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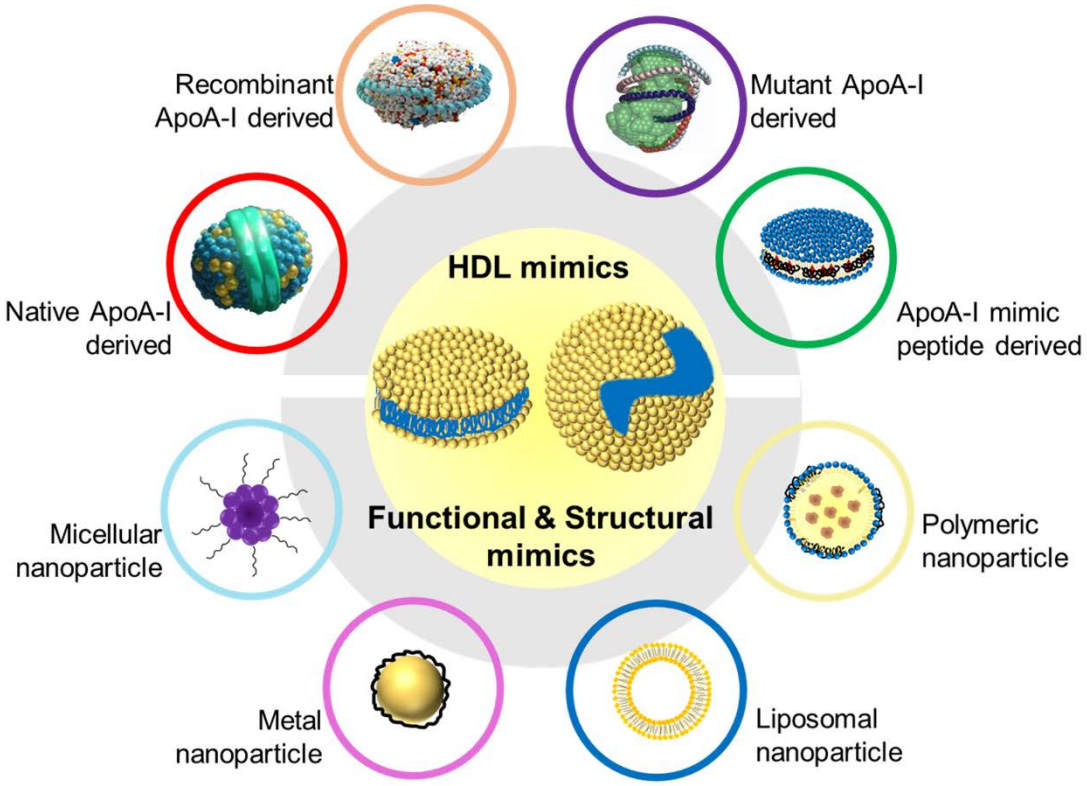
Abstract

Despite the ability of current efficacious low-density lipoprotein-cholesterol–lowering therapies to reduce total cardiovascular disease (CVD) risks, CVD still poses major risks for morbidity and mortality to the general population. Because of the pleiotropic endothelial protective effects of high-density lipoproteins (HDL), the direct infusion of reconstituted HDL (rHDL) products, including MDCO-216, CER001 and CSL112, have been tested in clinical trials to determine whether direct infusion of rHDL can reduce coronary events in CVD patients. In addition to these rHDL products, in the past two decades, there has been an increased focused on designing artificial HDL-mimicking nanotherapeutics to produce complementary therapeutic strategies for CVD patients beyond lowering of atherogenic lipoproteins. Although recent reviews have discussed the developments of artificial HDL-mimicking nanoparticles as therapeutics for CVD have been comprehensively, there has been little assessment of “plain” or “drug-free” HDL-mimicking nanoparticles as therapeutics alone. In this review, we will summarize the clinical outcomes of rHDL products, examine recent advances in other types of artificial HDL-mimicking nanotherapeutics, including polymeric nanoparticles, cyclodextrins, micelles, metal nanoparticles, etc., and potential new approaches for future CVD interventions. Moreover, success stories, lessons, and interpretations of the utility and functionality of these HDL-mimicking nanotherapeutics will be an integral part of this article.

Graphical/Visual Abstract and Caption



The types of HDL mimics and HDL-mimicking nanoparticles

1. Introduction

According to recent statistics and reports, there were an estimated 17.6 million CVD deaths in 2019 (Abdullah et al., 2018, Riaz et al., 2019, Sacks & Jensen, 2018). These formidable numbers call attention to the critical need to develop novel therapeutic strategies to supplement current LDL-lowering treatments. Numerous epidemiological studies have demonstrated a strong inverse association between HDL cholesterol (HDL-C) and risk of CVD, thus initiating the search for HDL-based interventions (Fotakis et al., 2019, Khera et al., 2017, Kingwell & Chapman, 2013).

HDL are endogenous, nanosized, protein-lipid particles that circulate in blood and play a role in the transport and metabolism of triglycerides, phospholipids, and cholesterol (Lüscher et al., 2014, Rader & Tall, 2012). The most established functional properties associated with HDL are its atheroprotective activities, such as its antioxidant (Brites et al., 2017) and anti-inflammatory capabilities (Fotakis et al.,

2019), endothelial cell maintenance functions (Lüscher et al., 2014), and its role in mediating cholesterol efflux. Its role in the promotion of reverse cholesterol transport (RCT) and cellular cholesterol efflux is commonly considered to be HDL's most crucial anti-atherogenic property, leading the research community to investigate whether direct infusion of reconstituted apolipoprotein A-I (ApoA-I), the major protein component of HDL, can reduce coronary events in humans with CVD (Khera et al., 2017). The infusion of rHDL in animals and early clinical imaging trials reported evidence of plaque regression (Rader, 2018, Rader & Tall, 2012). A series of major ApoA-I-derived rHDL mimetics, including ETC-216/MDCO-216 (ApoA-I Milano) (Nicholls et al., 2018), CER-001 (recombinant, wild-type ApoA-I) (Andrews et al., 2017, Nicholls et al., 2018), and CSL111/CSL112 (native ApoA-I isolated from human plasma) (Gibson et al., 2021), have completed human clinical trials with discrete clinical performance and/or are still under investigation. In comparison, using synthetic peptide analogs of ApoA-I's amphipathic helices offers an easier and more cost friendly approach to the preparation processes for fabricating ApoA-I-derived rHDL therapies. For instance, ETC-642 is an ApoA-I mimetic peptide–phospholipid complex that has completed early-stage clinical development and reduced cardiovascular events (Di Bartolo et al., 2011, Di Bartolo et al., 2011).

In addition to conventional rHDL, various HDL-mimicking nanoparticles (Chen et al., 2020) have been developed for CVD including liposomes, inorganic or polymeric nanoparticles, cyclodextrins, micelles, metal nanoparticles and lipid-conjugated core scaffold nanoparticles. These HDL-mimicking nanoparticles have exhibited similar biofunctions to native HDL, including RCT, antioxidant and anti-thrombotic effect, etc. (Gupta et al., 2021, Kuai et al., 2016). Moveover, HDL-mimicking nanoplatforms have been shown to provide several advantages over the traditional rHDL strategy (Kornmueller et al., 2019). Foremost, the surface of nanoparticles can be decorated with ligands specific to disease sites or targets, allowing for improvement of therapeutic efficacy without a notable change in cytotoxicity to normal tissue (Chuang et al., 2020).Furthermore, the preparation process of rHDL requires expensive raw materials like apolipoproteins, challenging the wide availability of the final product. Meanwhile, advances in chemical synthesis have made HDL-mimicking nanoparticles more simple and efficient nanoplatforms to well fulfill the functionality of native HDL.

In this review, we will discuss both traditional rHDL products (ApoA-I/ApoA-I mimetic peptides complexed with phospholipid) along with other types of HDL-mimicking nanoparticles. These HDL mimetics, termed "artificial HDL-mimicking nanotherapeutics", possess structures and/or functions similar to native HDL. There have been many reported developments regarding modifications of artificial HDL-mimicking nanotherapeutics that focus on the use of these nanoparticles as drug delivery platforms in many different applications. Here, we will instead focus on the critical elements of "plain" or "drug-free" artificial HDL-mimicking nanotherapeutics in cardiovascular intervention, emphasizing innovative technologies published within the last decade.

2. HDL metabolism, RCT process and its cardiovascular protective effects

HDL is an endogenous lipoprotein particle with a density between 1.063 and 1.21 g/mL and a size ranging from 5 to 17 nm in diameter. HDL's composition is a very heterogenous, but mainly consists of phospholipids and amphipathic alpha-helical apolipoprotein A-I (ApoA-I). Depending on the state of maturation or synthesis, HDL takes on many forms *in vivo.* It can exist as lipid-poor small discoidal particles while also existing as larger spherical particles rich in cholesterol esters and other hydrophobic cargo. (Chen et al., 2020).

The most popular mechanistic hypothesis underlying the antiatherogenic effects of HDL is the concept of reverse cholesterol transport (Rodriguez et al., 2019, Singh et al., 2007). Namely, HDL effluxes cholesterol from peripheral cells, such as arterial macrophage foam cells, and transports it to the liver for biliary excretion, leading to a reduced burden of coronary atherosclerosis in humans (Jomard & Osto, 2020). **Figure 1** summarizes the important HDL metabolism steps in circulation. ApoA-I, the primary structural protein of HDL, is secreted predominantly by the liver and the small intestine (Fisher et al., 2012, Ikonen, 2008). Phospholipids and free cholesterol transported via ATP-binding cassette (ABC) transporter A1 (ABCA1) rapidly lipidate the protein. The stability of ApoA-I and the formation of nascent HDL particles (discoidal form) is dependent on this initial lipidation. The primary function of nascent HDL is to remove cholesterol and phospholipids from peripheral tissues. More specifically, nascent HDL particles enter circulation and efflux cellular cholesterol from atherosclerotic plaque-associated lipid-

laden macrophages via ABCA1 to form cholesterol-rich HDL (Navdaev et al., 2020). HDL further matures when cholesterol is esterified to cholesterol ester (CE) by lecithin-cholesterol acyltransferase (LCAT). The hydrophobic CE sequesters into the core of HDL particles, leading to the formation of a spherical mature HDL and reduction of free cholesterol levels on the particle surface (Waksman et al., 2010). This reduction of free cholesterol levels in critical for maintaining the concentration gradient of free cholesterol between cells and plasma to prevent net efflux of cholesterol from reaching equilibrium. Mature HDL can internalize more excess cholesterol effluxed by ATP-binding cassette transporter G1 (ABCG1) to become larger and more mature. Mature HDL can then mediate the removal of cholesterol, promoting RCT, via two different methods. In the plasma compartment, mature HDL can transfer CE to low-density lipoproteins (LDL) in exchange for triglycerides (TG), a process favored by cholesteryl ester transfer protein (CETP). Cholesterol-loaded LDL is then taken up by hepatocytes through the LDL receptor. Secondly, HDL can interact with scavenger receptor-B1 (SR-B1) which uptakes CE and other lipids from mature HDL into hepatocytes for elimination. HDL's ability to remove excess cholesterol from atherosclerotic plaque-associated lipid-laden macrophages has been recognized as its primary mechanism of action for protection against atherosclerosis and reduction of CVD risks (Chen et al., 2020, Nicholls et al., 2018, Sacks & Jensen, 2018, Singh et al., 2007). Clinical outcomes have demonstrated that elevated serum concentrations of HDL are beneficial in preventing CVD (Sacks & Jensen, 2018). Therefore, the development of artificial HDL particles that preserve these above-mentioned inherent features of native HDL represents a promising therapeutic approach for CVD (Chen & Frishman, 2016, Di Bartolo et al., 2018, Lüscher et al., 2014, Rader, 2018, Singh et al., 2007, Tardif, 2010, Waksman et al., 2010).

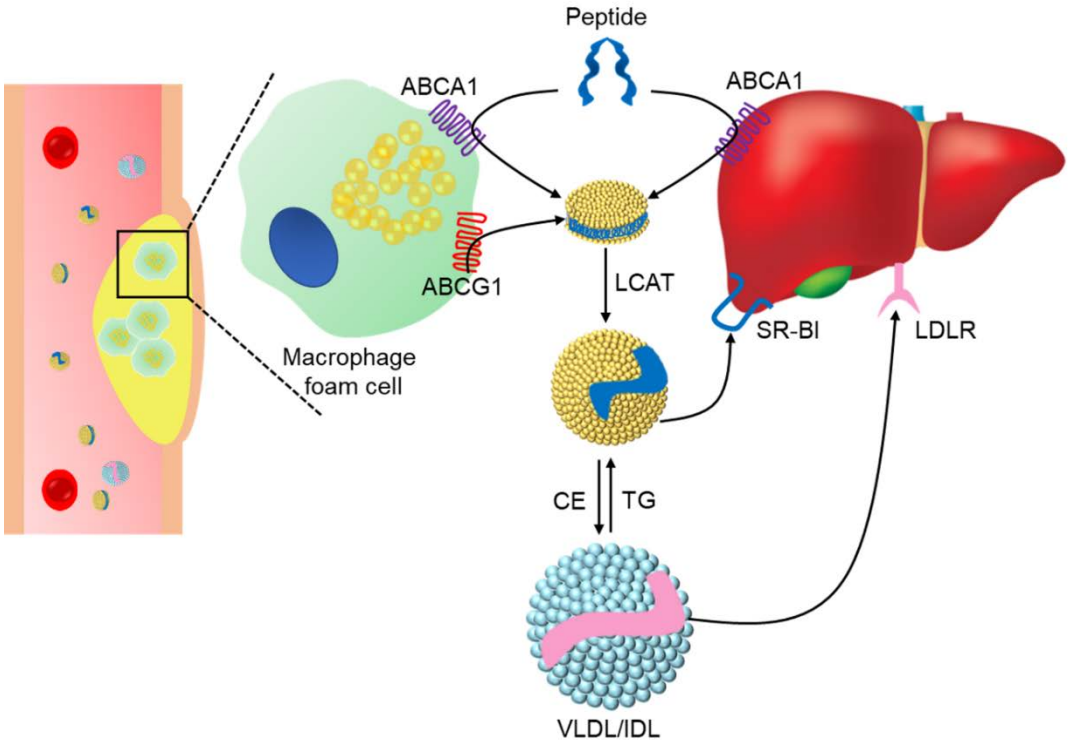


Figure 1. Current concepts in HDL maturation and its potential relationship to atherosclerosis.

In addition to maturation during the RCT process, HDL can also be remodeled and produced by other enzymes and proteins, such as hepatic lipase, phospholipid transfer protein, and endothelial lipase (Trajkovska et al., 2017). Hepatic lipase (HL) can hydrolyze phospholipids and triglycerides on HDL, converting lipid-rich spherical HDL (HDL_2) to smaller spherical HDL particles (HDL_3). It is also believed that HL plays a role in the catabolism of HDL, in which lipids become detached from mature HDL to gradually produce smaller particles until ApoA-I is either cleared by the kidney or returns back to begin the RCT pathway once again (Deeb et al., 2003). Phospholipid transfer protein (PLTP) can transfer

phospholipids between HDL and other lipoproteins and participate in HDL conversion, in which there are alterations to the size and composition of HDL. Two different proposed theories explain the method in which HDL conversion occurs when PLTP binds. The first proposed method is that multiple small lipid-poor ApoA-I/phospholipid complexes dissociate from mature HDL particles and form a new, HDL particle. The second proposition is the fusion of two HDL particles, creating an unstable intermediate, in which excess phospholipids would dissociate and fuse with ApoA-I to form a nascent HDL particle, while the rest of the HDL particle would stabilize, resulting in a new, mature HDL particle (Albers et al., 2012). Lastly, endothelial lipase (EL) can bind to HDL, its preferred substrate, and hydrolyze phosphatidylcholine lipids, which can then be used in the biosynthesis of lipids when taken up by cells (Yu et al., 2017). All these processes contribute to the existence of different forms of HDL particles *in vivo.*

3. Overview of ApoA-I-based HDL infusions in clinical trials

3.1 rHDL therapies tested in clinical trials

Several ApoA-I-based reconstituted HDL therapies have been developed for intravenous administration in humans, which include three major ApoA-I–based approaches (ETC-216/MDCO-216, CER-001, and CSL111/CSL112). A critical issue is that these three approaches differ considerably in composition (**Table 1**). Regarding the protein composition, ETC-216/MDCO-216 contains recombinant ApoA-I Milano, CER-001 contains recombinant wild-type human ApoA-I, and CSL111/CSL112 contains native ApoA-I isolated from human plasma. The differences in phospholipids used to reconstitute ApoA-I are also significant among these rHDL products (**Table 1**). The type and amount of ApoA-I and phospholipids have been demonstrated to influence the ability of HDL to enhance cellular cholesterol efflux. Thus, differences in composition may considerably impact the relative functionality and efficacy of these products in humans (Karalis & Jukema, 2018, Rader, 2018, Singh et al., 2007).

