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Leading Opinion

Detecting the functional complexities between high-density lipoprotein mimetics

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ABSTRACT

High-density lipoprotein (HDL) is a key regulator of lipid homeostasis through its native roles like reverse cholesterol transport. The reconstitution of this natural nanoparticle (NP) has become a nexus between nanomedicine and multi-disease therapies, for which a major portion of HDL functionality is attributed to its primary scaffolding protein, apolipoprotein A1 (apoA1). ApoA1-mimetic peptides were formulated as cost-effective alternatives to apoA1-based therapies; reverse-4F (r4F) is one such peptide used as part of a nanoparticle platform. While similarities between r4F- and apoA1-based HDL-mimetic nanoparticles have been identified, key functional differences native to HDL have remained undetected. In the present study, we executed a multidisciplinary approach to uncover these differences by exploring the form, function, and medical applicability of engineered HDL-mimetic NPs (eHNPs) made from r4F (eHNP-r4F) and from apoA1 (eHNP-A1). Comparative analyses of the eHNPs through computational molecular dynamics (MD), advanced microfluidic NP synthesis and screening technologies, and *in vivo* animal model studies extracted distinguishable eHNP characteristics: the eHNPs share identical structural and compositional characteristics with distinct differences in NP stability and organization; eHNP-A1 could more significantly stimulate anti-inflammatory responses characteristic of the scavenger receptor class B type 1 (SR-B1) mediated pathways; and eHNP-A1 could outperform eHNP-r4F in the delivery of a model hydrophobic drug to an *in vivo* tumor. The biomimetic microfluidic technologies and MD simulations uniquely enabled our comparative analysis through which we determined that while eHNP-r4F is a capable NP with properties mimicking natural eHNP-A1, challenges remain in reconstituting the full functionality of NPs naturally derived from humans.

1. Introduction

High-density lipoprotein (HDL) is an endogenous nanoparticle (NP) with anti-inflammatory properties and carrier capabilities. With an average diameter of 7–17 nm, HDL naturally transitions between spherical and discoidal configurations with the encapsulation and removal of cholesterols or other hydrophobic molecules from its hydrophobic core [1]. The impact of HDL and its transitioning structure is largely attributed to the functions of its

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primary protein component, apolipoprotein A1 (apoA1), which serves both as a scaffold to bind free lipids and cholesterols and as an agonist for anti-inflammatory processes like reverse cholesterol transport (RCT) from macrophages and the production of endothelial nitric oxide (NO) [1]. The most common receptors involved in the apoA1-stimulated regulatory processes include scavenger receptor class B type 1 (SR-B1), ATP-binding cassette transporter subfamily A member 1 (ABCA1), and ATP-binding cassette transporter subfamily G member 1 (ABCG1); while ABCA1 and ABCG1 are primarily attributed to RCT from macrophages, SR-B1 has been shown to be involved in both pathways of RCT and endothelial NO production [2]. Being a natural transporter for hydrophobic materials with the added anti-inflammatory benefits, HDL became a prime candidate for transporting therapeutic and diagnostic, or theranostic, agents for a variety of applications [3,4]. These applications have been applied to engineered NPs mimicking the form and function of HDL, allowing for the additional benefit of controlling the NP composition. Simplifying the complex lipidome and proteome of HDL to apoA1 and phospholipids, the development of HDL-mimetic NPs first started with direct proteo-lipidic self-assembly or the sodium cholate dialysis methods to reconstitute HDL (rHDL) [5], and has more recently incorporated microfluidic methods for a single-step assembly approach [6].

The engineering of HDL-mimetic NPs should involve a combination of computational and experimental approaches to comprehensively understand the form and function of the resulting NPs. For instance, the discoidal structure of HDL is often presented in a double-belt configuration, which has been commonly implemented in computational models of HDL self-assembly and is used to explain the structural stability of HDL [7–9]. Engineering HDL-mimetics has also resulted in the development of many apoA1-mimetic peptides for synthetic theranostic platforms to be used against diseases like cardiovascular disease (CVD), Alzheimer's disease, diabetes, and cancer [10,11]. Compared to apoA1, which has more than 240 amino acids, apoA1-mimetic peptides are not sequence homologous and are 18–22 amino acids in length to make the class-A amphipathic helical motif from the apoA1 lipid binding domain (Fig. S1) [10]. These mimetic peptides are also relatively cost-effective synthetic alternatives to apoA1, the purification and use of which requires difficult and expensive tests to ensure that human plasma (from which natural apolipoproteins are purified) is infection and endotoxin free [12]. Of the mimetic peptides studied, reverse-4F (r4F) has shown promising results *in vivo* in combatting the pathogenesis of atherosclerotic mouse models, and in the delivery of drug therapeutics [11,13–16]. The development of r4F was spurred by the varied properties among the 4F and r4F mimetic peptides, of which r4F was engineered to combine the bioavailability of 4F with different lipid-binding and anti-inflammatory properties that were demonstrated in mouse models [13]. NPs formulated with r4F have already shown HDL-like structures and reactivity with the receptor SR-B1 for various theranostic applications [11,16]. However, a direct comparison of r4F to apoA1 in the context of NP form, function, and drug delivery potential has yet to be conducted.

