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Immobilization of avidin on (001) GaAs surface

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ABSTRACT To reliably immobilize different biomoieties on surfaces of III-V semiconductors is one of the most critical issues in the development of biodetector devices based on the optical/electronic properties of these materials. Herein we demonstrate the successful immobilization of avidin, a robust and well-studied protein, on a (001) GaAs surface. The immobilization was investigated via specific binding to biotin, which was connected to the GaAs surface through commercially available long- or short-chain amino group terminated alkanethiols ($HS(CH_2)_{11}NH_2$ or $HS(CH_2)_2NH_2$), or through a biotinylated thiol synthesized in our laboratory. The immobilization performance was evaluated by photoluminescence and fluorescence microscopy measurements. We found that the biotinylated thiol mixed with a diluent thiol provides the highest avidin immobilization efficiency.

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1 Introduction

In the quest for the development of various biosensors and biochips, a great deal of attention has been focused on seeking efficient methods to immobilize probing biomolecules on the surface of quantum dots (nanocrystals) and solid substrates, such as Au [1–7], Si/SiO_2 [8, 9], polymers [10–13] and indium tin oxide (ITO) [14]. Biomolecules of interest can be directly immobilized on solid substrates by covalent linkage using carbodiimide chemistry [15, 16]. However, the direct immobilization approach cannot control the molecular orientation. It has been reported that randomly oriented streptavidin films bind with biotin-DNA less efficiently when compared to biotin-DNA assembled on molecularly ordered streptavidin films [16]. The consequence is lower efficiency of the target DNA capture and lower sensitivity in the hybridization analysis achieved with randomly oriented streptavidin films. To maximize the uptake of probe biomolecules and consequently capture more target moieties for detection, the most often employed configuration consists of the substrate/biotin/avidin/biotinylated probe/target architecture [3, 8, 9, 16, 17].

GaAs is an important material for electronic and photonic devices because of its high electron mobility and a direct band gap structure [18], which result in a strong photoluminescence (PL) signal from this material. The high sensitivity of GaAs PL emission to the physical and chemical state of its surface offers the potential advantage of this material in the development of a new generation of biosensors and biochips. This advantage is expected to be further enhanced by implementing optical detection based on GaAs quantum dots or nanocrystals. Clearly, the ability to efficiently immobilize targeted biomolecules on the surface of GaAs is of paramount importance for the successful development of novel biosensors based on GaAs and, potentially, on other III-V semiconductors.

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We have recently reported [19, 20] photoluminescence and attenuated total reflection (ATR) Fourier transform infra-red (FTIR) spectroscopy data demonstrating the superior efficiency of long-chain thiols, in comparison to short-chain thiols, for passivating the surface of (001) GaAs. These findings have also been corroborated by X-ray photoelectron spectroscopy (XPS) [21] as well as aging effects measurements [22]. In this report, we demonstrate the successful immobilization of avidin on the surface of (001) GaAs that was functionalized with a long-chain biotinylated thiol.

2 Experimental

The following chemicals were purchased from Aldrich: cysteamine (T2: $HS(CH_2)_2NH_2$), N-hydroxysuccinimidobiotin (NHS-biotin), 10×Tris buffer solution (pH = 7.4) and dimethyl sulfoxide (DMSO, for molecular biology). 11-amino-1-undecanethiol (T11: $HS(CH_2)_{11}NH_2$) was purchased from Dojindo Molecular Technologies Inc. Avidin (from egg white) and avidin fluorescein conjugate were products from Molecular Probes. Biotinylated thiol (TB: 2-(11-biotinylamino-undecanoylamino)-3-mercapto-propionic acid) and a thiol diluent (TD: 2-(11-acetylamino-undecanoylamino)-3-mercapto-propionic acid) were synthesized in our laboratory following the procedure of solid-phase synthesis of peptides. The chemical structures of TB and TD are shown in Fig. 1. The nominally undoped epi-ready (001) GaAs wafer (n-type at room temperature) was purchased from Atomergic Chemical Corp.

The (001) GaAs wafer was cleaned in an ultrasonic bath sequentially with OptiClear, acetone and isopropanol for

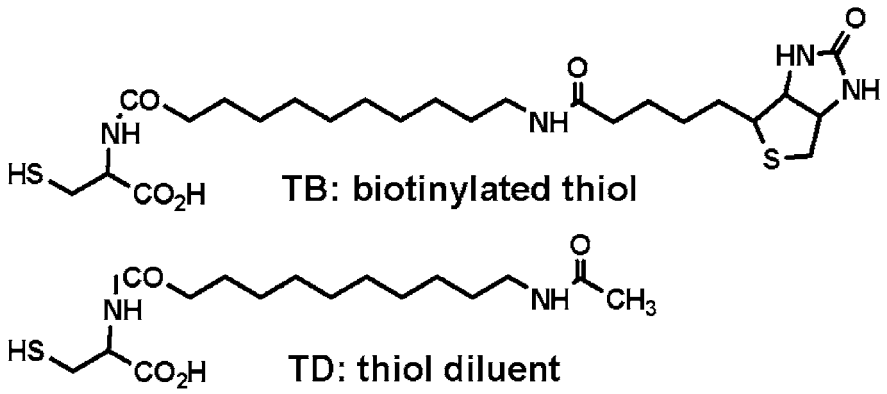


FIGURE 1 Chemical structure of biotinylated thiol (TB) and thiol diluent (TD)

