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Comparison of cholesterol transport capacity of peptide- and polymer-based lipid Nanodiscs

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ABSTRACT

Apolipoprotein-based, synthetic high-density lipoprotein (sHDL) nanodiscs have been extensively studied as a potential therapeutic agent for cardiovascular disease due to their ability to promote reverse cholesterol transport. Recently, polymer-based nanodiscs have been made possible with the development of novel polymeric materials such as styrene-maleic anhydride copolymer (SMA). While the polymer-based nanodiscs resemble the discoidal structure of sHDLs, their functional similarity with sHDL has not been investigated. In the present study, we compared the SMA-based and peptide-based sHDL nanodiscs focusing on their cholesterol mobilization effects. Results showed that SMA-based nanoparticles presented similar particle size and *in vitro* cholesterol efflux effect to those of sHDL nanodiscs. However, SMA nanodiscs induced less cholesterol mobilization *in vivo*, possibly due to insufficient cholesterol esterification by lecithin:cholesterol acyltransferase.

Introduction

Nanodiscs are discoidal, nanoscale particles characterized by lipid bilayer encircled by stabilizing agent.[1] Nanodiscs not only serve as powerful tools for membrane protein isolation, but also present potential for therapeutic applications due to their small particle sizes, well-defined structure, and capacity to deliver wide varieties of therapeutic agents.[2,3] Synthetic high-density lipoprotein (sHDL) nanodisc composed of apolipoproteins or their mimetic peptides is one of the most studied nanodiscs for therapeutic applications.[4] Due to the compositional and structural resemblance of sHDL to natural pre-beta HDL particles, sHDL nanodiscs have been found to exert several HDL-like functions, especially cholesterol transport functions.[4] Clinical studies confirmed that sHDL infusion could greatly elevate serum cholesterol levels, indicating increased cholesterol mobilization and accelerated reverse cholesterol transport (RCT) following sHDL administration.[5] The RCT-enhancing effects of sHDLs have inspired research interest in developing sHDL nanodiscs to treat cardiovascular diseases such as atherosclerosis.[6] While apolipoprotein or its mimetic peptide is considered to be key components of sHDL,[7] protein-free HDL mimetics have also been previously developed. For example, protein free lipid micelles were previously reported to increase cholesterol efflux and promote cholesterol mobilization *in vivo* in a similar manner to sHDL.[8,9] Given the significant manufacturing costs of apolipoprotein and mimetics, such protein-free HDL mimetics would present as appealing alternative for apolipoprotein sHDL nanodiscs.

In the past decade, several amphiphilic polymeric materials with alternating hydrophilic and hydrophobic unit, such as styrene-maleic acid (SMA) and diisobutylene-maleic acid (DIBMA), have been developed.[10,11] Previous research suggested that those polymers could form nanodiscs with lipids in a manner similar to that of apolipoproteins,[12,13] although some recent studies challenged the conventional understanding regarding the particle structure and homogeneity of SMA-lipid complexes.[14] Despite the controversy, polymer nanodiscs are widely

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Table. 1
Particle size of different nanodiscs measured by DLS (n = 3, Mean ± SD).

Nanodisc	*Z*-average (d. nm)	Polydisperse Index
22A-eSM	10.30 ± 0.89	0.177 ± 0.015
SMA:eSM = 0.5:1	269.3 ± 32.35	0.327 ± 0.036
SMA:eSM = 1:1	71.43 ± 12.97	0.139 ± 0.034
SMA:eSM = 1.5:1	10.89 ± 0.08	0.241 ± 0.020
SMA:eSM = 2:1	9.88 ± 0.26	0.222 ± 0.032
SMA:eSM = 3:1	10.19 ± 0.44	0.284 ± 0.046

used in membrane protein isolation research. Moreover, several drug delivery systems utilizing those polymers have been proposed to deliver anti-cancer and antibiotic agents.[15] However, despite the presumed structural similarity between apolipoprotein-based sHDL nanodisc and polymer-based nanoparticles, the functional comparison between the two nanoparticles has not been studied. To fill this gap, in the present study, apolipoprotein (ApoA-I) mimetic peptide 22A-based sHDL nanodiscs and SMA-based nanoparticles were prepared. The biological functions of two nanoparticles, especially the cholesterol transport functions, were compared *in vitro* and *in vivo*.