The applications promised in NP therapies show broad reaches inclusive of imaging agents for targeted areas in the body and treatments for various diseases. With an extensive library of possibilities, different methods have been used to synthesize NPs based on lipids, polymers, proteins/peptides, and their combinations [17]; the most common method is to use bulk benchtop mixing to enhance NP self-assembly, or the spontaneous organization of NP precursors. A major challenge associated with this bulk synthesis technique is the resultant high batch-to-batch variation of the NP physicochemical properties. To address this challenge, microfluidic technology has brought a new synthesis strategy presenting a continuous production platform for highly uniform NPs assembled through controlled mixing patterns (diffusive or convective means) [18], which facilitate precursor microscale interactions that were not achievable through traditional macroscale (centimeter to millimeter) benchtop synthesis techniques.

Additional critical barriers to translational studies for nanomedicine arise from the inability of conventional experimental model systems to conduct mechanistic studies of NP interactions with cells in pathophysiologically relevant microenvironments. Microfluidic technologies also offer a method through which to screen NP interactions with a biological microenvironment through *in vitro* microengineered physiological systems. These *in vitro* platforms, otherwise known as “organ-on-a-chip” systems, are capable of more accurately replicating the physiological tissue environment over traditional *in vitro* assays through the inclusion of key parameters needed for appropriate cellular functionality [19]. In the case of NPs delivered intravenously, studies examining the interactions between NPs and blood-vascular components are of great importance. Substantial research has presented 3D co-culture systems of vascular endothelial cells (ECs) and mural cells (e.g., smooth muscle cells, pericytes, fibroblasts) with a lumen structure that improves the physiological relevance over non-lumen based models [20]. With such a platform commonly used to study endothelial responses such as permeability and angiogenesis [21,22], probing the capacity of HDL-mimetics to exert hallmark properties of HDL, such as stimulating the production of endothelial NO [23], is a critical step in their comparative analysis.

In this work, we conducted a comparative analysis between the use of apoA1 and r4F in HDL-mimetics. We first developed and implemented a novel microfluidic platform with which to synthesize engineered HDL-mimetic NPs (eHNPs) made from r4F and apoA1. Through the combined results of experimental and computational NP characterization techniques, our findings initially drew parallels across the various physical and chemical properties shared between the eHNPs including NP structure, size, stability, and composition. We then applied an innovative translational nanomedicine approach by pairing a microengineered vasculature system to screen eHNP function *in vitro* with a murine model to examine eHNP drug delivery to vascularized tumors *in vivo*. Our initial comparisons included eHNP interactions with cells that commonly interact with HDL; we then probed eHNP interactions with macrophages and ECs in the context of prevalent diseases such as CVD and cancer for the purposes of delivering incorporated theranostic agents. Analyzing the computational and experimental results unraveled distinct differences between the eHNPs that were tied to NP stability and the mimicry of hallmark HDL functions linked to the SR-B1 pathway.

2. Results and discussion

2.1. eHNPs share similarities in form, but they have distinct temporal differences

The engineering of NPs benefits from incorporating both computational and experimental methods to comprehensively understand the similarities and differences between different NP formulations. Comparisons between eHNP-A1 and eHNP-r4F made through molecular dynamics (MD) simulations showed similar discoidal structures for the self-assembled NPs (Fig. 1A). Transmission electron microscopy (TEM) micrographs also showed discoidal structures for both eHNP-A1 (Fig. 1B) and eHNP-r4F (Fig. 1C), the stacking structures of which were artifacts of the negative staining techniques in preparing the eHNPs for TEM imaging [24]. The synthesis of the eHNPs was accomplished using our new microfluidic platform that contains a micropost array to induce the