

Nanodevices for Treatment of Hyperlipidemia.

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Introduction: Atherosclerosis, the leading cause of death in the developed world, is responsible for more than half of the yearly mortality in the United States. One of the most important risk factors for atherosclerosis is hyperlipidemia, characterized by elevated blood levels of low-density lipoproteins (LDLs). Excessive LDLs can accumulate in the vicinity of a small vascular injury, forming so-called fatty streaks, the first manifestation of atherosclerotic plaque. Hyperlipidemia is often related to the lack of LDL-receptors in hepatocytes, which consequently couldn't recognize LDLs and make impossible their further metabolism. Current strategies to treat hyperlipidemia include treatment by statins, which decrease cholesterol synthesis by the organism resulting in increased uptake of dietary cholesterol carried by LDLs. Other approaches focus on inducing increased LDL uptake by hepatocytes. Such treatments result in up to 50% decrease in blood LDL level. However, in many cases it is desirable to further decrease this index. This necessitates development of new strategies for **periodically cleaning up** excessive LDLs. We propose to use biodegradable polymeric nanoparticles to enhance delivery of low-density lipoproteins to liver. During endocytosis, LDLs fuse with lysosomes in hepatocytes and are digested by lysosomal acid hydrolases. Polymeric nanoparticles, on the other hand, are known to be actively uptaken by Kupffer cells in liver with the half life of several minutes. We propose to use monoclonal antibody to a major LDL component, human apolipoprotein B-100, covalently attached to biocompatible polylactide nanoparticles. After injection into circulation, such nanoparticles will adsorb LDLs via antibody-antigen interactions, and these complexes will be quickly uptaken by Kupffer cells (**Figure 1**). The Kupffer cells attempt to digest the uptaken material by directing it to lysosomes, thus providing a biochemical pathway similar to that for normally uptaken LDLs.

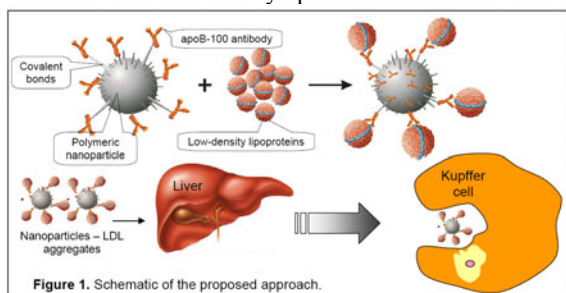


Figure 1. Schematic of the proposed approach.

Methods: Monoclonal antibody to human apolipoprotein-B (Apo-B, Meridian Life Science Inc., ME) was covalently attached to polybutylcyanoacrylate (PBCA) and polylactic acid (PLA) nanoparticles synthesized by us. PBCA nanoparticles were prepared by a fabrication procedure in acidic polymerization medium using Dextran 70 as a surfactant and stabilizer. The nanoprecipitation method was employed for the formation of PLA nanoparticles. Briefly, poly-(D,L-lactide) was dissolved in DMF. Nanoparticles were formed by adding the polymer solution to water, a non-

solvent, with ultrasonic treatment for 30 min. PLA NPs were purified by centrifugation (30 min, 3000g) and washed 2 times with HPLC water. Particle size (100 ± 20 nm) was determined using Atomic Force Microscopy (Veeco Dimensions 3100) and Dynamic Light Scattering (Brookhaven 90Plus). Sulfo-HSAB cross-linkers (Pierce Biotechnologies, Rockford, IL) were used to covalently bound antibody to human apolipoprotein-B to nanoparticles. Protein was first fluorescently labeled using AlexaFluor® Succinimidyl Ester dye, and then conjugated to nanoparticle. Protein-nanoparticle conjugates were separated from unreacted proteins by centrifugation. The amount of enzyme covalently attached to nanoparticles was quantified using fluorescently labeled antibody. When mixed with low-density lipoprotein solution, Ab-nanoparticle conjugates cause immunoprecipitation. In attempt to avoid precipitation, we prepared a series of dilutions of the initial nanoparticle suspension. We found the critical concentration of the anti-ApoB100-nanoparticle conjugates, which does not result in visible immunoprecipitation in the presence of 500 mg/dl LDL solution. To confirm that LDL-antibody interactions did take place for this system, we determined particle size in the suspension dynamic light scattering (DLS). Presence of the particles with the average diameter of ~ 818 nm confirms the formation LDL-anti-apoB100-nanoparticle complexes (particle size for anti-apoB100-coated nanoparticles in suspension is ~ 140 nm, while particle size for pure LDL controls is ~ 20 nm, as measured by DLS). Thus, we demonstrated that anti-apoB100 nanoparticles can effectively absorb LDLs and their concentration can be adjusted in such a way that resulting LDL-anti-apoB100-nanoparticle complexes are less than $1\ \mu\text{m}$ in diameter.

Results: Uptake of fluorescently labeled LDL-conjugate complexes by Kupffer cells was studied using a fluorescence microscope. Cells were isolated by incubating mixed non-parenchymal cells, which were obtained by collagenase digestion of the rat liver, with CD68-conjugated superparamagnetic polystyrene beads. Kupffer cell cultures were treated with fluorescently-labeled LDL-conjugate complexes. Suspension of fluorescently labeled free LDLs was used as a control. After washing, cells were investigated with the microscope to determine whether or not binding to macrophages had occurred. It was found that LDL-nanoparticles conjugates were uptake by Kupffer cells. After that live/dead cell assay kit was used to check viability of treated Kupffer cells.

Conclusions: In conclusion, we found that antibody to human apolipoprotein-B can be covalently attached to 100 nm PBCA and PLA nanoparticles without considerable changes in receptor-binding ability. Antibody-nanoparticle conjugates reacted with low-density lipoprotein forms ~ 800 nm aggregates and such aggregates can be uptake by Kupffer cells.

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