Materials and methods

Detailed materials and methods can be found in the supplementary information.

Results/discussion

SMA-eSM nanoparticles with different lipid-to-polymer weight ratios were prepared, and their particle sizes were compared with 22A-eSM

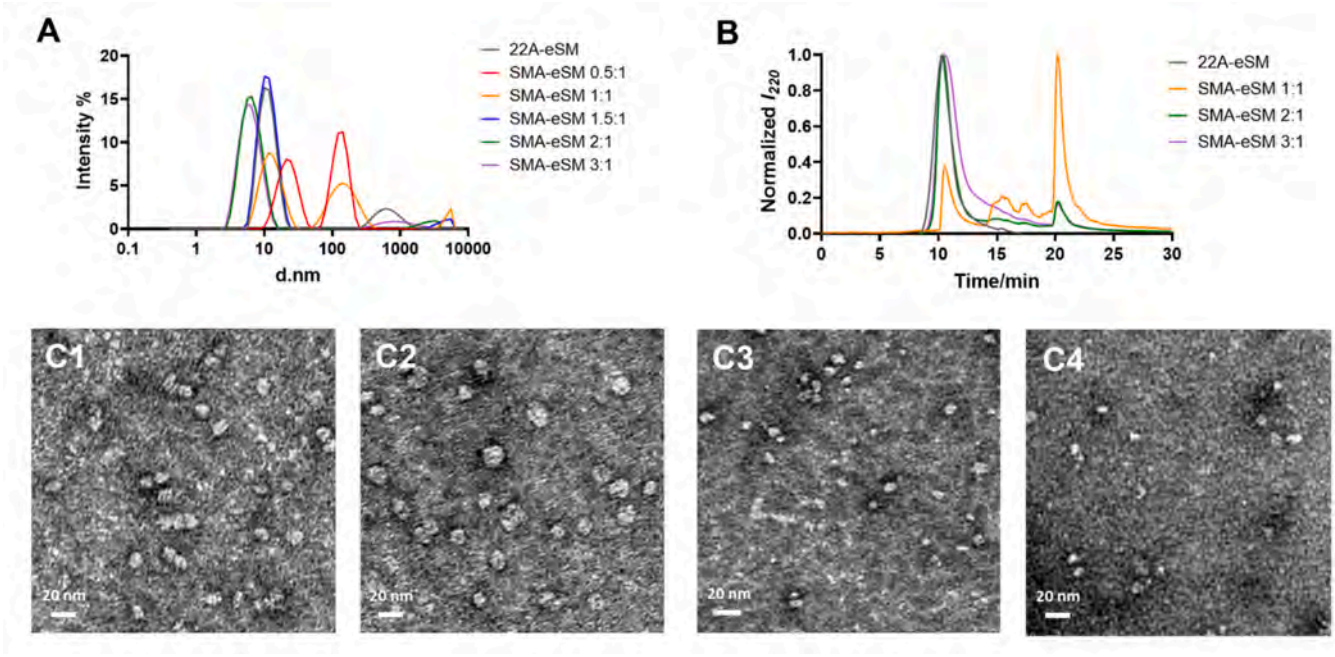

Fig. 1. Particle size distribution of different nanoparticles analyzed by DLS (A) and SEC (B). (C) Representative TEM images of 22A-eSM (C1), SMA-eSM 1:1 (C2), SMA-eSM 2:1 (C3) and SMA-eSM 3:1 (C4) nanodiscs.