Apolipoprotein Milano (ETC-216/MDCO-216), developed by US biopharmaceutical company Esperion Therapeutics, is a recombinant ApoA-I Milano/phospholipid complex synthetic variant of HDL. In 2003, its phase II clinical trial was carried out in 57 patients with Acute Coronary Syndrome (ACS). Patients were randomized to five-weekly infusions of either ETC-216 or placebo (Saline). Intravascular ultrasound (IVUS) was performed to measure the changes in the percentage of atheroma volume within two weeks of patients experiencing ACS (baseline) and after each weekly infusion (Nissen et al., 2003). Results showed that five weeks of treatment with ETC-216 decreased the atheroma volume by a significant 4.2% ($p<0.01$). Subsequently, MDCO-216, a refined and purified form of ETC-216, was developed with the ability to promote cholesterol efflux capacity without any adverse effects on immune function (Nissen et al., 2003). However, the phase III trial (MILANO-PILOT) completed in 2018 demonstrated that although MDCO-216 infusion increased cholesterol efflux, it did not yield an incremental benefit to the patients with coronary disease. (ClinTrial.gov identifier NCT02678923) (Nicholls et al., 2018).

CER-001, bioengineered by US biopharmaceutical company Cerenis Therapeutics, is a bio-engineered complex of recombinant wild-type human ApoA-I and sphingomyelin (Zheng et al., 2016). In 2014, a phase II trial (CHI-SQUARE trial) was conducted where 504 patients with ACS were randomized to receive 6 weekly infusions of either CER-001 (3, 6, or 12 mg/kg) or placebo (ClinTrial.gov identifier NCT01201837). Although CER-001 failed to produce a significant reduction in coronary atherosclerosis as assessed by IVUS (Tardif et al., 2014), reanalysis in anatomically matched arterial segments revealed plaque regression achieved at the lowest dose arm (3 mg/kg), especially in patients with high plaque burden at baseline (Kootte et al., 2015). In 2018, a phase II CARAT trial was then conducted to evaluate the effect of CER-001 infusions (3 mg/kg) in patients with ACS and a high coronary plaque burden (ClinTrial.gov identifier NCT2484378). The CARAT trial showed no benefit after ten weekly infusions of CER-001 compared with placebo. In patients with high coronary plaque burden, no regression of atherosclerosis was noted (Nicholls et al., 2018, Zheng et al., 2020).

CSL111, developed by global biotherapeutics leader CSL Behring, is a reconstituted HDL-particle comprised of soybean phosphatidylcholine and human ApoA-1 (Shaw et al., 2008). In 2007, a phase II ERASE trial was conducted in which 183 patients were randomly assigned to 4 weekly infusions of placebo, 40 mg/kg of CSL-111 or 80 mg/kg of CSL-111 (Tardif et al., 2007). A high incidence of

transaminase elevations with the 80 mg/kg treatment led to prematurely discontinuation of this arm. There was a significant change from baseline in atheroma volume (−3.4%; P>0.001) in the 40 mg/kg group, but this difference did not reach statistical significance (P=0.48) compared with placebo (ClinTrial.gov identifier NCT00225719). **CSL-112,** a second-generation derived from CSL-111, was then developed to further increase the tolerant dose in humans and is still in clinical development today (Gille et al., 2018, Gibson et al., 2016, Tricoci et al., 2015). In a phase IIa clinical trial, no patients developed anti-CSL-112 or apoA-I antibodies, suggesting that CSL-112 does not cause an immunogenic response (Triococi et al., 2015). Furthermore, CSL-112 has also completed a large phase II safety study in 1200 patients receiving four weekly infusions of either 2 g or 6 g of ApoA-I protein reconstituted to form HDL. In this study, CSL-112 was found to be well tolerated in patients, lacking any signs of hepatic or renal toxicity or any other safety concerns (Gibson et al., 2016). A phase 3 AEGIS-II trial was initiated in 2017, in which more than 17,000 subjects are enrolled from approximately 1,000 sites around the world to evaluate whether CSL112 reduces cardiovascular events in high-risk patients (Gibson et al., 2021).

Table 1. Characteristics and clinical updates of apolipoprotein A-I-based rHDL infusion therapeutics

rHDL product	ApoA-I source	Phospholipid	Protein to phospholipid ratio	Clinical trials	Patients enrolled	Status	Change in percent atheroma volume (PAV, %) from baseline	Cholesterol efflux capacity (%)
ETC-216	Recombinant ApoA-I Milano	DPPC	1:2.7 weight ratio	Milano, Phase 2	57	Completed	-1.06% (treatment) versus 0.14% (placebo), p=0.02	NA
MDCO-216		POPC	1:1.1 weight ratio	MILANO-PILOT, Phase 3, NCT02678923	126	Completed	-0.21% (treatment) versus -0.94% (placebo), p=0.07	80.4% increase
CER-001	Recombinant wild type ApoA-I	SPM and DPPG at molar ratio of 32:1	1:2.7 weight ratio	CHI SQUARE, Phase 2, NCT01201837	507	Completed	-0.02% (treatment) versus 0.02% (placebo), p=0.86	NA
				CARAT, Phase 2, NCT2484378	293	Completed	-0.09% (treatment) versus -0.41% (placebo), p=0.15	44% increase
CSL111	Native ApoA-I isolated from human plasma	Mixed PC isolated from soy	1:150 molar ratio	ERASE Phase 2, NCT00225719	183	Completed	-3.4% (treatment) versus -1.6% (placebo), p=0.48	15% increase
CSL112			1:55 molar ratio	AEGIS-I, Phase 2, NCT02108262	1258	Completed	NA	300% increase
				AEGIS-II, Phase 3, NCT03473223	17400	Ongoing	NA	NA

Abbreviations: DPPC, Dipalmitoyl phosphatidylcholine; DPPG, 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; SPM, sphingomyelin; PC, Phosphatidylcholine; CHI-SQUARE, Can HDL Infusions Significantly Quicken Atherosclerosis Regression; CARAT, CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial; ERASE, Effect of rHDL on Atherosclerosis-Safety and Efficacy; AEGIS-I trial, Apo-I Event Reducing in Ischemic Syndromes I; AEGIS-II, ApoA-I Event reducing in Ischemic Syndromes II, NA, not applicable.

3.2 Challenges associated with ApoA-I based products

ApoA-I-based rHDL products present serious challenges such as expansive and complex manufacturing processes and the need to produce a high quantity of very pure protein due to the relatively high ApoA-I doses required to achieve therapeutic benefit. The current dose of ApoA-I in CSL-112, an ApoA-I-based rHDL product undergoing phase 3 clinical trial, is 6 g per infusion administered four times in weekly intervals. Thus, the course of therapy will require 24 g of ApoA-I protein, When considering the number of patient worldwide who currently suffer from a cardiovascular-related disease, the feasibility of acquiring this high amount of protein greatly diminishes. Treating just a million patients will require 24 tons of protein. For plasma purified ApoA-I, it will be difficult to secure sufficient volumes of human plasma. For ApoA-I produced by recombinant technologies, the cost of manufacturing and manufacturing process complexity remains high due to the hydrophobic nature of ApoA-I, relatively low expression levels and high endotoxin binding tendencies. Because of the levels of endotoxin, host cell protein and host cell DNA impurities are limited for each protein infusion by the WHO and FDA guidelines. Thus, large doses of ApoA-I necessitates the production of highly pure protein. The hydrophobic nature of ApoA-I leads to relatively low expression titers in recombinant processes and a need for an extensive purification process to remove impurities, resulting in an expansive protein product. Ultimately, rHDL will be challenging to produce in a scalable and economically feasible manner.

4. HDL-mimicking artificial nanotherapeutic development

4.1 ApoA-I mimetic peptide-based applications and modification research

4.1.1: ApoA-I mimetic peptides

Due to the challenges such as the cost and difficulty in purification posed by ApoA-I-based rHDL products, there has been considerable interest in designing shorter peptides that mimic the functionalities of full-length ApoA-I proteins (Ditiatkovski et al., 2017, Karalis & Jukema, 2018, Leman et al., 2014, Zhao et al., 2014). ApoA-I mimetic peptides do not share primary amino-acid homology but do possess the secondary helix-like structure of ApoA-I. ApoA-I mimetic peptides are therefore less costly to produce, which is beneficial for drug development. Over the past few decades, multiple ApoA-I mimetic peptides, either alone or complexed with phospholipids as therapeutic agents, have been designed and tested in human clinical trials, but none have been FDA-approved yet. In this review, we discuss three mimetic peptides, including 4F, Fx-5A and ETC-642, all of which have been widely tested and are the most clinically advanced. Their characteristics are summarized (**Table 2)**.

ETC-642 is an ApoA-1 mimetic peptide (ESP24218 or 22A) produced by Esperion complexed with two naturally occurring phospholipids, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and sphingomyelin (Di Bartolo et al., 2011). In 2002, 28 patients with stable atherosclerosis were given a single-dose infusion in a phase I clinical study of ETC-642 to test the safety and tolerability of the peptide. A 4-week observation period established that ETC-642 was safe and well-tolerated at 1 to 30 mg/kg doses (Kuai et al., 2016). Nonetheless, in 2006, Esperion was acquired by Pfizer, resulting in termination of the development of ETC-642 with unknown considerations. There are no planned or ongoing clinical studies in humans at this moment.

4F is an amphipathic peptide that has two enantiomers (D-4F and L-4F), which promote the remodeling of endogenous HDL. 4F induces the formation of pre-β HDL and exerts anti-inflammatory properties likely through the sequestration of oxidized lipids (Kruger et al., 2005, Ou et al., 2003). D-4F was found to be well tolerated in phase I clinical trials in 2008 (ClinTrial.gov identifier NCT00907998). L-4F was also tested in phase I clinical trials in 2010 and was found to be well tolerated when administering an infusion with the dose range of 3 to 100 mg for seven daily doses and 10 and 30 mg subcutaneous doses for 28 days (ClinTrial.gov identifier NCT00568594). However, there was no notable improvement of the HDL on inflammatory markers. Subsequent animal studies showed that the main anti-inflammatory action of both D-4F and L-4F peptides is induced by blocking the absorption and/or production of oxidized lipids in the gut (Watson et al., 2011).

Fx-5A is a peptide-phospholipid complex produced by assembling sphingomyelin with the 5A peptide, a bihelical ApoA-I mimetic peptide. The ability of this synthetic peptide-phospholipid complex to collect cholesterol effluxed by the ABCA1 transporter makes it very effective in enhancing the first step of the

RCT pathway (Tabet et al., 2010). Administration of Fx-5A for 13 weeks reduced the progression of atherosclerosis in apoE$^{-/-}$ mice compared with saline control. In addition, Fx-5A exerted anti-inflammatory and antioxidant properties in a rabbit model (Bourdi et al., 2018). Furthermore, Fx-5A was well tolerated at doses of 8, 25 and 75 mg/kg/day in cynomolgus monkeys. Therefore, Fx-5A was considered a strong candidate for further drug development. The administration of FX-5A is being tested in patients with CVD for five weekly infusions to examine the safety profile and pharmacokinetics (ClinTial.gov identifier NCT04216342)

Table 2. Characteristics and clinical update of those ApoA-I mimetic peptides

ApoA1-mimetic peptide	Primary sequence	Company	Administration	Formulation	Development phase	Outcomes
ETC-642	P-V-L-D-L-F-R-E-L-L-N-E-L-L-E-AL- K-Q-K-L-K	Esperion	Parenteral	Peptide/SPM/DPPC, 1/3.75/3.75 molar ratio	Phase 1. NCT not identified	Positive
4F	Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F- K-E-A-F-NH 2	Novartis	Oral/parenteral	A lyophilized powder in a sterile trehalose-phosphate buffer	Phase 1. NCT00907998 NCT00568594	Negative
Fx-5A	D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K- E-A-F-P-D-W-A-K-A-A-Y-D-K-A-AE-K-A-K-E-A-A	KineMed Inc.	Parenteral	5A/egg SPM; 1:8 molar ratio	Phase 1. NCT04216342	Ongoing

Abbreviations: DPPC, Dipalmitoyl phosphatidylcholine; SPM, sphingomyelin; PC, Phosphatidylcholine.

4.1.2 Differences in phospholipid composition

Despite significant emphasis on the impact of protein composition on rHDL products, i.e., native or recombinant ApoA-I, ApoA-I mutants, and mimetic peptides, the influences of HDL phospholipid composition on the final HDL products have not been systematically investigated. Studies have shown that the ability of rHDL to promote cholesterol efflux is influenced by the amount and type of phospholipid in the product, and therefore, the differences in phospholipid composition could have important implications on the relative functionality of these rHDL products. One study from our lab showed that the ApoA-I mimetic peptide 5A, when complexed with either SM or POPC, exhibited similar *in vitro* cholesterol efflux by ABCA1. Notably, there was a higher ABCG1- and SR-BI-mediated efflux observed with 5A-SM relative to 5A-POPC ($P<0.05$). When administrating Sprague-Dawley rats with 30 and 100 mg/kg doses of 5A-POPC-HDL or 5A-SM-HDL by intravenous infusion, statistically significant differences in the amount of mobilized free cholesterol (FC) were observed for 30 and 100 mg/kg doses of 5A-POPC and 5A-SM at almost all time points ($P < 0.05$, noted in **Figure 2A-2B**). The mobilized cholesterol in its entirety in the HDL fraction, as shown in **Figure 2C**, was analyzed using chromatographic separation of plasma lipoproteins 30 min post rHDL dosing. Injection of 5A-SM in animals resulted in a 3-fold higher mobilization of plasma cholesterol than did injection of 5A-POPC. While both rHDL exhibited anti-inflammatory properties, higher inhibition of TNF-α, IL-6, and IL-1 release was seen with 5A-SM infusion as compared to 5A-POPC ($P<0.05$). Both rHDL treatments (3/week for 6 weeks) showed a reduction in total plaque area in ApoE -/- mice, but only 5A-SM significantly reduced plaque area over placebo control and baseline ($P<0.01$). These results (shown in **Figure 2D-2E**) indicate that the type of phospholipid used to synthesize rHDL has a significant influence on rHDL's anti-inflammatory and anti-atherosclerosis properties (Schwendeman et al., 2015).

In order to systematically determine the effect of different phospholipid compositions of rHDL on a nanoparticle's overall pharmacokinetic and pharmacodynamic behavior *in vivo*, we constructed a set of rHDL by keeping the peptide component (22A) constant and varying phospholipids of different chain lengths and saturation, including 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), listed in increasing transition temperature order. Results suggested that rHDL constructed with variable phospholipid compositions had significant differences in the pharmacokinetics of phospholipid, with DSPC-rHDL demonstrating the longest half-life, 6.0 hours, relative to 1.0 hour for POPC-rHDL. Furthermore, there was a 6.5-fold increase between these two rHDL in the area under the curve for mobilized cholesterol. The data supported the idea that rHDL composed of lipids with higher transition temperatures, those with longer chain lengths and fewer degrees of saturation, showed a greater half-life and slower clearance due to their higher physical stability than the rHDL composed of phospholipids with lower transition temperatures. Increased mobilized cholesterol ability could attribute to longer circulation times of the rHDL containing lipids with higher transition temperatures. Therefore, consideration of the phospholipid component is imperative when designing rHDL for CVD treatment due to its crucial impact on cholesterol mobilization *in vivo* (Fawaz et al., 2020).

