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Differentiation of oxidized low density lipoproteins by nanosensors

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Abstract

Oxidized low density lipoprotein (oxLDL) is considered a biomarker for acute heart attack in patients with coronary artery disease (CAD). LDL cholesterol in the circulatory system can undergo oxidative modification to oxidized LDL (oxLDL), leading to the development of CAD. We tested whether indium oxide (In_2O_3) nanowires network- and carbon nanotube network-based field effect transistors (FETs) were able to differentiate the LDL cholesterol between the reduced (native LDL) and the oxidized state (oxLDL). LDL samples isolated from human plasma were exposed to In_2O_3 FETs, and conductivities and gating characteristics were obtained as current versus drain-source voltage (I_D–V_{DS}), and current versus gate-source voltage (I_D–V_{GS}). A higher conductivity was observed in the LDL sample containing 15.1% oxLDL relative to the sample containing 4.4% oxLDL. The results were validated by high performance liquid chromatography (HPLC). Next, carbon nanotube network-based FETs conjugated with anti-copper oxLDL antibody were exposed to the LDL samples. Distinct conductivities between nLDL and oxLDL were also observed from the I_D versus time domain curve in the presence of bovine serum albumin (BSA), demonstrating nano-scale sensors as potential lab-on-a-chip devices for detection of oxLDL cholesterol.

Keywords: Oxidized low density lipoprotein (LDL); Indium oxide (In_2O_3) nanowires; Field effect transistor; Carbon nanotubes

1. Introduction

Cardiovascular diseases are the emergent global health issue largely owing to the accelerating prevalence in the developing nations [1]. Oxidized LDL cholesterol (oxLDL) is known to initiate the development of coronary artery disease [2]. Elevated serum level of oxLDL predicts acute heart attack or coronary syndromes [3–5]. High performance liquid chromatography (HPLC) has been the mainstay to fractionate the percentage of oxLDL in LDL samples from the human plasma [6]. However, the development of nano-scale sensors provides a potential lab-on-chip capability to detect oxLDL cholesterol in a small amount of LDL samples.

Nanowire-based field effect transistors (FETs) have been demonstrated in biochemical applications. The development of nanowires has been based on a family of oxides harboring interesting optical and electrical properties. These binary oxide nanowires include GeO_2, Ga_2O_3, MgO_4 and SiO_2 [7]. Indium oxide (In_2O_3) represents a wide band gap transparent conductor with a direct and indirect band gaps at ∼3.6 and at ∼2.6 eV, respectively [8,9]. This unique property provides a broad spectrum of applications from solar cells to liquid crystal displays [10]. In addition to the application of chemical sensors [11], boron-doped silicon nanowire-based FETs have also been capable of detecting calcium ion (Ca^{2+}) [12]. Also, the tin-doped indium oxide thin film (In_2O_3: Sn, ITO)

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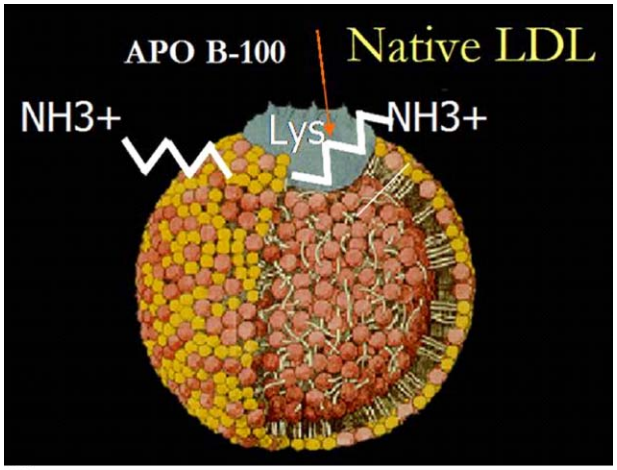
has been applied for flat panel displays in biomedical instruments by virtue of its high electrical conductivity and optical transparency [13].