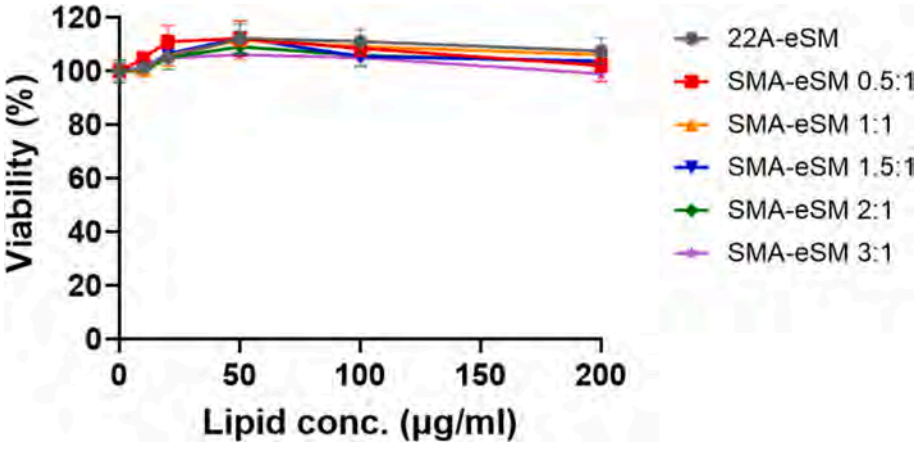

Fig. 2. Viability of RAW264.7 cells after incubation with different nanodiscs for 24 h (n = 6, Mean ± SD).

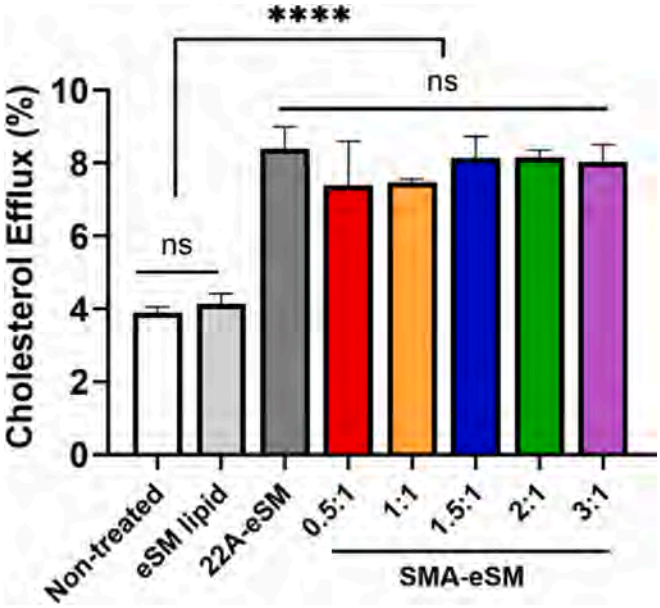

Fig. 3. Cholesterol efflux capacities of different nanodiscs on [^{3}H]-cholesterol loaded RAW 264.7 cells (n = 3, Mean ± SD. ****$p < 0.0001$).

sHDLs. Egg sphingomyelin (eSM) was selected to be the lipid component in both nanoparticles based on its potent cholesterol efflux and transport capacity in previously reported HDL mimetics.[16] As shown in Table 1 and Fig. 1, the averaged intensity-based particle sizes (*Z*-average) of eSM-SMA nanoparticles decrease as the polymer-to-lipid ratio increases. Dynamic light scattering (DLS) results suggested the presence of large particles in SMA-eSM 0.5:1 and 1:1. As intensity data are strongly biased towards large particles, the exact percentage of such large particles is low, which explained the lack of large particles in transmission electron microscopy (TEM) images. A more uniform particle size distribution was observed at SMA to eSM ratio of 2:1 or higher, although small amount of free SMA was also observed in size exclusion chromatography (SEC).

Peptide-based sHDL nanodiscs have been suggested to have favorable safety profiles in previous studies.[17] At the same time, the membrane solubilizing activity of SMA may pose safety concerns for SMA-based nanoparticles. To address this concern, the cytotoxicity of SMA-eSM nanodiscs was evaluated on RAW264.7 cells in comparison with 22A-eSM sHDLs. As shown in Fig. 2, none of the nanodiscs induced significant cytotoxicity on RAW264.7 cells, which allowed further *in vitro* and *in vivo* investigations.

