NPs made with various cores and phospholipids can also be formulated with Vit D3. Work to realize this construct is underway.

4. CONCLUSIONS

It is clear that HDL NPs will be the foundation of a new class of topical eye drops (Fig. 6) that have: (i) stability; (ii) minimal adverse side effects; (iii) tissue regenerative capabilities; and, (iv) anti-inflammatory properties. In addition to its use as a monotherapy, HDL NPs are ideal for incorporating small molecules (e.g. Vit D3) and nucleic acids (e.g. miR-146a) to modulate lipid metabolism and deliver drugs to enhance wound healing and reduce inflammation. These features endow HDL NPs with great translational potential and exemplify the impact that nanoparticles can have in medicine. Specifically, an HDL NP eye drop should be effective in treating dry eye or tear gland insufficiency, whose underlying etiology is inflammation [107]. Since destruction of the stem cell niche due to persistent inflammation is thought to be an underlying cause of limbal stem cell deficiency (see, [108] and references therein), an HDL NP eye drop should be helpful in modulating and/or avoiding limbal stem cell deficiency (LSCD). Nitrogen and sulfur mustards (NM and SM) are devastating compounds that have been used as chemical warfare agents. Recently, it has been shown that Vit D3 treatment protected against SM toxicity and prevented SM-induced mortality [109]. The eye is the most susceptible part of the body to the effects of mustard exposure, particularly SM [110-115]. Following exposure, severe and extensive inflammation is triggered, which also can result in phenotypes mimicking LSCD [116]. We opine that HDL NPs and HDL NP-Vit D3 eye drops have great potential as an extremely innovative therapy to comprehensively address corneal mustard keratopathy (CMK) without the side-effects associated with steroidal treatments. Corneal keratopathy occurs in more than 70% of diabetic patients, which manifested in part, as persistent epithelial defects, and recurrent erosions [117, 118]. Therefore, HDL NP eye drops might have prophylactic value in patients with diabetes. Finally, HDL NPs either alone or complexed with small molecules should be useful in a wide variety of ocular surface diseases such as chemical and thermal injury, long-term contact lens wear, severe chronic rosacea, Stevens-Johnson syndrome, atopic keratoconjunctivitis, bacterial keratitis and graft versus host disease, to name a few.

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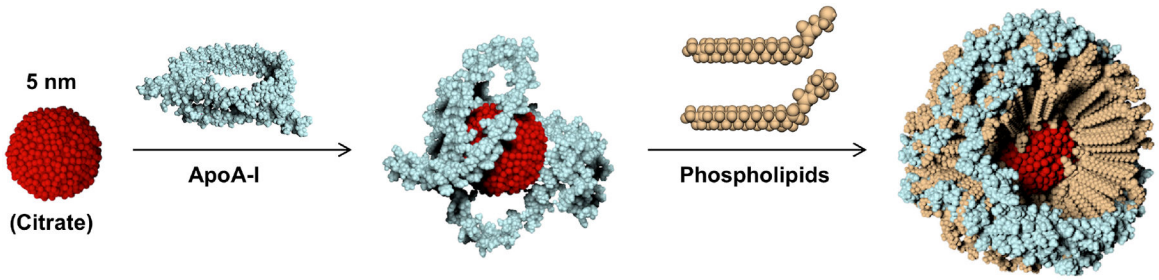

Figure 1: Bioengineered synthetic HDL NPs using inorganic (gold) core scaffold.

Synthesis scheme for HDL NPs made using a 5 nm diameter citrate stabilized gold nanoparticle scaffold (red) surface-functionalized with apoA-I (blue) and a phospholipid layer (tan).