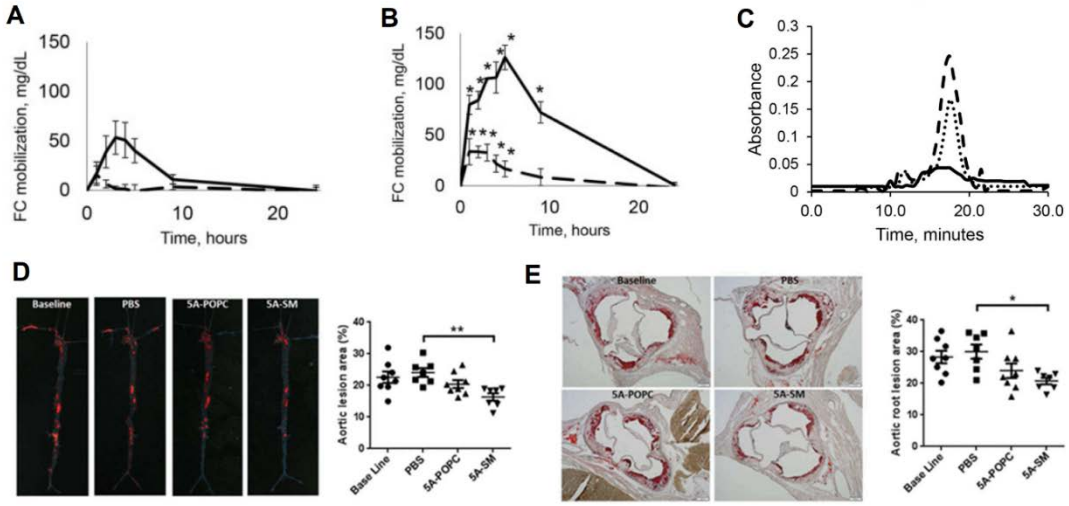


Figure 2. "Dose response of cholesterol mobilization following 5A-POPC and 5A-SM infusions at 30 mg/kg (dashed line) and 100 mg/kg (solid line) in normal rats. Unesterified cholesterol or FC mobilization by 5A-POPC (**A**) and 5A-SM (**B**). Infusion of 5A-POPC (dotted line) and 5A-SM (dashed line) leads to rapid cholesterol mobilization in the HDL subfraction 30 min post dose relative to baseline (solid line). Lipoproteins were separated by gel filtration chromatography and cholesterol levels were analyzed post fraction collection. Peaks at 12, 14, and 18 min represent VLDL, LDL, and HDL, respectively (**C**). Effect of 5A-POPC and 5A-SM rHDL on atherosclerosis regression in ApoE−/− mice. Aortas were dissected and plaque areas were visualized by Oil Red O staining. Representative lesion images and corresponding quantitative analyses of the aortas (**D**) and the aortic root cross-sections (**E**). N = 7-8 animals per group. (*) Denotes statistically significant differences with P values of at least <0.05; (**) indicates P values of <0.01". Reprinted with permission from (Schwendeman et al., 2015). Copyright 2015, Elsevier.

4.1.3 PEGylated HDL

Despite initial clinical successes, the reported half-life of endogenous HDL in humans is only 3.3 days (Jomard & Osto, 2020). Because of the rapid elimination of reconstituted or synthetic HDL (sHDL), following administration, the time in which sHDL reaches and remains above therapeutically efficacious plasma levels is quite limited. This rapid elimination of sHDL in circulation requires either frequent dosing, which highly compromises patient compliance, or high doses that may lead to off-target toxicity.

Pedersbæk et al. studied the effect of PEGylation of the lipids on the circulation of biomimetic HDL (b-HDL) by comparing apolipoprotein A-I (ApoA-I)-based b-HDL versus mono-PEGylated b-HDL (PEG b-HDL) both *in vitro* and *in vivo*. The results showed that PEGylation of the b-HDL scaffold only had minimal effect on the biological fate of the lipids (**Figure 3A**). Both b-HDL and PEG b-HDL had similar biological fates (Pedersbæk et al., 2021). In contrast, Murphy et al. demonstrated that reconstituted phospholipid/pegylated PEG-ApoA-I particles (PEG-rHDL) markedly increased plasma half-life and enhanced antiatherogenic properties *in vivo* compared with rHDL (Murphy et al., 2013).

Our lab modified the surface of sHDL with polyethylene glycol (PEG) to extend sHDL circulation *in vivo*. We incorporated various PEG modifying amounts (2.5, 5, and 10%) and different chain lengths (2 and 5 kDa) of PEG-modified lipids in sHDL's lipid membrane (Li et al., 2018). Incorporating PEG did not impact the ability of sHDL to promote cholesterol efflux, nor did it reduce cholesterol uptake of sHDL by the liver cells. By either incorporating more PEG or using PEG with longer chain lengths, the half-life of sHDL was significantly extended. The half-life of 5 and 10% sHDL- PEG 2k was 8.62 and 9.96 hours respectively, while 5% sHDL-PEG 5K was 12.5 hours for, notably longer compared to 4.59 hours for unmodified sHDL. The area under the curve for the phospholipid of sHDL was also increased ($p < 0.05$), but we did not observe an increase for the apolipoprotein A-I peptide component of sHDL. These observations suggests that sHDL is remodeled by endogenous lipoproteins *in vivo*. For the phospholipids of sHDL, the AUC and half-life grew with increasing percentages of PEG incorporated into the sHDL lipid membrane and increased with longer PEG chain lengths at the same surface density of the polymer. The extended phospholipid circulation led to a higher mobilization of free cholesterol in the plasma, a biomarker for enhancement of RCT (**Figure 3B-3C**). The area under the curve for cholesterol mobilization increased around 2-4-fold ($p < 0.05$), where longer PEG chains and higher molar percentages of incorporated PEGylated lipids achieved greater increases. Therefore, a longer circulation time and AUC for the phospholipids of PEGylated sHDL lead to a longer duration and greater ability of sHDL to mobilize cholesterol. (Li et al., 2018). Overall PEGylated sHDL displayed improvements in PK, increased cholesterol mobilization and longer pharmacodynamic effects relative to non-PEGylated sHDL.

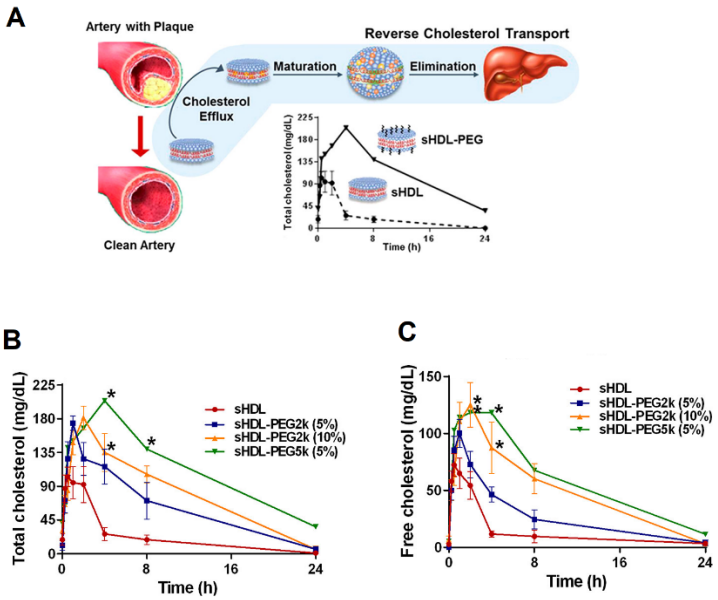


Figure 3. "PEGylation of sHDL led to beneficial changes (increase in mobilized cholesterol) in sHDL particle pharmacokinetic and pharmacodynamic behaviors (**A**). Pharmacodynamic assessment after IV administration of sHDL particles modified with different amount of PEGylated lipids. The level of total cholesterol (**B**) and free cholesterol (**C**) in rat serum were determined by commercially available kits. Data are shown as statistically significant differences of TC and FC changes for each group compared with sHDL group with $p < 0.0001$". Reprinted with permission from (Li et al., 2018). Copyright 2018, American Chemical Society.

4.2 PLGA-based HDL-mimicking nanoparticles

Polymer-based nanoparticles, such as nanoparticles composed of poly (lactic-co-glycolic acid) (PLGA), have been found to be advantageous in therapeutic applications due to their biodegradability, long circulation half-life, and ability to modify the surface for targeted activity. These properties are favorable as a decrease in the rapid clearance from the site of action increases the ability to achieve a rise in localized therapeutic concentration at the target site, and this, in turn, reduces the toxic side effects of the therapeutic agent. In recent years, polymer-based nanoparticles have become of high interest for drug delivery purposes and have been ultilized as imaging and diagnostics tools in the cardiovascular space (Kuai et al., 2016, Sanchez-Gaytan et al., 2015). Here, we will discuss the use of PLGA nanoparticles and cyclodextrin as targeted therapies and imaging tools for atherosclerosis and CVD.

Poly (lactic-co-glycolic acid) (PLGA) is a polymer commonly used in nanoparticles to achieve an extended-release of a drug over time, due to the slow degradation of PLGA into glycolic and lactic acid. PLGA can be incorporated into HDL's hydrophobic core, allowing for the encapsulation and slow release of drugs. For example, Sanchez-Gaytan et.al. formulated PLGA-HDL that was shown to mimic HDL activity, including uptake by macrophages and cholesterol efflux capabilities *in vitro*, and colocalization with macrophages in atherosclerotic plaque in the aorta of ApoE -/- mice (Sanchez-Gaytan et al., 2015). PLGA-HDL contains a hydrophobic PLGA core coated with phospholipids and ApoA-I protein at a lipid to protein ratio similar to endogenous HDL. The particle size ranges from 60-120 nm, determined by the flow rate of the microfluidics technology during production (**Figure 4A**). In the drug release experiment using Nile Red hydrophobic dye as a model drug, it was found that within 24 hours, 60% of the dye had been released, and within 5 days, 90% of the dye had been released, confirming the slow-release capabilities of the nanoparticle. Moreover, cholesterol was effluxed to a similar extent to native HDL at concentrations of 20 and 50 ug/mL PLGA-HDL in human macrophage-like THP-1 cells (**Figure 4B-4C**). DiR dye was used to label PLGA-HDL and the particle was administered via tail vein injection to ApoE -/- mice at a concentration of 10 mg/mL. PLGA-HDL was shown to colocalize with macrophages in the aortas of the ApoE -/- mice, showing preferential targeting of the atherosclerotic plaque. All-together, the slow-release profile, cholesterol efflux, and ability of the

particle to localize in atherosclerotic plaque areas shows promising potential for PLGA-HDL to be an effective therapeutic for atherosclerosis (**Figure 4D-4E**).

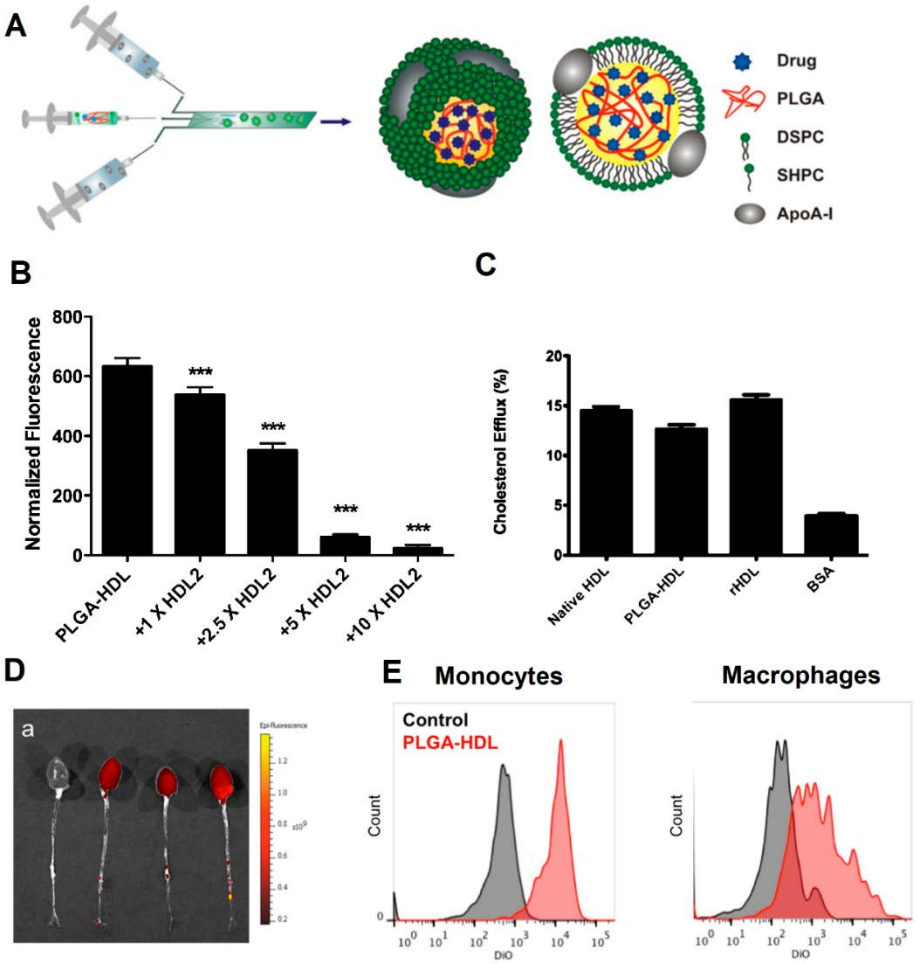


Figure 4. (**A**) "Schematic depiction of the synthesis of PLGA–HDL by microfluidic technology. PLGA–HDL nanoparticles target atherosclerotic plaques. (**B**) The preferential interaction of PLGA–HDL with macrophages was confirmed with flow cytometry analysis. Mean fluorescence of macrophages, pancreatic endothelial cells, smooth muscle cells, and hepatocytes incubated with PEG–PLGA NP (white) and PLGA–HDL (black). (**C**) Cholesterol efflux assay of native-HDL, PLGA–HDL, and microfluidic-synthesized HDL on human macrophage-like THP-1 cells at 50 μg/mL. (**D**) Fluorescence imaging of excised aortas of ApoE-KO mice injected with placebo or PLGA–HDL nanoparticles. (**E**) Fluorescent activated cell sorting of digested aortas injected with PLGA–HDL; the label DiR is mainly associated with monocytes and macrophages in the aorta". Figures combined and reprinted with permission from (Sanchez-Gaytan et al., 2015). Copyright 2015, American Chemical Society.

Macrophage apoptosis is a major contributor to atherosclerotic plaque instability. These unstable, or vulnerable plaques can then rupture, leading to thrombosis. In order to detect macrophage apoptosis and identify vulnerable plaque early on, Marrache et.al. synthesized TPP-HDL-ApoA-I-QD NPs, composed of a PLGA and cholesteryl oleate biodegradable core lined with quantum dots, decorated with a phospholipid bilayer coat containing ApoA-I memetic peptide, 4F, and triphenylphosphine (TPP) cations, which are used to detect mitochondrial membrane potential collapse, the initiating phase of macrophage apoptosis (Marrache & Dhar, 2013). The average particle size was 123 nm, roughly ten times the size of HDL. In RAW macrophages, confocal microscopy showed that TPP-HDL-ApoA-I-QD NPs accumulated in healthy cells but were undetected in apoptotic cells due to the lack of electrochemical proton gradient across the membrane present in healthy cells. Using flow cytometry, they also showed that TPP-HDL-ApoA-I-QD NPs could differentiate between apoptotic and healthy cells. TPP-HDL-ApoA-I-QD NPs also had cholesterol-binding properties *in vitro*, and the particle was able to reduce total cholesterol levels by 30% and triglyceride levels by 24% *in vivo*. All in all, these activities

indicate the therapeutic and imaging capabilities of TPP-HDL-ApoA-I-QD NPs on vulnerable plaque in atherosclerosis.