5 min each. The wafer was then etched in concentrated HCl for 1 min and, subsequently, rinsed with deionized water. After drying in nitrogen flow, the wafer was immersed in a 5 mM solution (T2, T11 or TB) of ethanol and 5% aqueous ammonia that was degassed with nitrogen flow for 2 h. TB was mixed with TD with the molar ratio of 1 : 1 and the total concentration was 5 mM. The solution was heated to 55 °C with continuous N_2 purging. After the 18-h exposure, the wafer was rinsed with hot isopropanol, methanol and water, and finally blow dried with nitrogen. Biotinylated surfaces were obtained either directly from TB or by the reaction between surface amino groups (from T2 or T11) and NHS-biotin (200 μg/ml in DMSO) for 3 h. Exposure to avidin was carried out by immersing the wafer in 200 μg/ml avidin in Tris buffer (pH 7.4) for 2 h, which was followed by rinsing with Tris buffer and deionized water and drying with nitrogen flow.

The PL response from (001) GaAs, which was measured at room temperature in the band gap emission region of GaAs ($\lambda \approx 870$ nm), was used to monitor each step of the surface modification with various thiols, a biotinylated thiol, a NHS-biotin and an avidin. The PL signal was excited with a laser operating at 532 nm and measured with a PL mapper (Philips PLM-150). The signal was collected with an IR array of InGaAs detectors. Typically, a PL map was collected at a 100-micrometer step, which for a 5 mm × 5 mm sample gave the intensity result based on the average from 2500 points. We found that the cleaning and etching procedure resulted in an up to three-fold increase of the PL signal from the investigated samples. Consequently, each time two samples were prepared with etched surfaces: one for coating with a thiol or avidin, and another for reference. PL measurements for both samples were carried out after nominally the same time elapsed from their etching; typically after 24 to 26 h. Aging effects in etched, thiolated and biotin-coated GaAs samples have been reported elsewhere [22].

Fluorescence from immobilized avidin–fluorescein conjugates was observed with a fluorescence microscope (Leica DMRX Microsystems Digital Imaging with DC300 camera). The fluorescein chromophore emits at 518–523 nm and we used a blue light source emitting at 450–490 nm to excite this material.

3 Results and discussion

Various procedures that we investigated for avidin immobilization have been schematically depicted in Fig. 2. Our method of depositing thiols on etched surfaces of (001) GaAs is similar to that reported by Hou et al. [23]. But, we have compared the PL signal enhancement using an etched, instead of oxidized, GaAs sample as reference. We believe that this is a more adequate approach to illustrate the influence of thiols and other ad-molecules on PL from GaAs, as the etching alone resulted in up to three-fold enhancement of the PL signal compared with highly oxidized GaAs.

The results of PL intensity measurements for various interfaces are summarized with the bar chart in Fig. 3. It can be seen that a direct coating of (001) GaAs with avidin has resulted in the increase of the PL signal by up to 2.5 times (case a). Avidin is positively charged in a Tris buffer solution; thus, it is reasonable to expect that it could influence the electric field of the surface state dependent depletion width near the GaAs surface. For the investigated n-type GaAs material, this could take place if the adsorbed molecule (avidin) released an electron and effectively returned a surface-trapped charge to the semiconductor bulk and reduced the surface electric field. The resulting contraction in the depletion width could explain the increase of the PL signal [24].

A short-chain thiol (T2) deposition (case b in Fig. 3) did not result in any measurable PL enhancement. A significant PL enhancement was also not observed following the deposition of biotin on such a surface. It is well known that the sulfur in alkanethiols can ef-

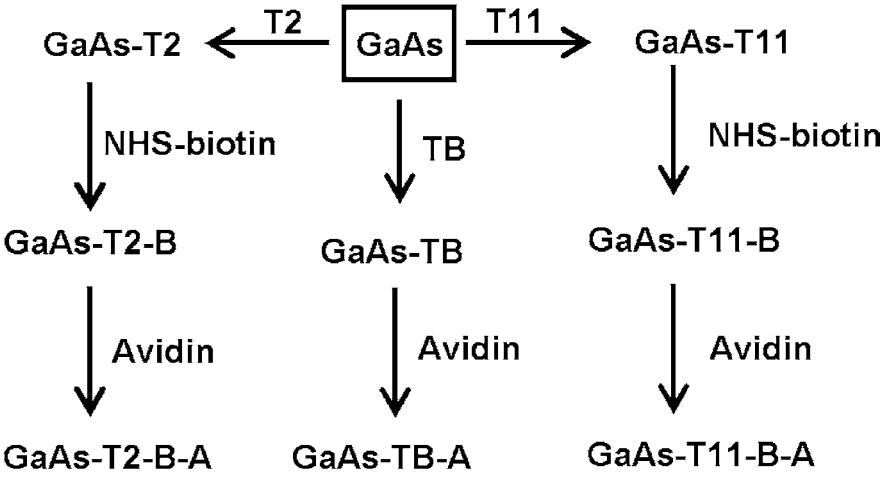


FIGURE 2 Procedures to immobilize avidin on the surface of (001) GaAs (T2: $HS(CH_2)NH_2$, T11: $HS(CH_2)_{11}NH_2$, B: biotin, A: avidin)