One of the pivotal biofunctions of sHDL nanodiscs is to enhance cellular cholesterol efflux, which is considered to be the rate-limiting step for reverse cholesterol transport. The cholesterol efflux capacity of sHDL nanodiscs is commonly attributed to the interaction between ApoA-I moieties and cholesterol transporters such as ATP-binding cassette transporters A1 (ABCA1).[7] However, as shown in Fig. 3, the polymer-based SMA-eSM nanodiscs induced similar increases in cholesterol efflux on [^{3}H]-cholesterol laden macrophages to 22A-eSM sHDLs, indicating the ApoA-I independent cholesterol efflux capacity of SMA-eSM nanodiscs. Similar cholesterol efflux capacity has been reported on other lipid nanoparticles without ApoA-I moiety such as liposomes and micelles.[9] The ApoA-I independent cholesterol efflux may be attributed to the passive diffusion of cholesterol from the cell membrane to cholesterol receptors in the media, where lipid vehicles such as SMA-eSM nanodiscs may promote cholesterol efflux by serving as an additional sink for cholesterol.[18] No difference was observed between different SMA-eSM nanoparticles, despite the fact that SMA-eSM 3:1 presented slightly higher cholesterol loading capacity (Supplementary Fig. 1). This is possibly due to other factors affecting passive diffusion of cholesterol such as the rate of cholesterol transport to cell membranes. The plain eSM suspension, however, did not increase cholesterol efflux compared to the non-treated group. The lack of cholesterol efflux capacity of lipid suspension may be attributed to the large particle size of lipid aggregation which limits the collision with cholesterol.[19]

To investigate the *in vivo* cholesterol mobilization effects of different nanodiscs, the SMA-eSM (SMA:eSM = 2:1) and 22A-eSM nanodiscs were administered in rats at the same lipid concentration. The total cholesterol, free cholesterol, cholesterol ester, and phospholipid concentrations are shown in Fig. 4. Both nanodiscs induced significant increases in plasma cholesterol and phospholipid concentrations in 24 h following administration. The elevation of plasma phospholipid level after administration of different nanodiscs was comparable. However, 22A-eSM sHDL led to a greater increase in plasma total cholesterol compared to SMA-eSM nanodiscs (Fig. 4A), suggesting a greater cholesterol mobilization *in vivo*. The inferior cholesterol mobilization effect of SMA-eSM may be attributed to the inefficient cholesterol esterification, which is evidenced by the greater free cholesterol increase and higher free cholesterol to total cholesterol ratios in rats treated with SMA-eSM (Fig. 4B-D). Catalyzed by lecithin cholesterol acyl transferase (LCAT), cholesterol esterification is believed to be essential for the efficient transport of cholesterol by lipoproteins and for maintaining free cholesterol concentration gradient between serum and cell membranes.[20] As LCAT activation requires binding of apolipoproteins such as ApoA-I or ApoE,[21] it is likely that the inefficient cholesterol esterification by SMA-eSM is due to the lack of LCAT activation activity.

HDL mimetics are known to undergo remodeling process after entering blood circulation, where they interact with endogenous HDLs and other lipoproteins.[22] To investigate the remodeling process, isolated HDL was incubated with different nanoparticles for 30 min, followed by SEC analysis. In contrast to 22A-eSM nanodiscs, SMA-eSM quickly led to the formation of smaller-sized particles, likely due to the lipid dissolution effects of SMA. The composition and bioactivity of the newly formed lipoprotein particles, especially their cholesterol transport capacities, would warrant further investigation.

For preliminary safety evaluation, the aspartate transaminase (AST) and alanine aminotransferase (ALT) levels were determined. Compared to the 22A-eSM sHDL treated group, rats administered with SMA-eSM nanodiscs presented elevated ALT at 8 h and increased AST at 24 h post-injection (Supplementary Fig. 2).

Overall, SMA-based and 22A-based nanodiscs showed similar particle characteristics and cellular safety profiles. In terms of cholesterol mobilization effects, both nanodiscs showed comparable cholesterol efflux capacities *in vitro*. However, the *in vivo* cholesterol mobilization capacity of SMA-eSM nanoparticles is limited due to insufficient cholesterol esterification, suggesting the indispensable role of ApoA-I moiety in LCAT activation. The quick remodeling with endogenous lipoproteins, as well as the potential adverse effects, may need to be considered for future development of SMA-based nanomedicine.