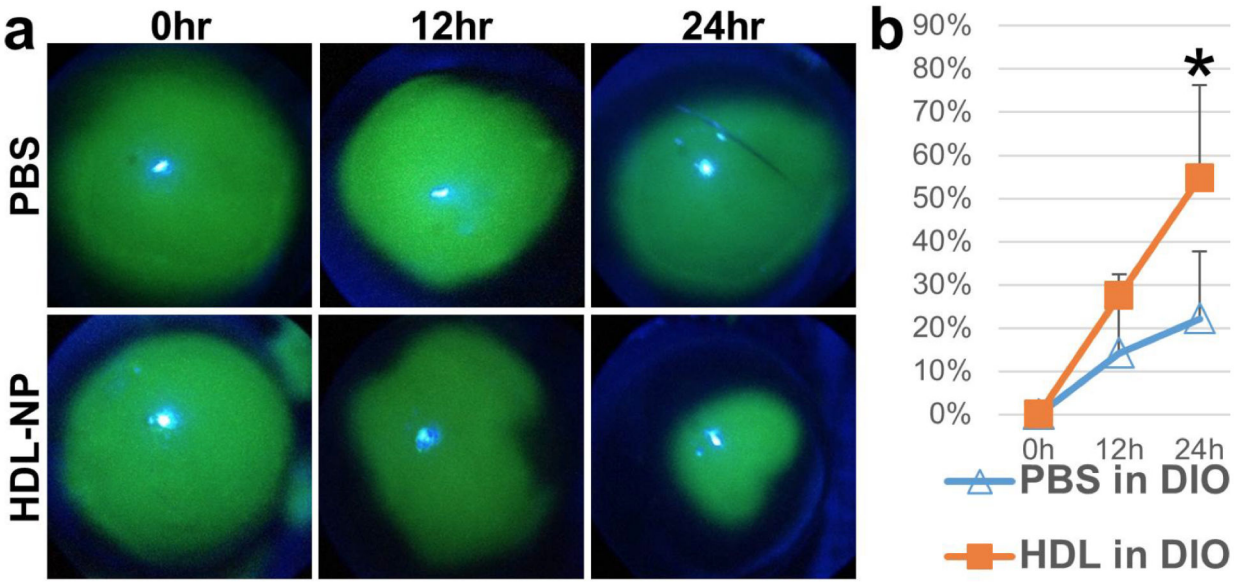

Figure 2: Therapeutic effect of HDL NPs on wound healing of mouse corneal epithelium.

Corneal images (a) and epithelial corneal wound closure percentage (b) in DIO mouse corneas treated with HDL NPs or control. Green fluorescence represents corneal wound. N=8. *p<0.05. The figure is taken from Junyi Wang *et al.* with permission [23].