Early detection of vulnerable plaque could enhance the outcomes for atherosclerosis patients by adjusting the therapeutic approach and acquiring the right treatment plan for the patient. In a more recent study, Banik et. al. optimized TPP-HDL-ApoA-I-QD NPs by modifying the cholesteryl oleate and PLGA composition to target of both intracellular cholesterol metabolism pathways and extracellular cholesterol removal (Banik et al., 2017). The resulting nanoparticle was optimized based on size and cholesterol binding kinetics. It contains 40% cholesteryl oleate and is named T-CO_{40}-HDP-NP. DSPE-PEG serves as the phospholipid component while L-4F serves as the ApoA-I mimetic protein in T-CO_{40}-HDP-NP, which differs from TPP-HDL-ApoA-I-QD NP. T-CO_{40}-HDP-NP was able to localize in the mitochondria of macrophages and remove cholesterol from RAW 267.4 macrophage cells and smooth muscle cells in a cholesterol efflux assay. Furthermore, T-CO_{40}-HDP-NP was reported to accumulate primarily in the aorta and liver tissues and significantly reduced triglycerides and cholesterol levels in Balb/c albino mice. After a twice-a-week treatment of 10 mg/kg T-CO_{40}-HDP-NP for seven weeks, ApoE -/- mice showed a reduction in triglycerides, total cholesterol and LDL compared to controls, little foam cell presence along the endothelium, and decreased TNF-α cytokine levels. Due to its mitochondrial targeting and HDL mimetic activities, T-CO_{40}-HDP-NP help maintain cholesterol balance intracellularly and extracellularly, having potential as a therapeutic to reduce atherosclerotic plaque formation.

4.3 Cyclodextrin-based HDL-mimicking nanoparticles

Like HDL, cyclodextrins (CD) have a hydrophilic outer surface and a hydrophobic central core compartment. This cyclic oligosaccharide has been mainly used to increase the aqueous solubility and increase the bioavailability and stability of drugs. It has also been found to be useful as a “plain” or “blank” therapeutic entity. Most importantly, cyclodextrin has been used in Niemann-Pick Type C (NPC) disease to remove cholesterol and decrease cholesterol accumulation, in hopes to restore the cholesterol homeostasis in the late endosomes/lysosomes and reduce the onset of neurodegeneration and the pathogenesis of NPC (Rosenbaum et al., 2010, Vite et al., 2015). Studies have also shown that cyclodextrins can be beneficial for atherosclerosis. For example, in a study by Zimmer et al. cyclic oligosaccharide 2- hydroxypropyl-β-cyclodextrin (CD) was found to be able to balance cholesterol and immune responses in the vasculature by promoting cholesterol solubility and enhancing LXR activity, leading to an increase in cholesterol efflux (Zimmer et al., 2016). ApoE -/- mice fed a high cholesterol diet for eight weeks and treated with 2 g/kg CD twice a week had a decrease in atherosclerotic lesions in the aortic root, reduction in cholesterol crystal (CC) in plaque areas, and a decrease in proinflammatory cytokine levels. It was also shown that CD was able to reduce established plaque in ApoE -/- mice, decreasing atherosclerotic plaque area by 45% after twice per week injections for four weeks. CD also increased macrophage cholesterol efflux capacity and increased expression of ABCA1 and ABCG1 (LXR target genes) in macrophages loaded with CC and lowered IL-6, TNF-a, and IL-1β cytokines that modulate LXR. Downregulation of NLRP3 inflammasome genes was also reported *ex vivo* in human atherosclerotic plaques. Additionally, CD facilitated RCT in bone marrow-derived macrophages from wild-type mice. Overall, CD appears to mimic HDL’s anti-atherosclerotic properties which include removal of excess cholesterol and decrease in proinflammatory cytokine expression, while also modulating LXR activities. The enhancement of LXR leads to an increase in transcription of key cholesterol shuffling vehicles, and in turn, an increase in cholesterol efflux, thus making it beneficial for atherosclerosis treatment.

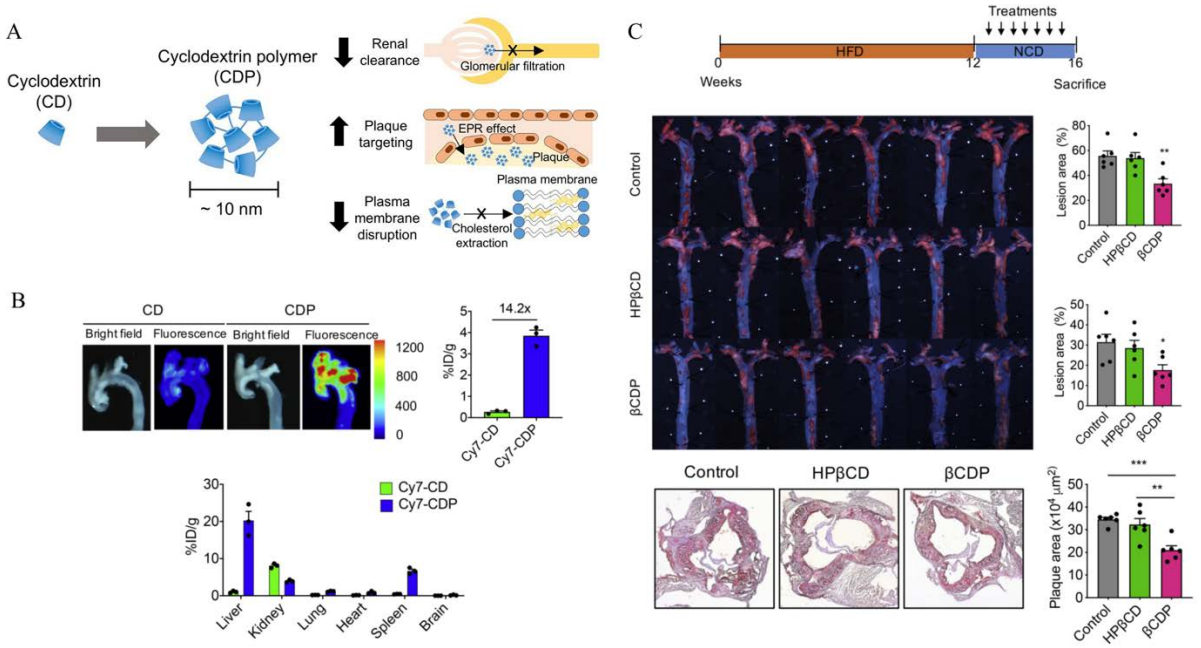


Figure 5. (**A**) "Schematic presentation of cyclodextrin polymer for effective and safe treatment of atherosclerosis. (**B**) Preferential accumulation of CDP in atherosclerotic plaques. Representative *ex vivo* bright-field and fluorescence images of the dissected aorta. Quantification of fluorescence in the dissected aorta and major organs as measured by NIR fluorescence imaging system. (**C**) βCDP showed an improved anti-atherosclerotic efficacy of compared to HPβCD in mice". Reprinted with permission from (Kim et al., 2020). Copyright 2020, Elsevier.

α-CD is another cyclodextrin that has been studied in the cardiovascular space. α-CD is a soluble cyclic fiber that contains six glucose molecules that form a hydrophobic cavity. This cavity has a high affinity for lipids. The original rationale for using α-CD as a therapeutic for cardiovascular disease is that it would interfere with cholesterol or triglyceride absorption, mimicking the activity of other soluble fibers. In fact, it has been shown that 1 g of α-CD has the capacity to bind as much as 9 g of dietary fat. α-CD is also generally recognized as safe (GRAS) by the FDA, making it a great therapeutic candidate. In a previous study, LDLR -/- mice fed a western diet for 14 weeks that were treated with 2.1% α-CD had a 15.3% decrease in plasma cholesterol as compared to the control. This led to the double-blind clinical study trial to study the effects of α-CD on serum lipids. In this clinical trial study, Amar et al. treated 75 healthy patients for 12-14 weeks with either placebo or α-CD (6 grams oral a day; two 1 g tablets per meal) with a one-week washout period between arms (Amar et al., 2016). There were no significant differences in total cholesterol or other lipid or lipoprotein levels between placebo or α-CD treated patients, which diverges from expectations. Although no changes were observed in LDL particle number, a 10% reduction in small LDL particle number was seen in patients treated with α-CD as compared to placebo. Small LDL has been reported to be more proatherogenic than large LDL particles because smaller LDL is able to pass through the vessel wall and is more likely to become oxidized and contribute to foam cell and atherosclerotic plaque formation. The ability of α-CD to decrease small LDL particle numbers and its safety and tolerability opens up opportunities for additional studies and modifications to better target atherosclerosis pathologies. Kim et al. showed that at equivalent doses, cyclodextrin polymer (CDP) significantly inhibited plaque growth and displayed outstanding plaque targeting efficacy (**Figure 5A-5C**) compared to monomeric hydroxypropyl-beta-cyclodextrin (HPβCD in a mouse model of atherosclerosis (Kim et al., 2020).

4.4 Gold/metal-based HDL-mimicking nanoparticles

Metal-based nanoparticles act as drug delivery carriers, diagnostic tools, and radiation-based anticancer therapies (Ceresa et al., 2014, Klębowski et al., 2018). They became of interest in the medical and therapeutic space due to their high biocompatibility, stability, and tailorability. These properties make metal-based nanoparticles ideal for mimicking endogenous HDL and allows for the possibility of achieving the 5-17 nm in diameter in size and discoidal or spherical shape of the particle.

In the cardiovascular space, metal-based nanoparticles, especially gold nanoparticles (Au-NP), have been utilized as both therapy-based and imaging tools in RCT pathways and analysis of atherosclerotic plaque.

Au-NPs can be used to enhance RCT activity, leading to the reduction of atherosclerotic plaque formation. For example, Thaxton et. al. formulated an HDL mimetic gold-based nanoparticle (HDL-Au NP) that can bind cholesterol in an *in vitro* assay, titrating NBD-cholesterol in a 5 nM HDL-Au NP solution to create a binding isotherm from the fluorescent signal (Thaxton et al., 2009). HDL-Au NP consists of a 5 nm gold core scaffold, surrounded by 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (PDP-PE), and amine- functionalized lipid, 1-2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) phospholipids, and ApoA-I protein (**Figure 6A**). The resulting HDL-Au NP was determined to be 17.9 nm in size. The average number of proteins and phospholipids per particle, 3 and 83 respectively, corresponded well to amounts found in endogenous HDL. The Kd of binding was determined to be 3.8 nM. The ability of HDL-Au NP to bind cholesterol shows early potential of this nanoparticle to be of use in enhancing RCT and improving atherosclerosis outcomes.

Moreover, in an experiment designed to examine how different lipids and addition of ApoA-I affects the self-assembly interactions of Au with these components, Lai et.al. were able to show that their Au-NP was able to activate LCAT and form CE. This Au-NP consists of a gold core functionalized with DPPC/MPDP Pe/2-MPT at a 175:175:175 ratio and 3 ApoA-I proteins. It was found that CE distributed near the core of the Au-NP, mimicking endogenous HDL (Lai et al., 2017). This is important because the packaging of CE near the core maintains a concentration gradient that allows for more cholesterol to bind at the nanoparticle's surface during reverse cholesterol transport (**Figure 6B**). They confirmed that ApoA-I incorporation in the Au-NP was necessary to activate LCAT and form CE, highlighting the importance of HDL's primary protein in the functional activity of gold nanoparticles.

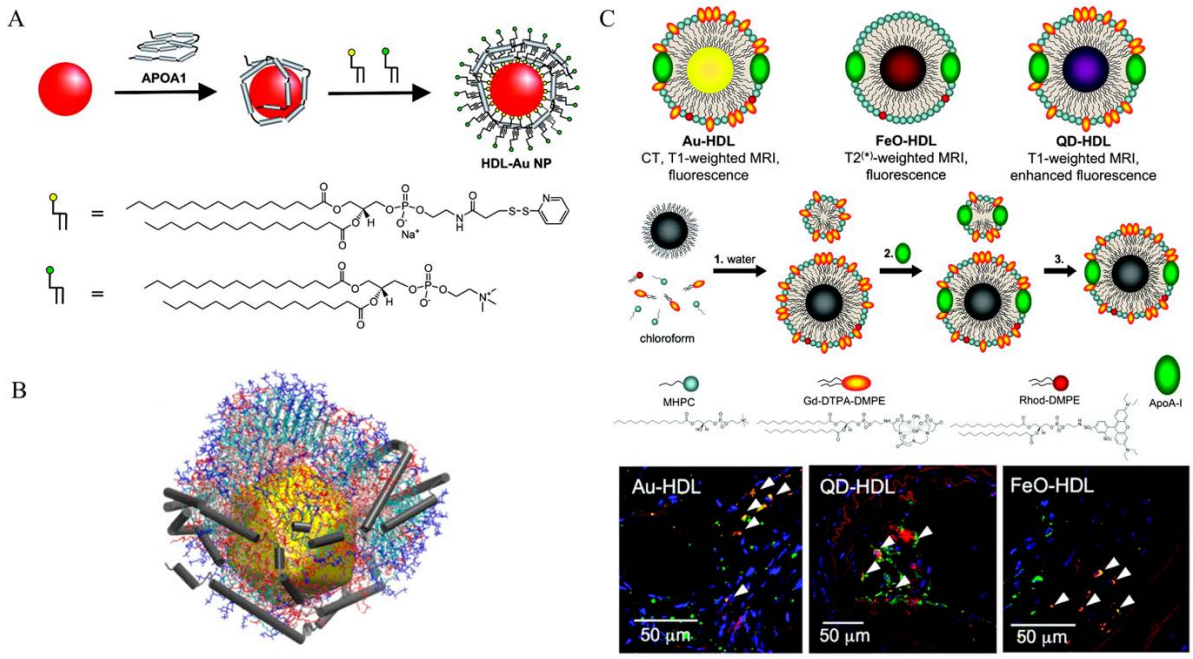


Figure 6. (**A**) "Synthesis of biomimetic HDL using a Au NP core for use as a therapeutic. Au NPs of 5 nm in diameter were surface-functionalized with ApoA-I and then with two phospholipids, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[3-(2-pyridyldithio) propionate] (yellow) and 1-2-dipalmitoyl-sn-glycero-3-phosphocholine (green)". Reprinted with permission from (Thaxton et al., 2009) Copyright 2009, American Chemical Society. (**B**) "Self-assembly of a mixture of DPPC/MPDP PE/2-MPT (175:175:175) on three apoA-I loaded AuNPs". Reprinted with permission from (Lai et al., 2017). Copyright 2017, American Chemical Society. (**C**) "Representative synthesis of the three nanocrystal HDLs for imaging atherosclerotic plaque and their imaging of atherosclerosis. Confocal microscopy images of aortic sections of mice injected with nanocrystal HDL. Red is nanocrystal HDL, macrophages are green, and nuclei are blue. Yellow indicates colocalization of nanocrystal HDL with macrophages and is indicated by arrowheads". Reprinted from (Cormode et al., 2008). Copyright 2008, American Chemical Society.