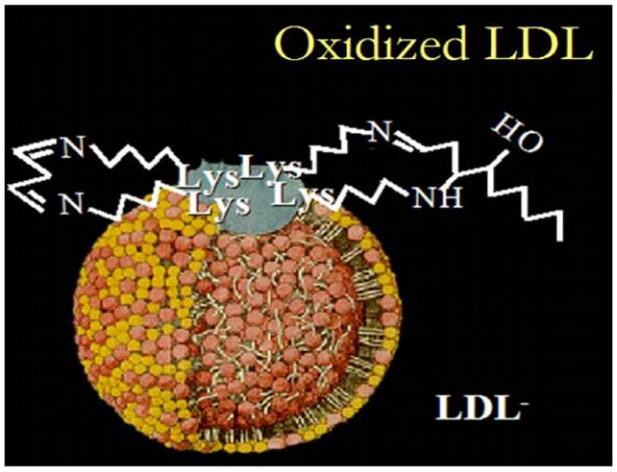
Recently, In_2O_3 nanowires-based FETs have been demonstrated as a sensitive chemical sensor for NO_2 and NH_3 [14]. In_2O_3 functions as the n-type semiconductor as a result of oxygen vacancy doping [15]. The level of doping is inversely related to oxygen concentration. When an oxygen vacancy is present, a vacancy level appears in the band gap. The vacancy level is composed of In-5 sp orbital hybridized with O-2p orbital which exhibits a strong In–In interaction. Occupation of electrons to the oxygen vacancy results in a stronger In–In bonding strength. Thus, the change in In_2O_3 conductance is due to the electron transfer between the nanowire and the target molecules.

In this context, the redox property of LDL cholesterol permits the nanosensors to accumulate or deplete electrons. The individual LDL cholesterol is approximately 22 nm in diameter with a molecular weight of 2300 kDa (kilo-Dalton). The LDL cholesterol contains an Apo B-100 lipoprotein (550 kDa) [16] which is distinct from high density lipoprotein (HDL). The Apo B-100 lipoprotein contains lysine residues which undergo oxidation in blood or arterial walls; thereby, converting reduced state of LDL (nLDL) to the oxidate state (oxLDL) (Fig. 1). These oxLDL particles trigger vascular oxidative stress and recruitment of inflammatory cells into the vessel walls. The transmigration of these cells, specifically, monocytes, from blood into arterial walls is a crucial event in initiating coronary artery disease [17]. Therefore, the level of circulating oxLDL in human plasma is considered as an emergent marker to predict acute coronary syndromes.

We hereby investigated the effects of reduced nLDL and oxidized LDL cholesterol on the conductivities of In_2O_3 nanowires and carbon nanotubes. The LDL sample containing 15.1% oxLDL particles increased the conductance of the n-type In_2O_3 nanowire-based FET relative to the sample containing 4.4% oxLDL as demonstrated by the current versus drain-source voltage (I_D–V_{DS}) and current versus gate-source voltage (I_D–V_{GS}) plots and validated by high performance liquid chromatography. The identical LDL samples were exposed to the p-type carbon nanotube network. The LDL sample containing 15.1% oxLDL particles decreased the conductance of the p-type carbon nanotube network relative to the sample containing 4.4% oxLDL, demonstrating a complementary response by the $I_D - V_{DS}$ [18]. Furthermore, the carbon nanotube network-based FETs were conjugated with anti-copper oxLDL antibodies and were exposed to the LDL samples in the presence of bovine serum albumin (BSA), a ubiquitous protein carrier in the blood. The LDL sample containing 15.1% oxLDL particles demonstrated high peaks in the I_D versus time plot. Despite the high sensitivity of nanowire-based FETs [19]; device-to-device variations exist among individual transistors. The development and application of high density carbon nanotube network-based FETs which were conjugated with antibodies specific for oxLDL enhanced the selective detection of oxLDL cholesterol.



(a)



(b)

Fig. 1. Native vs. oxidized LDL particles. (a) Native LDL represents ~95% of total LDL particles. Oxidative modification of LDL involves alterations in both the protein and lipid components of the LDL particles. Progressive oxidation of the apoprotein is associated with the loss of specific amino acid residues sensitive to oxidation, such as lysine, tyrosine and cysteine [28]. (b) OxLDL or LDL^-, which is found in plasma in vivo, is characterized by its electronegativity and oxidative status [6]. OxLDL represents 0.2–8% of total LDL particles, and is strongly associated with an increased risk of atherosclerosis [29]. Three possible sources of oxLDL are: (1) oxidation of LDL trapped in the arterial wall; (2) ingestion of oxidants or generation from postprandial lipoprotein remnants [30]; and (3) oxidation in plasma [31].

2. Materials and methods

Bovine serum albumin and methoxypolyethylene glycol imidazolyl carbonyl were purchased from Sigma–Aldrich. Copper-induced oxLDL antibodies were purchased from Biodesign Co, MA. LDL samples were obtained from fasting adult human volunteers under institutional review board approval at the University of Southern California Atherosclerosis Research Unit, Department of Medicine.