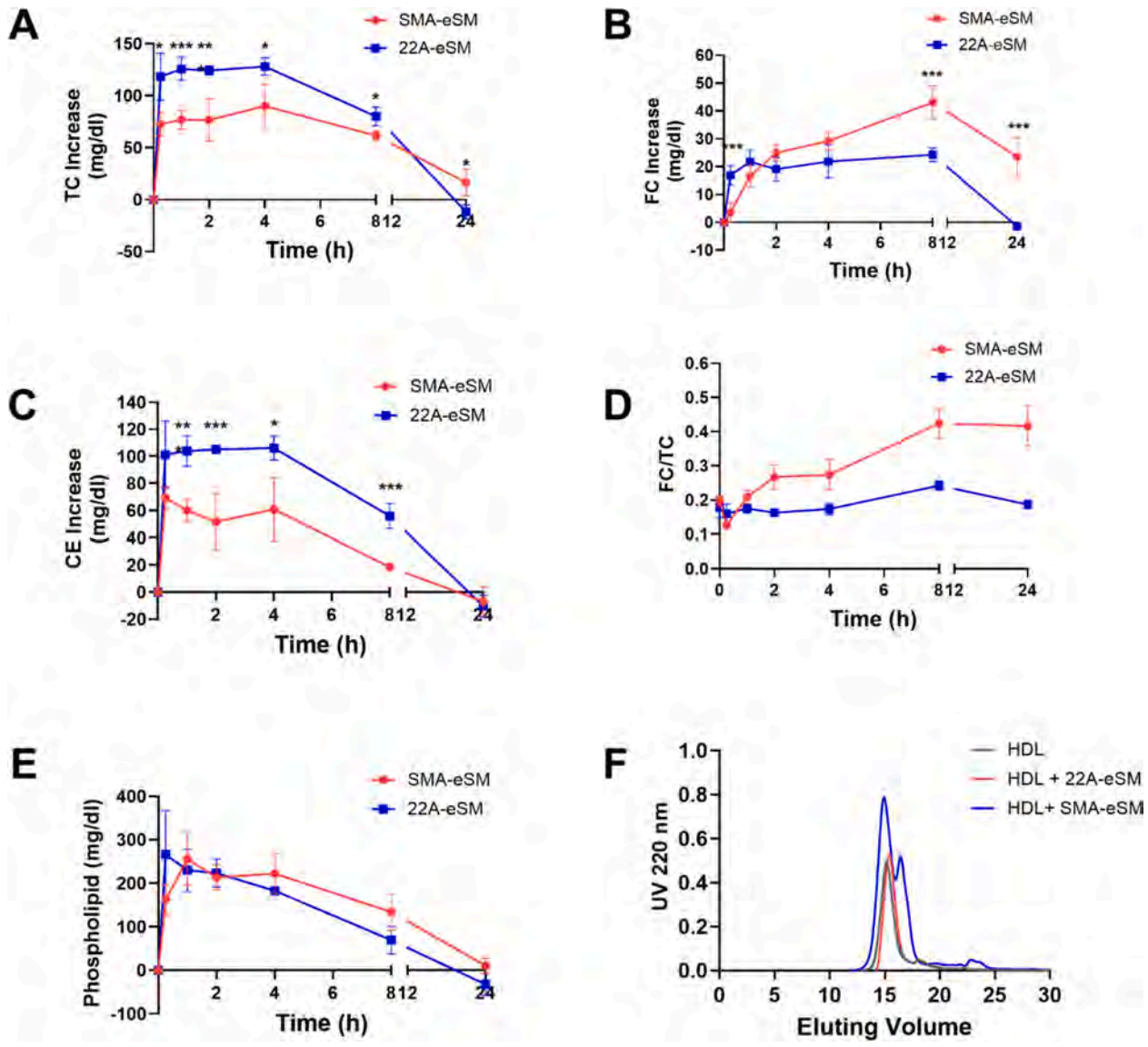

Fig. 4. Plasma total cholesterol (TC) increases (A), free cholesterol (FC) increases (B), cholesterol ester (CE) increases (C), free cholesterol percentage (D), and phospholipid increases (E) after iv administration of SMA-eSM or 22A-eSM nanodiscs. (F) *In vitro* remodeling with human HDL was analyzed by SEC. (n = 3–4, Mean ± SD. $*p < 0.05$, $**p < 0.01$, $***p < 0.005$, $****p < 0.001$).

CRediT authorship contribution statement

Minzhi Yu: Writing – original draft, Investigation, Formal analysis. **Saatvik Vaishnav:** Writing – original draft, Investigation. **Kristen Hong Dorsey:** Investigation. **May Thazin Phoo:** Investigation. **Antonela Rodriguez:** Investigation. **Anna Schwendeman:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Conflict of interest

Dr. Schwendeman declares financial interests for board membership, as a paid consultant, for research funding, and/or as equity holder in EVOQ Therapeutics. The University of Michigan has a financial interest in EVOQ Therapeutics, Inc.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nano.2024.102795.

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