Metal-based nanoparticles have various uses that span beyond the enhancement of HDL's RCT capabilities, such as acting as contrast agents for medical imaging and targeting atherosclerotic plaque areas, providing insight on atherosclerosis progression and vulnerability. In a series of imaging experiments, Cormode et al. were able to show the utility of gold particles (Au), iron oxides (FeO) and quantum dots (QD) in molecular imaging (Cormode et al., 2008). These inorganic nanocrystals served as the hydrophobic core of HDL in the nanocrystal-core HDL particles. Along with ApoA-I, phospholipids including fluorescent and paramagnetic lipids, were incorporated onto the hydrophobic core to create an HDL mimetic nanoparticle. The size of Au-HDL, FeO-HDL, and QD-HDL was 9.7 nm, 11.9 nm and 12.0 nm respectively. In order to induce atherosclerotic plaque formation, ApoE -/- mice were fed a high fat-diet for ten weeks (**Figure 6C**). Following nanocrystal-core HDL particle administration, MRI imaging of the abdominal aorta detected particles in the aortic wall, and confocal microscopy showed the particles colocalized with macrophages. In a CT scan, Au-HDL showed a lower signal-to-noise ratio in the images of the aortas of mice when compared to saline and Au-PEG, which was used as a non-specific particle lacking ApoA-I. Moreover, the image showed two hotspots where Au-HDL accumulated, both areas of plaque with high macrophage content. This shows potential for Au-NP to be useful for CT imaging to provide insight on plaque with high macrophage content.

In a separate experimental study, Cormode et al. used the Au-HDL particle in combination with multicolor CT to identify macrophage burden, calcification and stenosis of atherosclerotic plaques from a single scan (Cormode et al., 2010). They discovered that distinction of Au-HDL, gold-based contrast agent, iodinated contrast agent, and calcium-rich matter was achievable using spectral CT and concentrations of these agents could be determined with high accuracy. As previously shown, TEM and confocal microscopy confirmed the ability of Au-HDL to accumulate in the aortas of ApoE -/- mice, primarily in the macrophage-rich areas. Due to the ability of spectral CT to distinguish between the Au-HDL in the macrophages, iodinated contrast agents, and calcified structures, this technique could allow for a detailed analysis of atherosclerotic plaque composition, stenosis of the artery, calcification, and inflammation. This information help diagnose and monitor atherosclerosis disease progression, leading to improved therapeutic outcomes for patients.

4.5 Micellar-based HDL-mimicking nanoparticles

Micelles are nanoparticles consisting of lipids or other amphiphilic molecules, such as polymers (Cabral et al., 2011, Lu et al., 2018). These nanoparticles can self-assemble in an aqueous solution. Like HDL, the lipids or amphiphilic molecules of micelles form a hydrophilic surface with a hydrophobic core but lack the presence of ApoA-I protein. Micelles also exist in a monolayer, which differs from HDL's lipid bilayer. The resulting particles usually range in size from 10-100 nm in diameter. Micelles have been shown to be very versatile, combining with other types of material such as gold, iron oxide etc. to create therapeutic entities. Here, we will discuss the use of micelles as therapeutics and detection tools for cardiovascular disease.

Clotting can occur in atherosclerotic plaques, where fibrinogen deposits in and on the surface of the plaque. Due to this fact, the fibrin deposited on plaques has the potential to serve as a target for therapeutics aiming to modulate or image plaque. To test this hypothesis, Peters et. al. created a fluorescent micelle containing a clot-binding peptide cysteine-arginine-glutamic acid-lysine-alanine (CREKA) that was able to target clotted plasma proteins of atherosclerotic plaque, showing ability of micelles to identity plaque prone to rupture, leading to thrombosis (Peters et al., 2009). These CREKA micelles were composed of a DSPE tail, PEG2000 spacer, and the CREKA peptide and infrared fluorophore forming the polar head (**Figure 7A**). The resulting particle had an average size of 17 nm in diameter and a half-life of 130 minutes. Fluorescein-labeled CREKA micelles were able to accumulate in the aorta of $ApoE^{-/-}$ mice. The highest fluorescence intensity was detected in regions more susceptible to atherosclerotic plaque formation, such as the lower aortic arch. CREKA micelles were also able to accumulate where plaques are most prone to rupture, which is in the shoulder regions of plaque. Moreover, no CREKA micelles were found to accumulate in the aortas of wildtype mice, further confirming the ability of CREKA micelles to specifically target atherosclerotic plaque. Finally, CREKA micelles were able to encapsulate hirulog, an anticoagulant, and deliver this drug to plaque areas in the aorta, increasing the antithrombin activity as compare to control. All in all, CREKA micelle's ability to

accumulate in diseased and rupture-prone plaque areas of the aorta shows potential utility as a “plain” or drug-carrying entity to tackle atherosclerotic plaque.

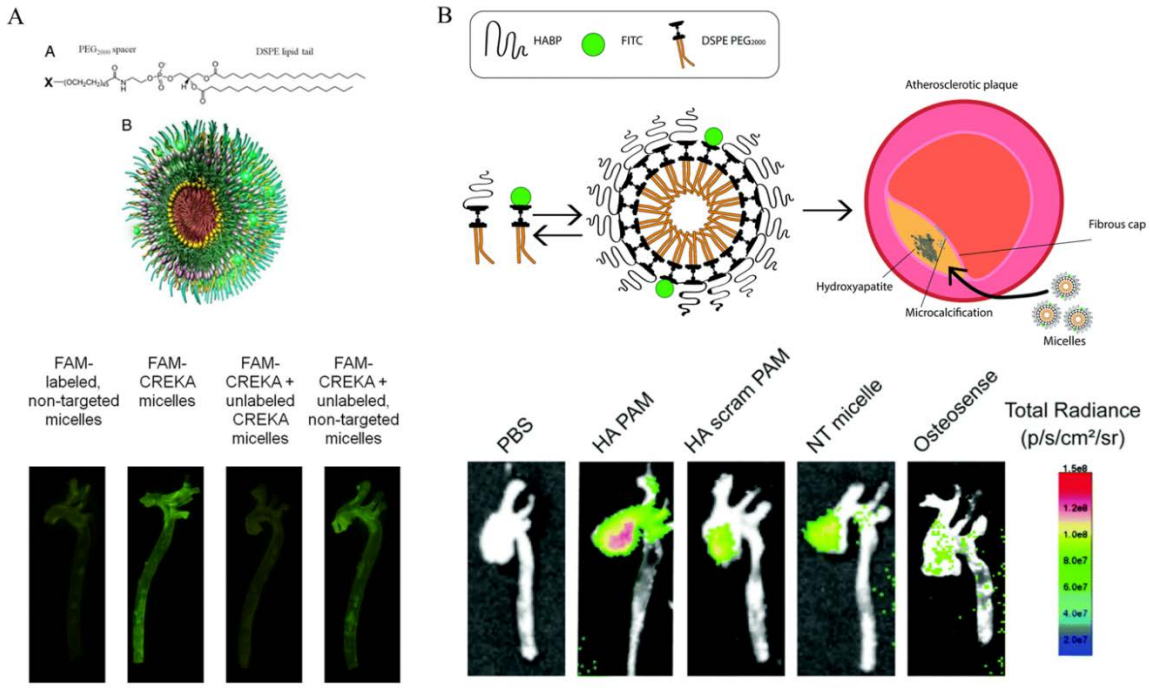


Figure 7. (**A**) “Construction of modular multifunctional CREKA micelles, made up of a DSPE tail, a poly (ethylene glycol) (PEG2000) spacer, and a variable polar head group (X) of CREKA. CREKA micelles can specifically target the aortic tree of atherosclerotic mice. Micelles were injected intravenously and allowed to circulate for 3 h. The aortic tree was excised after perfusion and imaged *ex vivo”*. Figures combined and reproduced with permission from (Peters et al., 2009) Copyright 2009, National Academy of Sciences. (**B**) “Schematic of HA PAM synthesis and targeting calcification found in atherosclerotic plaque”. Figures combined and reproduced with permission from (Chin et al., 2019). Copyright 2019, Royal Society of Chemistry.

It has been reported that patients with atherosclerosis who have calcification in their vasculature have a higher risk of developing thrombosis, myocardial infarction, and cardiovascular morbidity. This is due to the fact that vascular smooth muscle cells initiate calcification and the release of hydroxyapatite (HA), where HA can then aggregate on the outer fibrous caps of plaque, causing the plaque to become unstable and prone to rupture. Chin et. al. created a peptide-based micelle named HA PAM that targets HA, showing areas of atherosclerotic plaque vulnerability (Chin et al., 2019). HA PAM contains the HA binding peptide (HABP) used for targeting HA microcrystals, mixed with DSPE-PEG 2000 linked to fluorescent markers FITC or Cy7, and is about 8 nm in diameter in size. 83% of HA PAM bound to HA microcrystals at a concentration of 10 μM HA PAM, and the Kd was determined to be 6.26 μM. Additionally, HA PAM detected regions of calcification in plaque in the aorta of ApoE$^{-/-}$ mice, showing an increase in signal intensity in the brachiocephalic artery, a site prone to calcification. Furthermore, HA PAM bound to calcifications as small as 50 μm in calcified human tibial arteries (**Figure 7B**). In summary, HA PAM was able to identify calcified areas of atherosclerotic plaque, highlighting its capability to be used as an imaging tool, and potential as a theranostic tool with the incorporation of calcium mitigating therapeutics.

4.6 Lipid-conjugated core scaffold-based HDL-mimicking nanoparticles

Lipid-conjugated core scaffold used in HDL mimetic particle synthesis allows for the formation of a soft core that is able to pick up and drop off cholesterol and cholesteryl esters as it circulates. This is an important feature of HDL when facilitating RCT. Henrich et. al. formulated spherical HDL-like nanoparticles, named lipid conjugate HDL-like nanoparticles (LC HDL NPs), using different organic scaffolds to create his soft core, which were able to mimic the structure, RCT capabilities, and anti-inflammatory activities of mature, spherical HDL (Henrich et al., 2019). LC HDL NPs were formulated with ApoA-I, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) lipid, and one of the three different

organic scaffolds composed of a hydrophobic small molecule-phospholipid conjugate (PL_4) or amphiphilic DNA-linked PL_4 core. The resulting PL_4 HDL NP and 9-DNA-PL_4 HDL NP (DNA-PL_4 HDL NP) were spherical in morphology and found to be 13.8 ± 3.9 and 13.3 ± 4.6 nm in diameter in size, respectively. 18-DNA-PL_4 HDL NP was unable to conjugate properly and therefore was excluded from the rest of the study. LC HDL NPs were found to have similar secondary structures to endogenous human HDLs and a composition that closely resembles that of mature HDL, excluding cholesterol and cholesteryl esters components. PL_4 HDL NP and DNA-PL_4 HDL NP were able to facilitate cholesterol efflux (6.2 and 6.5%, respectively) at 100 nM protein, deliver cholesterol to hepatocytes within 30 minutes and allow LCAT to esterify both free and bound cholesterol. Lastly, at a concentration of 150 nM, both PL_4 HDL NP and DNA-PL_4 HDL NP dose-dependently reduced NF-κB activation by 31% and 16% respectively. Overall, the LC HDL NPs size, shape and compositional similarities to endogenous mature HDL, as well as its ability to uptake and transport cholesterol and reduce inflammatory activity promoted by NF-κB, makes it a plausible contender for future generations of HDL-mimetic therapeutics.

Conclusion

Overwhelming epidemiological evidence suggests that high levels of LDL-C and low levels of HDL-C correlate with increased CVD risk. However, therapeutic interventions that involved infusions of rHDL particles resulted in mixed outcomes. Some smaller Phase II studies involving imaging modalities indicated the reduction of atheroma burden in patients. However larger Phase 2 trials failed to show atheroma reduction and reduction of subsequent cardiovascular events. Some clinical failures were attributed to either poor clinical study design and execution or to the rHDL composition and low doses selected for clinical evaluation. However, these failures also resulted in the lipoprotein research community questioning potential benefits of rHDL infusions. While phase 2 failures resulted in the termination of clinical development of CER-001 and MEDCO-216, CSL-112 proceeded to a large Phase 3 clinical trial to show the reduction of CVD events in a large patient population. Thus, the jury is still out to determine if rHDL infusions will result in improved patient survival and the results of the CSL-112 AEGIS-II study will likely define the future of the entire field.

Additionally, the bench-to-bedside transition of synthetic HDL has been challenging due to the technical difficulties of purifying ApoA-I from human plasma and producing recombinant ApoA-I, and rapid elimination of rHDL *in vivo*. While the products furthest in clinical development closely resemble endogenous HDL composition with ApoA-I protein and phospholipids, other HDL-mimicking nanoparticles containing ApoA-I peptides or polymer-based biomaterials could offer advantages in costs and manufacturing scale-up. The potential success of a large Phase 3 clinical study for rHDL will likely renew research interest in the area of HDL biomimetics. Lipid nanoparticles have long been suggested as potential antiatherosclerosis agents for their capacity to facilitate reverse cholesterol transport in a similar way to native HDL. It was also reported that lipid nanoparticles could enhance cholesterol efflux through direct interactions with the cell membrane and facilitate cholesterol mobilization through their interactions with native lipoproteins, leading to plaque regression in atherosclerotic animal models. Thus, future focus shall be placed on those areas to better establish the overall functionality as well as safety of these HDL mimicking nanoparticles.

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References

Abdullah SM, Defina LF, Leonard D, Barlow CE, Radford NB, Willis BL, Rohatgi A, McGuire DK, de Lemos JA, Grundy SM, et al. Long-Term Association of Low-Density Lipoprotein Cholesterol With Cardiovascular Mortality in Individuals at Low 10-Year Risk of Atherosclerotic Cardiovascular Disease. *Circulation* 2018, 138:2315-2325.

Albers JJ, Vuletic S, Cheung MC. Role of plasma phospholipid transfer protein in lipid and lipoprotein metabolsim. *Biochim Biophys Acta* 2012, 1821(3): 345–357.

Amar MJA, Kaler M, Courville AB, Sampson M, Remaley A. Randomized double blind clinical trial on the effect of oral αlpha-cyclodextrin on serum lipids. *Lipids Health Dis* 2016, 15:115.

Andrews J, Janssan A, Nguyen T, Pisaniello AD, Scherer DJ, Kastelein JJ, Merkely B, Nissen SE, Ray K, Schwartz GG, et al. Effect of serial infusions of reconstituted high-density lipoprotein (CER-001) on coronary atherosclerosis: rationale and design of the CARAT study. *Cardiovasc Diagn Ther* 2017, 7:45-51.

Banik B, Wen R, Marrache S, Kumar A, Kolishetti N, Howerth EW, Dhar S. Core hydrophobicity tuning of a self-assembled particle results in efficient lipid reduction and favorable organ distribution. *Nanoscale* 2017, 10:366-377.

Bourdi M, Amar M, Remaley AT, Terse PS. Intravenous toxicity and toxicokinetics of an HDL mimetic, Fx-5A peptide complex, in cynomolgus monkeys. *Regul Toxicol Pharmacol* 2018, 100:59-67.

Brites F, Martin M, Guillas I, Kontush A. Antioxidative activity of high-density lipoprotein (HDL): Mechanistic insights into potential clinical benefit. *BBA Clin* 2017, 8:66-77.

Cabral H, Matsumoto Y, Mizuno K, Chen Q, Murakami M, Kimura M, Terada Y, Kano MR, Miyazono K, Uesaka M, et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. *Nat Nanotechnol* 2011, 6:815-823.

Ceresa C, Bravin A, Cavaletti G, Pellei M, Santini C. The combined therapeutical effect of metal-based drugs and radiation therapy: the present status of research. *Curr Med Chem* 2014, 21:2237-2265.

Chen J, Zhang X, Millican R, Creutzmann JE, Martin S, Jun HW. High density lipoprotein mimicking nanoparticles for atherosclerosis. *Nano Converg* 2020, 7:6.

Chen N, Frishman WH. High-Density Lipoprotein Infusion Therapy and Atherosclerosis: Current Research and Future Directions. *Cardiol Rev* 2016, 24:298-302.

Chin DD, Wang J, Mel de Fontenay M, Plotkin A, Magee GA, Chung EJ. Hydroxyapatite-binding micelles for the detection of vascular calcification in atherosclerosis. *J Mater Chem B* 2019, 7:6449-6457.