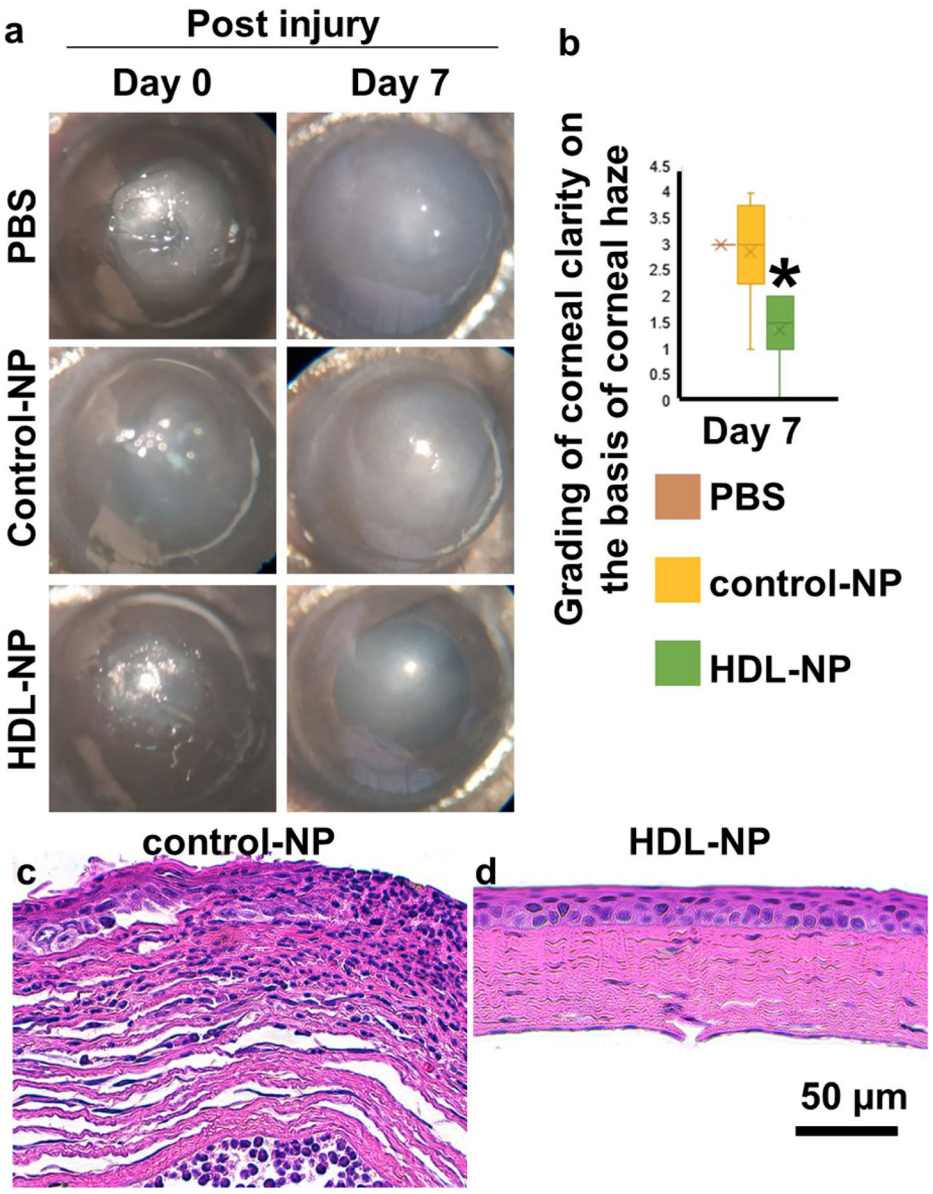

Figure 3: HDL NP treatment reduces inflammation after alkali burn.

Mice were treated with HDL NPs, control NPs, or PBS (topically) following 30s alkali burns. (a) Representative images. (b). Degree of haze. (c-d). H&E 7 days post burn. N=8. The figure is taken from Junyi Wang *et al.* with permission [23].