Chuang ST, Cruz S, Narayanaswami V. Reconfiguring Nature's Cholesterol Accepting Lipoproteins as Nanoparticle Platforms for Transport and Delivery of Therapeutic and Imaging Agents. *Nanomaterials (Basel)* 2020, 10.

Cormode DP, Roessl E, Thran A, Skajaa T, Gordon RE, Schlomka JP, Fuster V, Fisher EA, Mulder WJ, Proksa R, et al. Atherosclerotic plaque composition: analysis with multicolor CT and targeted gold nanoparticles. *Radiology* 2010, 256:774-782.

Cormode DP, Skajaa T, van Schooneveld MM, Koole R, Jarzyna P, Lobatto ME, Calcagno C, Barazza A, Gordon RE, Zanzonico P, et al. Nanocrystal core high-density lipoproteins: a multimodality contrast agent platform. *Nano Lett* 2008, 8:3715-3723.

Deeb SS, Zambon A, Carr MC, Ayyobi AF, Brunzell JD. Hepatic Lipase and dyslipidemia: interactions among genetic variants, obesity, gender, and diet. *J Lipid Res* 2003, 44:1279-1286.

Di Bartolo BA, Nicholls SJ, Bao S, Rye KA, Heather AK, Barter PJ, Bursill C. The apolipoprotein A-I mimetic peptide ETC-642 exhibits anti-inflammatory properties that are comparable to high density lipoproteins. *Atherosclerosis* 2011, 217:395-400.

Di Bartolo BA, Psaltis PJ, Bursill CA, Nicholls SJ. Translating Evidence of HDL and Plaque Regression. *Arterioscler Thromb Vasc Biol* 2018, 38:1961-1968.

Di Bartolo BA, Vanags LZ, Tan JT, Bao S, Rye KA, Barter PJ, Bursill CA. The apolipoprotein A-I mimetic peptide, ETC-642, reduces chronic vascular inflammation in the rabbit. *Lipids Health Dis* 2011, 10:224.

Ditiatkovski M, Palsson J, Chin-Dusting J, Remaley AT, Sviridov D. Apolipoprotein A-I Mimetic Peptides: Discordance Between In Vitro and In Vivo Properties-Brief Report. *Arterioscler Thromb Vasc Biol* 2017, 37:1301-1306.

Fawaz MV, Kim SY, Li D, Ming R, Xia Z, Olsen K, Pogozheva ID, Tesmer JJG, Schwendeman A. Phospholipid Component Defines Pharmacokinetic and Pharmacodynamic Properties of Synthetic High-Density Lipoproteins. *J Pharmacol Exp Ther* 2020, 372:193-204.

Fisher EA, Feig JE, Hewing B, Hazen SL, Smith JD. High-density lipoprotein function, dysfunction, and reverse cholesterol transport. *Arterioscler Thromb Vasc Biol* 2012, 32:2813-2820.

Fotakis P, Kothari V, Thomas DG, Westerterp M, Molusky MM, Altin E, Abramowicz S, Wang N, He Y, Heinecke JW, et al. Anti-Inflammatory Effects of HDL (High-Density Lipoprotein) in Macrophages Predominate Over Proinflammatory Effects in Atherosclerotic Plaques. *Arterioscler Thromb Vasc Biol* 2019, 39:e253-e272.

Gibson CM, Kastelein JJP, Phillips AT, Aylward PE, Yee MK, Tendera M, Nicholls SJ, Pocock S, Goodman SG, Alexander JH, et al. Rationale and design of ApoA-I Event Reducing in Ischemic Syndromes II (AEGIS-II): A phase 3, multicenter, double-blind, randomized, placebo-controlled,

parallel-group study to investigate the efficacy and safety of CSL112 in subjects after acute myocardial infarction. *Am Heart J* 2021, 231:121-127.

Gille A, D'Andrea D, Tortorici MA, Hartel G, Wright SD. CSL112 (Apolipoprotein A-I [Human]) Enhances Cholesterol Efflux Similarly in Healthy Individuals and Stable Atherosclerotic Disease Patients. *Arterioscler Thromb Vasc Biol* 2018, 38:953-963.

Gupta A, Sharma R, Kuche K, Jain S. Exploring the Therapeutic Potential of the Bioinspired Reconstituted High Density Lipoprotein Nanostructures. *Int J Pharm* 2021:120272.

Hendrich SE, Hong BJ, Rink JS, Nguyen ST, Thaxton CS. Supramolecular Assembly of High-Density Lipoprotein Mimetic Nanoparticles Using Lipid-Conjugated Core Scaffolds. *J Am Chem Soc* 2019, 141:9753-9757.

Ikonen E. Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol* 2008, 9:125-138.

Jomard A, Osto E. High Density Lipoproteins: Metabolism, Function, and Therapeutic Potential. *Front Cardiovasc Med* 2020, 7:39.

Karalis I, Jukema JW. HDL Mimetics Infusion and Regression of Atherosclerosis: Is It Still Considered a Valid Therapeutic Option? *Curr Cardiol Rep* 2018, 20:66.

Khera AV, Demler OV, Adelman SJ, Collins HL, Glynn RJ, Ridker PM, Rader DJ, Mora S. Cholesterol Efflux Capacity, High-Density Lipoprotein Particle Number, and Incident Cardiovascular Events: An Analysis From the JUPITER Trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin). *Circulation* 2017, 135:2494-2504.

Kim H, Han J, Park JH. Cyclodextrin polymer improves atherosclerosis therapy and reduces ototoxicity. *J Control Release* 2020, 319:77-86.

Kingwell BA, Chapman MJ. Future of high-density lipoprotein infusion therapies: potential for clinical management of vascular disease. *Circulation* 2013, 128:1112-1121.

Klębowski B, Depciuch J, Parlińska-Wojtan M, Baran J. Applications of Noble Metal-Based Nanoparticles in Medicine. *Int J Mol Sci* 2018, 19.

Kootte RS, Smits LP, van der Valk FM, Dasseux JL, Keyserling CH, Barbaras R, Paolini JF, Santos RD, van Dijk TH, Dallinga-van Thie GM, et al. Effect of open-label infusion of an apoA-I-containing particle (CER-001) on RCT and artery wall thickness in patients with FHA. *J Lipid Res* 2015, 56:703-712.

Kornmueller K, Vidakovic I, Prassl R. Artificial High Density Lipoprotein Nanoparticles in Cardiovascular Research. *Molecules* 2019, 24.

Kruger AL, Peterson S, Turkseven S, Kaminski PM, Zhang FF, Quan S, Wolin MS, Abraham NG. D-4F induces heme oxygenase-1 and extracellular superoxide dismutase, decreases endothelial cell sloughing, and improves vascular reactivity in rat model of diabetes. *Circulation* 2005, 111:3126-3134.

Kuai R, Li D, Chen YE, Moon JJ, Schwendeman A. High-Density Lipoproteins: Nature's Multifunctional Nanoparticles. *ACS Nano* 2016, 10:3015-3041.

Lai CT, Sun W, Palekar RU, Thaxton CS, Schatz GC. Molecular Dynamics Simulation and Experimental Studies of Gold Nanoparticle Templated HDL-like Nanoparticles for Cholesterol Metabolism Therapeutics. *ACS Appl Mater Interfaces* 2017, 9:1247-1254.

Leman LJ, Maryanoff BE, Ghadiri MR. Molecules that mimic apolipoprotein A-I: potential agents for treating atherosclerosis. *J Med Chem* 2014, 57:2169-2196.

Li D, Fawaz MV, Morin EE, Ming R, Sviridov D, Tang J, Ackermann R, Olsen K, Remaley AT, Schwendeman A. Effect of Synthetic High Density Lipoproteins Modification with Polyethylene Glycol on Pharmacokinetics and Pharmacodynamics. *Mol Pharm* 2018, 15:83-96.

Lu Y, Yue Z, Xie J, Wang W, Zhu H, Zhang E, Cao Z. Micelles with ultralow critical micelle concentration as carriers for drug delivery. *Nat Biomed Eng* 2018, 2:318-325.

Lüscher TF, Landmesser U, von Eckardstein A, Fogelman AM. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. *Circ Res* 2014, 114:171-182.

Marrache S, Dhar S. Biodegradable synthetic high-density lipoprotein nanoparticles for atherosclerosis. *Proc Natl Acad Sci U S A* 2013, 110:9445-9450.

Michael Gibson C, Korjian S, Tricoci P, Daaboul Y, Yee M, Jain P, Alexander JH, Steg PG, Lincoff AM, Kastelein JJ, et al. Safety and Tolerability of CSL112, a Reconstituted, Infusible, Plasma-Derived Apolipoprotein A-I, After Acute Myocardial Infarction: The AEGIS-I Trial (ApoA-I Event Reducing in Ischemic Syndromes I). *Circulation* 2016, 134:1918-1930.

Murphy AJ, Funt S, Gorman D, Tall AR, Wang N. Pegylation of high-density lipoprotein decreases plasma clearance and enhances antiatherogenic activity. *Circ Res* 2013, 113:e1-e9.

Navdaev AV, Sborgi L, Wright SD, Didichenko SA. Nascent HDL (High-Density Lipoprotein) Discs Carry Cholesterol to HDL Spheres: Effects of HDL Particle Remodeling on Cholesterol Efflux. *Arterioscler Thromb Vasc Biol* 2020, 40:1182-1194.

Nicholls SJ, Andrews J, Kastelein JJP, Merkely B, Nissen SE, Ray KK, Schwartz GG, Worthley SG, Keyserling C, Dasseux JL, et al. Effect of Serial Infusions of CER-001, a Pre-β High-Density Lipoprotein Mimetic, on Coronary Atherosclerosis in Patients Following Acute Coronary Syndromes in the CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial: A Randomized Clinical Trial. *JAMA Cardiol* 2018, 3:815-822.

Nicholls SJ, Puri R, Ballantyne CM, Jukema JW, Kastelein JJP, Koenig W, Wright RS, Kallend D, Wijngaard P, Borgman M, et al. Effect of Infusion of High-Density Lipoprotein Mimetic Containing Recombinant Apolipoprotein A-I Milano on Coronary Disease in Patients With an Acute Coronary Syndrome in the MILANO-PILOT Trial: A Randomized Clinical Trial. *JAMA Cardiol* 2018, 3:806-814.

Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, Eaton GM, Lauer MA, Sheldon WS, Grines CL, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *Jama* 2003, 290:2292-2300.

Ou J, Ou Z, Jones DW, Holzhauer S, Hatoum OA, Ackerman AW, Weihrauch DW, Gutterman DD, Guice K, Oldham KT, et al. L-4F, an apolipoprotein A-1 mimetic, dramatically improves vasodilation in hypercholesterolemia and sickle cell disease. *Circulation* 2003, 107:2337-2341.

Pedersbæk D, Krogager L, Albertsen CH, Ringgaard L, Hansen AE, Jønsson K, Larsen JB, Kjær A, Andresen TL, Simonsen JB. Effect of apoA-I PEGylation on the Biological Fate of Biomimetic High-Density Lipoproteins. *ACS Omega* 2021, 6:871-880.

Peters D, Kastantin M, Kotamraju VR, Karmali PP, Gujraty K, Tirrell M, Ruoslahti E. Targeting atherosclerosis by using modular, multifunctional micelles. *Proc Natl Acad Sci U S A* 2009, 106:9815-9819.

Rader DJ. Apolipoprotein A-I Infusion Therapies for Coronary Disease: Two Outs in the Ninth Inning and Swinging for the Fences. *JAMA Cardiol* 2018, 3:799-801.

Rader DJ, Tall AR. The not-so-simple HDL story: Is it time to revise the HDL cholesterol hypothesis? *Nat Med* 2012, 18:1344-1346.

Riaz H, Khan SU, Rahman H, Shah NP, Kaluski E, Lincoff AM, Nissen SE. Effects of high-density lipoprotein targeting treatments on cardiovascular outcomes: A systematic review and meta-analysis. *Eur J Prev Cardiol* 2019, 26:533-543.

Rodriguez A, Trigatti BL, Mineo C, Knaack D, Wilkins JT, Sahoo D, Asztalos BF, Mora S, Cuchel M, Pownall HJ, et al. Proceedings of the Ninth HDL (High-Density Lipoprotein) Workshop: Focus on Cardiovascular Disease. *Arterioscler Thromb Vasc Biol* 2019, 39:2457-2467.

Rosenbaum AI, Zhang G, Warren JD, Maxfield FR. Endocytosis of beta-cyclodextrins is responsible for cholesterol reduction in Niemann-Pick type C mutant cells. *Proc Natl Acad Sci U S A* 2010, 107:5477-5482.

Sacks FM, Jensen MK. From High-Density Lipoprotein Cholesterol to Measurements of Function: Prospects for the Development of Tests for High-Density Lipoprotein Functionality in Cardiovascular Disease. *Arterioscler Thromb Vasc Biol* 2018, 38:487-499.

Sanchez-Gaytan BL, Fay F, Lobatto ME, Tang J, Ouimet M, Kim Y, van der Staay SE, van Rijs SM, Priem B, Zhang L, et al. HDL-mimetic PLGA nanoparticle to target atherosclerosis plaque macrophages. *Bioconjug Chem* 2015, 26:443-451.

Schwendeman A, Sviridov DO, Yuan W, Guo Y, Morin EE, Yuan Y, Stonik J, Freeman L, Ossoli A, Thacker S, et al. The effect of phospholipid composition of reconstituted HDL on its cholesterol efflux and anti-inflammatory properties. *J Lipid Res* 2015, 56:1727-1737.

Shaw JA, Bobik A, Murphy A, Kanellakis P, Blombery P, Mukhamedova N, Woollard K, Lyon S, Sviridov D, Dart AM. Infusion of reconstituted high-density lipoprotein leads to acute changes in human atherosclerotic plaque. *Circ Res* 2008, 103:1084-1091.

Singh IM, Shishehbor MH, Ansell BJ. High-density lipoprotein as a therapeutic target: a systematic review. *Jama* 2007, 298:786-798.

Tabet F, Remaley AT, Segaliny AI, Millet J, Yan L, Nakhla S, Barter PJ, Rye KA, Lambert G. The 5A apolipoprotein A-I mimetic peptide displays antiinflammatory and antioxidant properties in vivo and in vitro. *Arterioscler Thromb Vasc Biol* 2010, 30:246-252.

Tardif JC. Emerging high-density lipoprotein infusion therapies: fulfilling the promise of epidemiology? *J Clin Lipidol* 2010, 4:399-404.

Tardif JC, Ballantyne CM, Barter P, Dasseux JL, Fayad ZA, Guertin MC, Kastelein JJ, Keyserling C, Klepp H, Koenig W, et al. Effects of the high-density lipoprotein mimetic agent CER-001 on

coronary atherosclerosis in patients with acute coronary syndromes: a randomized trial. *Eur Heart J* 2014, 35:3277-3286.

Tardif JC, Grégoire J, L'Allier PL, Ibrahim R, Lespérance J, Heinonen TM, Kouz S, Berry C, Basser R, Lavoie MA, et al. Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. *Jama* 2007, 297:1675-1682.

Thaxton CS, Daniel WL, Giljohann DA, Thomas AD, Mirkin CA. Templated spherical high density lipoprotein nanoparticles. *J Am Chem Soc* 2009, 131:1384-1385.

Trajkovska KT, Topuzovska S. High-density lipoprotein metabolism and reverse cholesterol transport: strategies for raising HDL cholesterol. *Anatol J Cardiol* 2017, 18:149-154.

Tricoci P, D'Andrea DM, Gurbel PA, Yao Z, Cuchel M, Winston B, Schott R, Weiss R, Blazing MA, Cannon L, et al. Infusion of Reconstituted High-Density Lipoprotein, CSL112, in Patients With Atherosclerosis: Safety and Pharmacokinetic Results From a Phase 2a Randomized Clinical Trial. *J Am Heart Assoc* 2015, 4:e002171.

Vite CH, Bagel JH, Swain GP, Prociuk M, Sikora TU, Stein VM, O'Donnell P, Ruane T, Ward S, Crooks A, et al. Intracisternal cyclodextrin prevents cerebellar dysfunction and Purkinje cell death in feline Niemann-Pick type C1 disease. *Sci Transl Med* 2015, 7:276ra226.

Waksman R, Torguson R, Kent KM, Pichard AD, Suddath WO, Satler LF, Martin BD, Perlman TJ, Maltais JA, Weissman NJ, et al. A first-in-man, randomized, placebo-controlled study to evaluate the safety and feasibility of autologous delipidated high-density lipoprotein plasma infusions in patients with acute coronary syndrome. *J Am Coll Cardiol* 2010, 55:2727-2735.

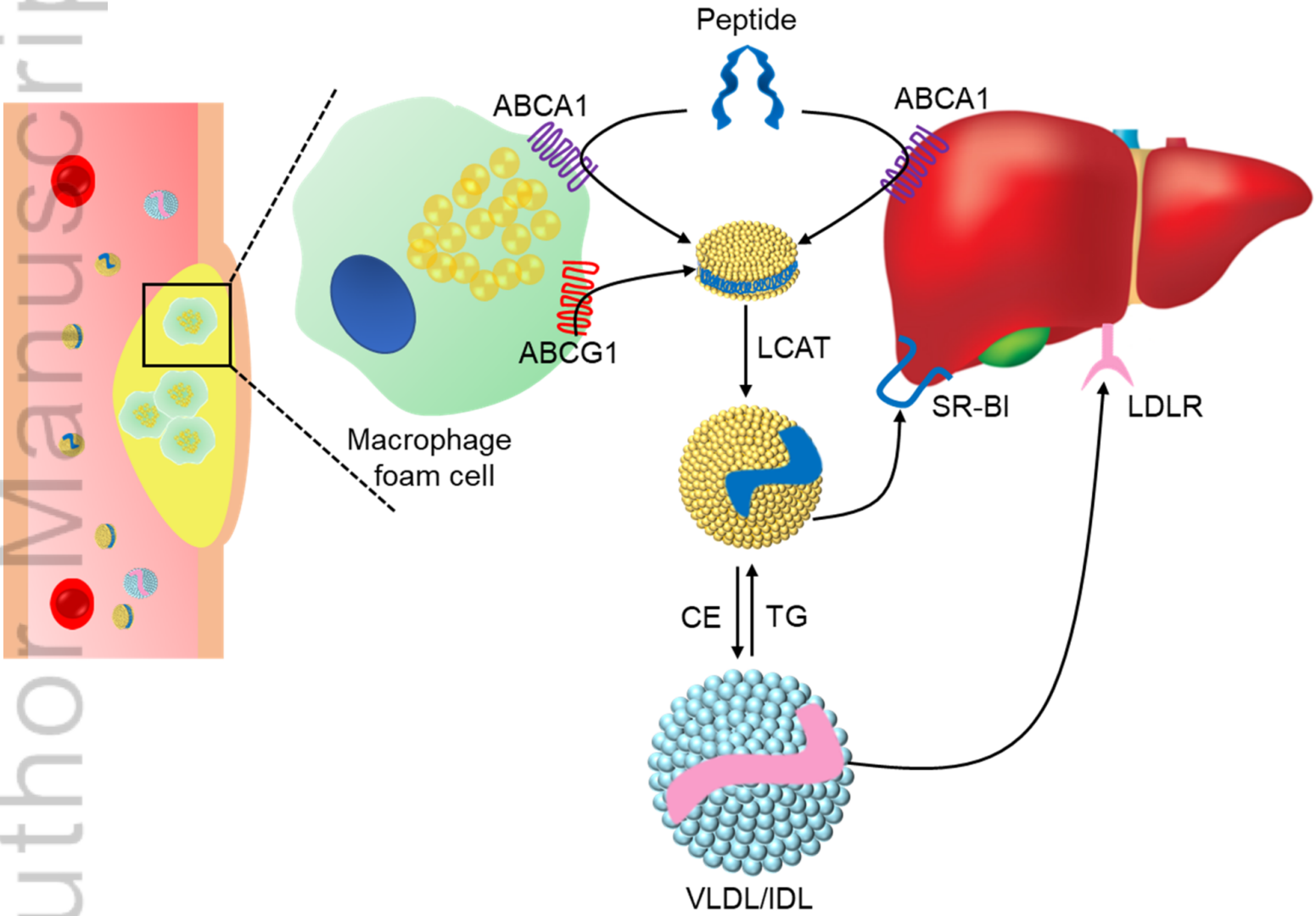
Watson CE, Weissbach N, Kjems L, Ayalasomayajula S, Zhang Y, Chang I, Navab M, Hama S, Hough G, Reddy ST, et al. Treatment of patients with cardiovascular disease with L-4F, an apo-A1 mimetic, did not improve select biomarkers of HDL function. *J Lipid Res* 2011, 52:361-373.

Yu JE, Han SY, Wolfson B, Zhou Q. The role of endothelial lipase in lipid metabolism, inflammation, and cancer. *Histol Histopathol* 2018, 33 (1): 1-10.

Zhao Y, Black AS, Bonnet DJ, Maryanoff BE, Curtiss LK, Leman LJ, Ghadiri MR. In vivo efficacy of HDL-like nanolipid particles containing multivalent peptide mimetics of apolipoprotein A-I. *J Lipid Res* 2014, 55:2053-2063.

Zheng KH, Kaiser Y, van Olden CC, Santos RD, Dasseux JL, Genest J, Gaudet D, Westerink J, Keyserling C, Verberne HJ, et al. No benefit of HDL mimetic CER-001 on carotid atherosclerosis in patients with genetically determined very low HDL levels. *Atherosclerosis* 2020, 311:13-19.

Zimmer S, Grebe A, Bakke SS, Bode N, Halvorsen B, Ulas T, Skjelland M, De Nardo D, Labzin LI, Kerksiek A, et al. Cyclodextrin promotes atherosclerosis regression via macrophage reprogramming. *Sci Transl Med* 2016, 8:333ra350.



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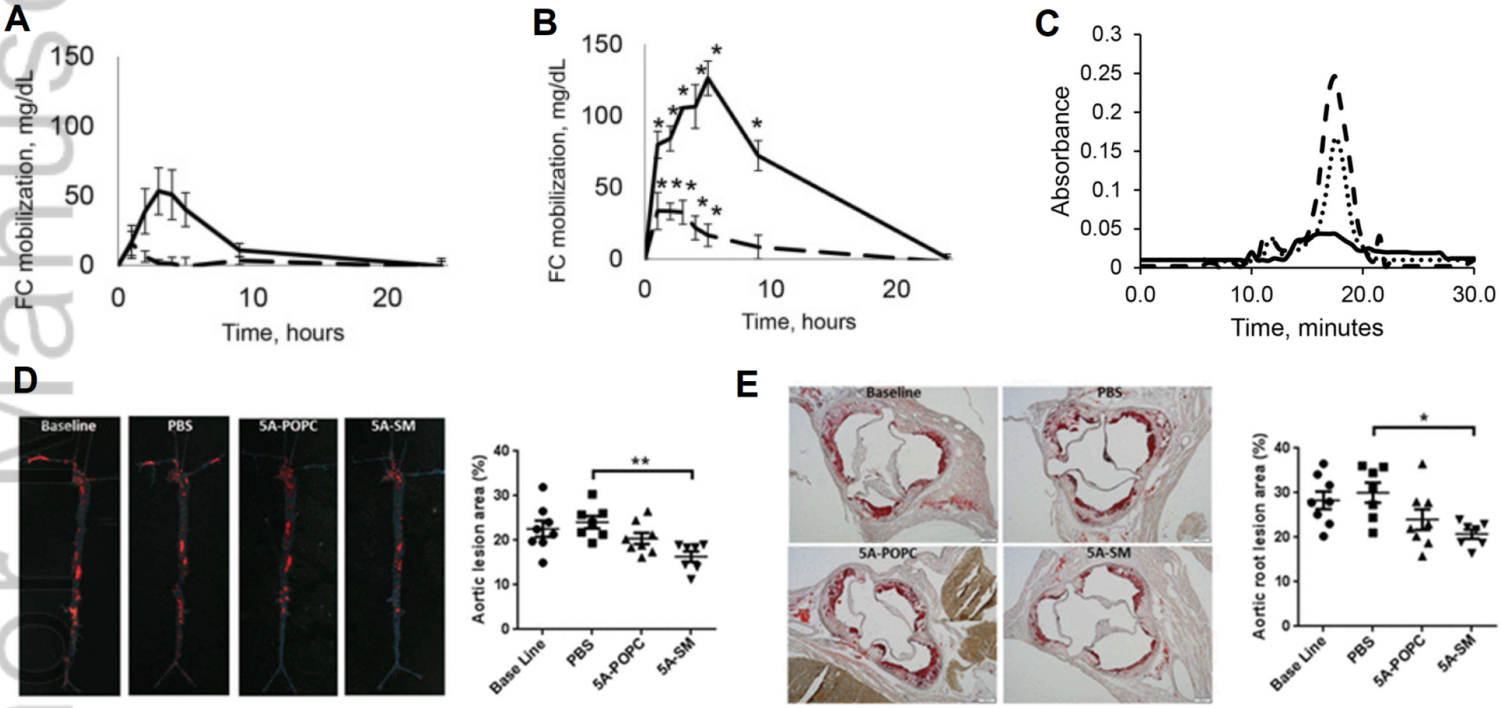

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Effect of Synthetic High Density Lipoproteins Modification with Polyethylene Glycol on Pharmacokinetics and Pharmacodynamics

Author: Dan Li, Maria V. Fawaz, Emily E. Morin, et al

Publication: Molecular Pharmaceutics

Publisher: American Chemical Society

Date: Jan 1, 2018

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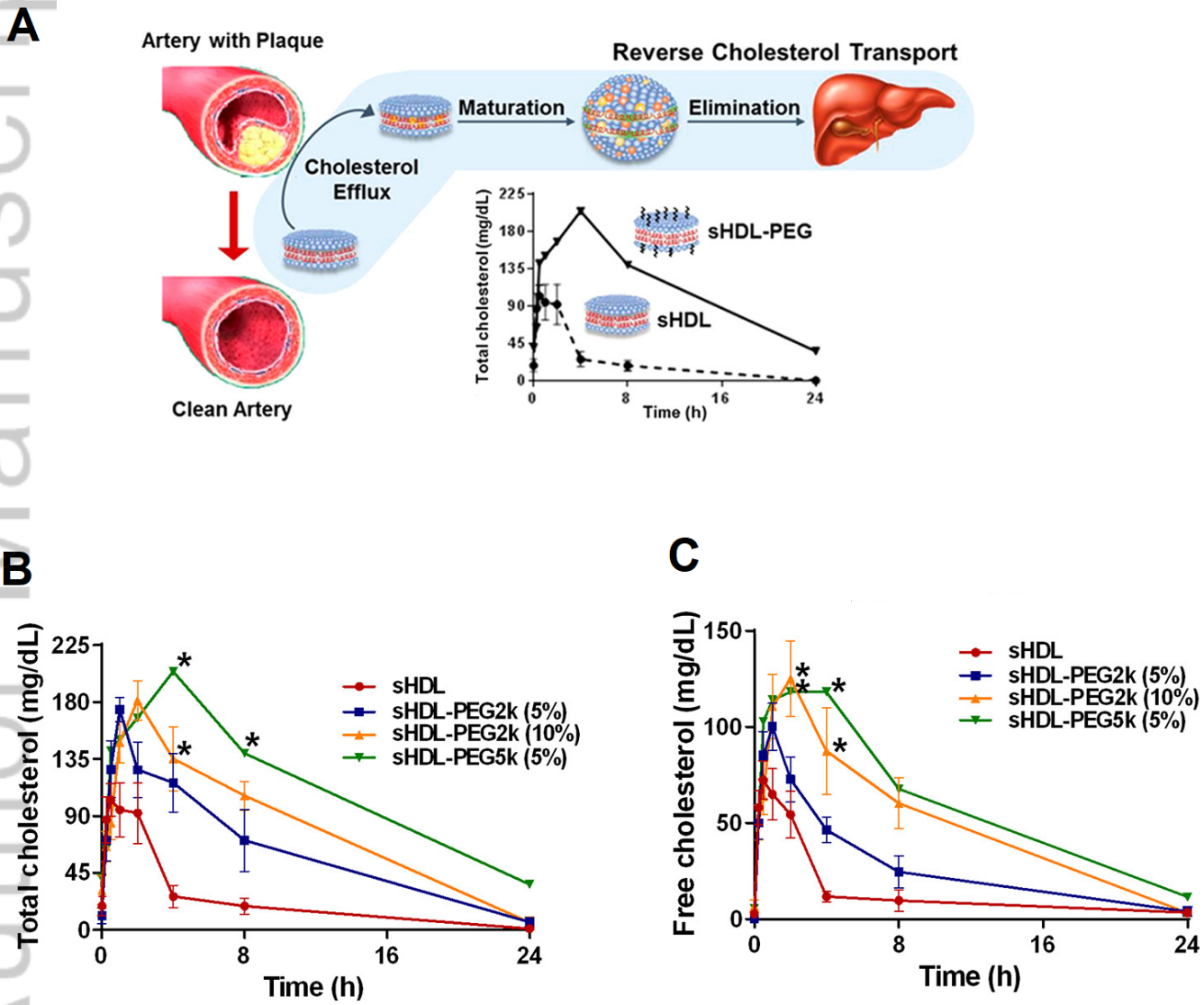

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HDL-Mimetic PLGA Nanoparticle To Target Atherosclerosis Plaque Macrophages

Author: Brenda L. Sanchez-Gaytan, Francois Fay, Mark E. Lobatto, et al
Publication: Bioconjugate Chemistry
Publisher: American Chemical Society
Date: Mar 1, 2015