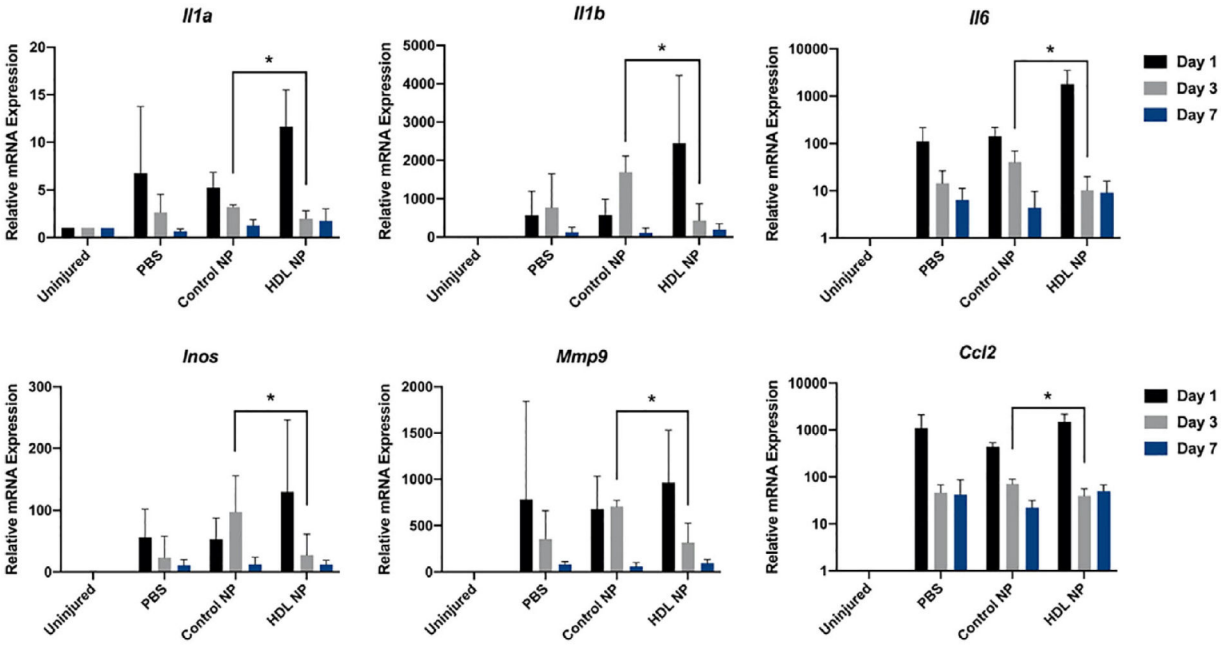

Figure 4: HDL NP has anti-inflammation activity.

Filter paper (1 mm) soaked in NaOH (1 m) was placed on the corneal surface of 6 week old WT mice for 30 s and then washed extensively with PBS. Corneas were topically treated with HDL NPs, control NPs (inert AuNP core, passivated with polyethyleneglycol (PEG)), or PBS daily for 7 d. Whole corneal tissues were dissected and total RNAs were isolated for RT-qPCR for inflammation-related genes at post injury day 1, 3, and 7 (N = 8). *$p < 0.05$. Unpaired t-tests were conducted. The figure is taken from Junyi Wang *etal.* with permission [23].

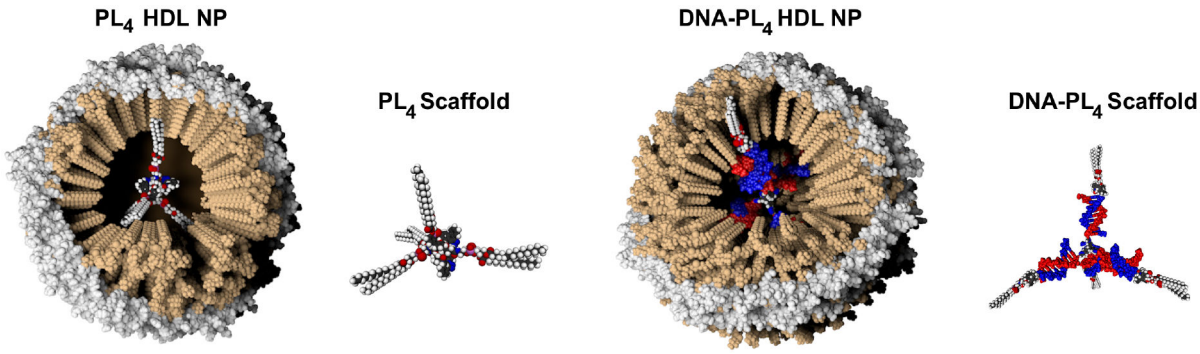

Figure 5: Bioengineered synthetic HDL NPs using organic (PL4 and DNA-PL4) core scaffold.
Organic tetrahedral phospholipid (PL4) or PL4 with bioprogrammable DNA "arms" (DNA-PL4) are used as scaffolds for ocHDL NPs. Synthesis of ocHDL NPs made using organic scaffolds proceeds similar to the ones made using AuNPs.

Figure 6: High-Density Lipoprotein Nanoparticle Eye Drop