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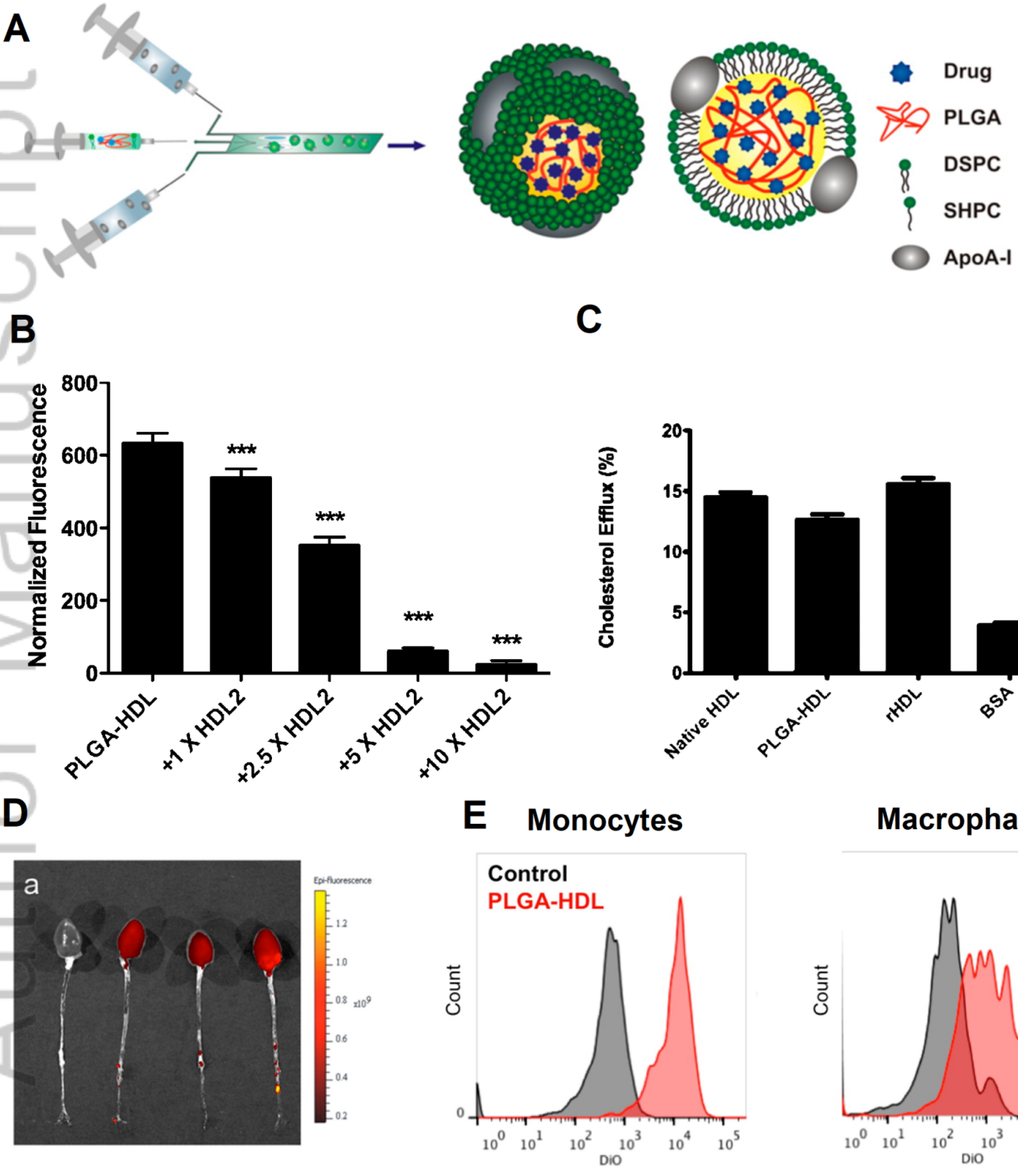

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Publisher	Wiley
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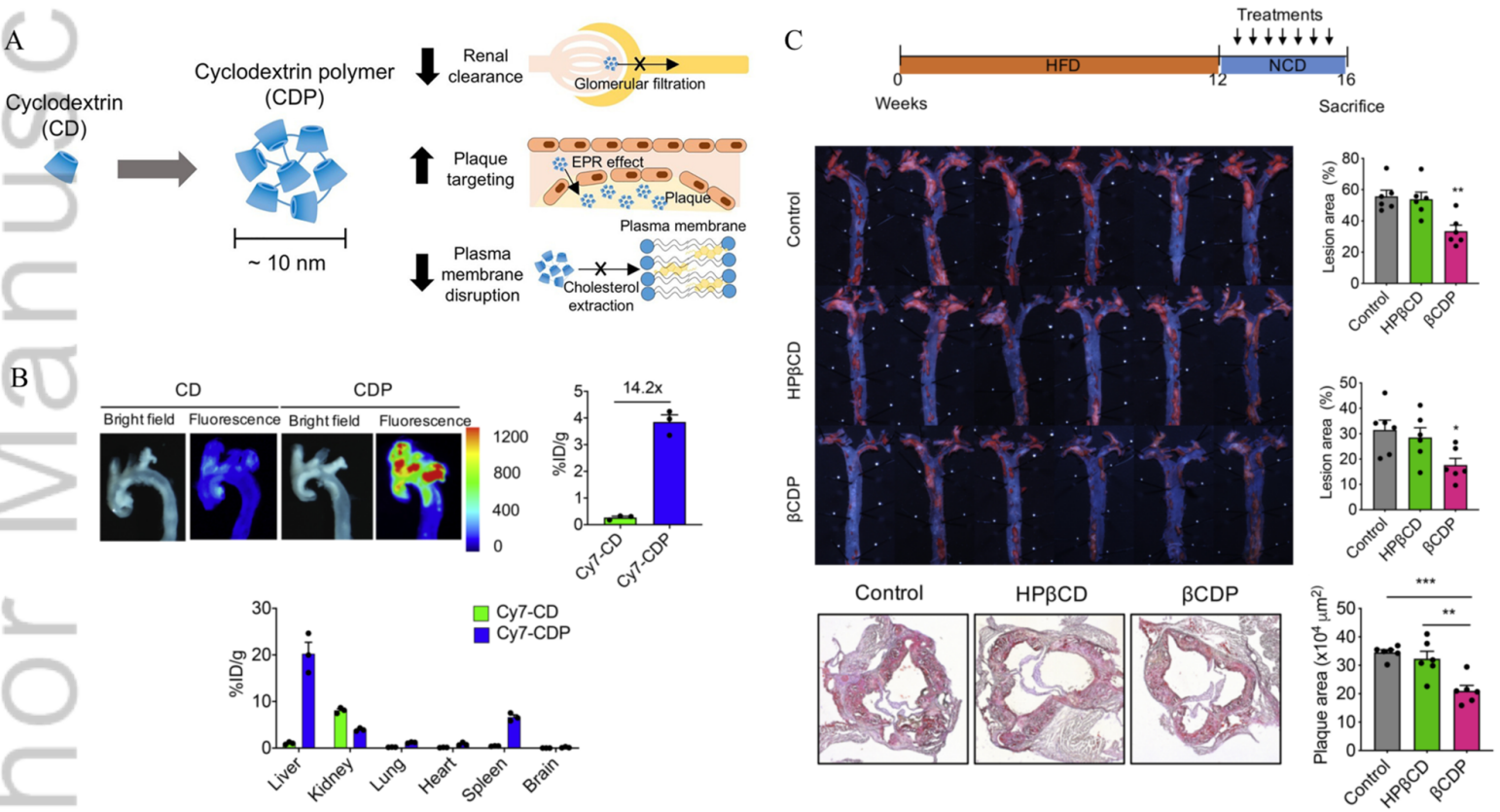

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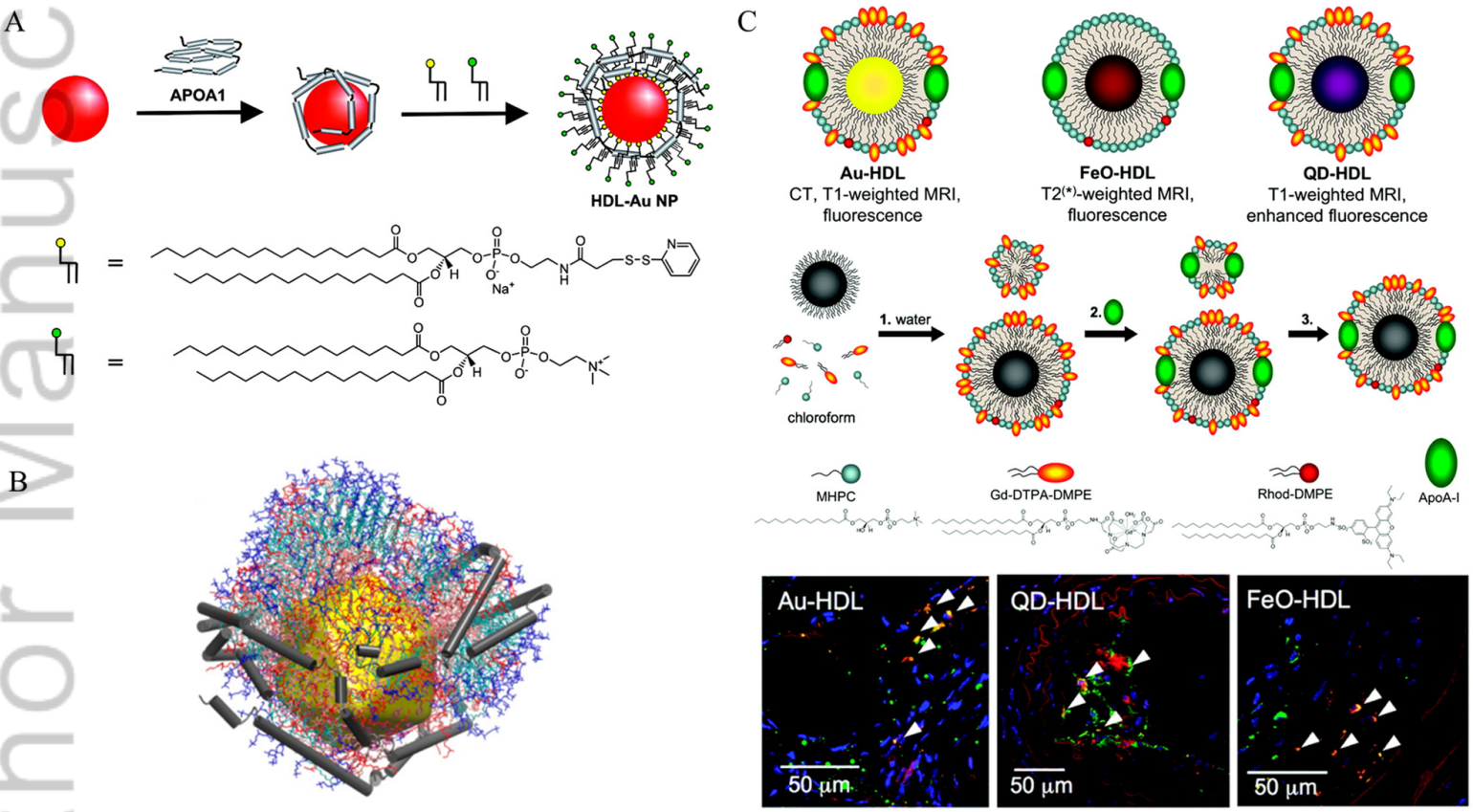

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Templated Spherical High Density Lipoprotein Nanoparticles

Author: C. Shad Thaxton, Weston L. Daniel, David A. Giljohann, et al
Publication: Journal of the American Chemical Society
Publisher: American Chemical Society
Date: Feb 1, 2009

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Molecular Dynamics Simulation and Experimental Studies of Gold Nanoparticle Templated HDL-like Nanoparticles for Cholesterol Metabolism Therapeutics

Author: Cheng-Tsung Lai, Wangqiang Sun, Rohun U. Palekar, et al

Publication: Applied Materials

Publisher: American Chemical Society

Date: Jan 1, 2017

Nanocrystal Core High-Density Lipoproteins: A Multimodality Contrast Agent Platform

Author: David P. Cormode, Torjus Skajaa, Matti M. van Schooneveld, et al
Publication: Nano Letters
Publisher: American Chemical Society
Date: Nov 1, 2008

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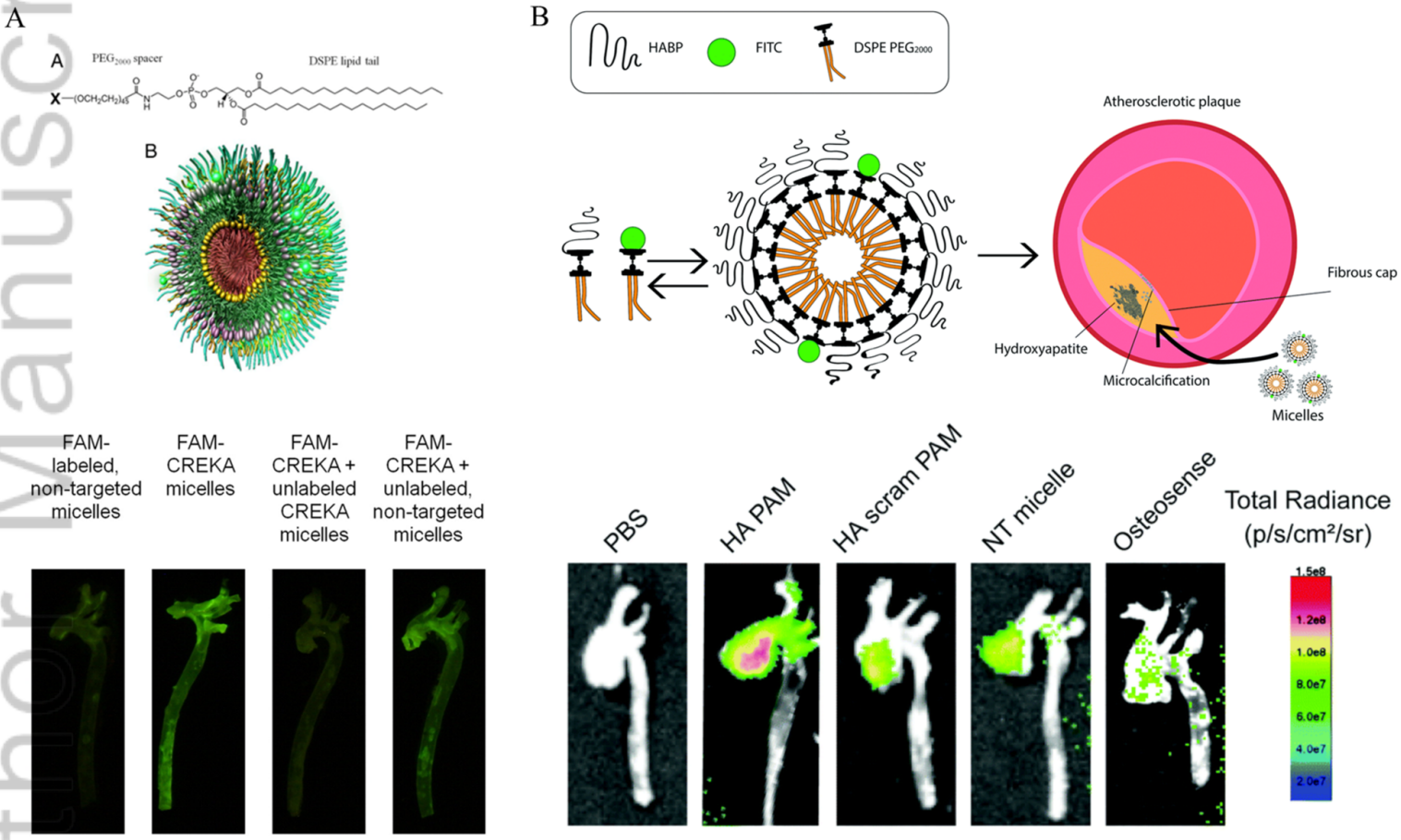

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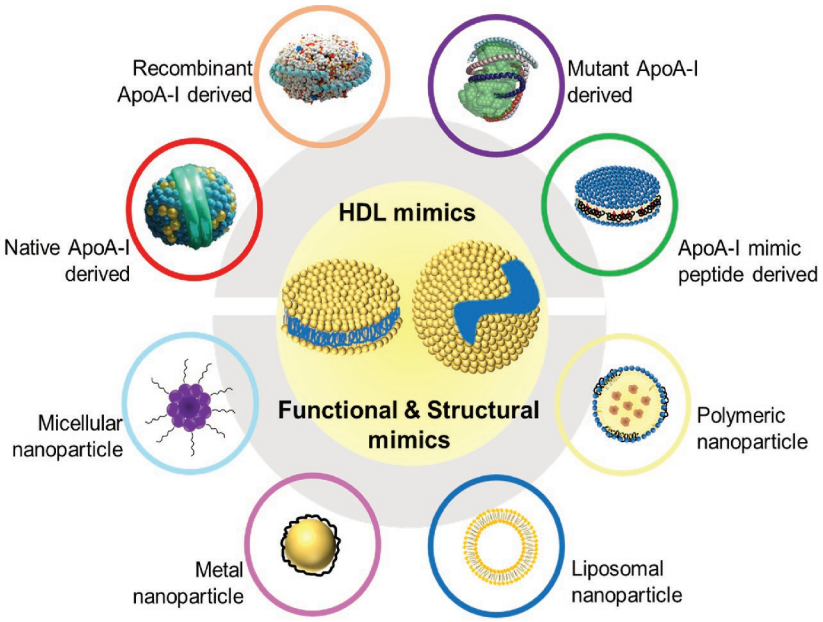

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