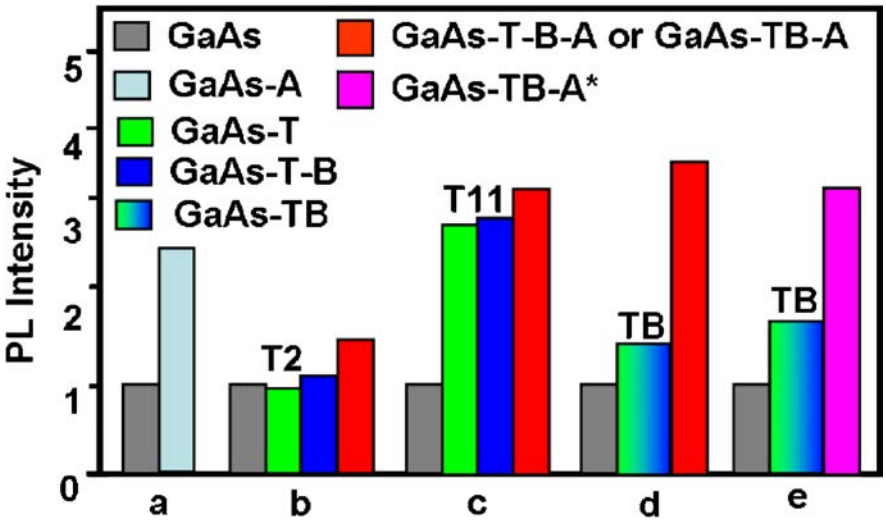


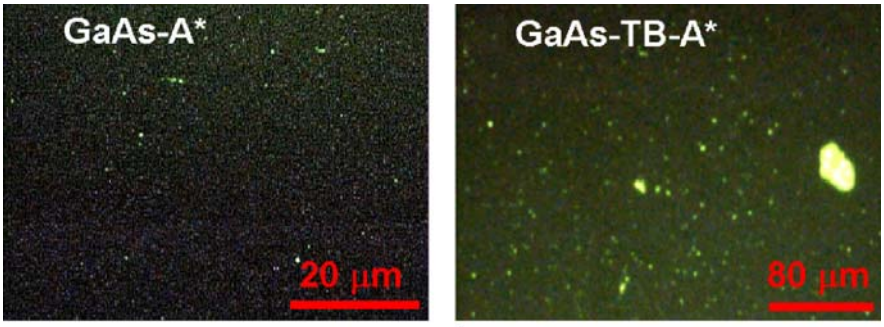
FIGURE 3 PL intensity from (001) GaAs having various interfaces on the surface; here 'T' in the figure description refers to T2 and T11 in sample sets b and c, respectively

fectively passivate the surface states in GaAs and unpin the surface Fermi level, therefore enhancing the photoluminescence signal [25–28]. However, based on the observation of the short-chain thiol–Au interface and the weak Van der Waals interaction between short alkyl chains [29], it is expected that the T2 thiol on a GaAs surface will not form a highly condensed thin film. Thus, such an alkanethiol will not efficiently neutralize the PL reducing surface states. Neither will it sufficiently protect the GaAs surface against poisonous reactions with ambient moisture and oxygen. After the surface amino groups ($-NH_2$) reacted with biotin, the biotinylated interface seemed to affect the surface more efficiently than T2 only, although only a small PL enhancement (10%) was observed. Deposition of avidin on top of such an interface further increased the PL signal to an overall intensity of 50% greater when compared with that of the reference GaAs wafer. It is possible that the origin of this enhancement is the same as for the case a in Fig. 3, i.e. due to avidin being directly adsorbed on the surface of GaAs that is partially covered with T2. However, it is also feasible that the avidin molecules trapped through the biotin–avidin interaction could lead to some enhancement of the PL signal.

A deposition of long-chain alkanethiols (T11) on (001) GaAs resulted in the up to three-fold enhancement of the PL signal (case c in Fig. 3). Due to the stronger Van der Waals interaction between the long alkyl chains, the T11 molecules tend to be highly packed and well ordered. For instance, the increased coverage of Au with sulfur has been observed for long-chain thiols [29]. It is reasonable to expect that a similar result would apply to the (001) GaAs surface. This would lead to a significant reduction of non-radiative recombination centers and, consequently, an enhancement of the PL signal. Also, long-chain thiols would form an efficient barrier to protect the GaAs surface from the interaction with ambient moisture and oxygen to a better extent than a thinner barrier produced by short-chain alkanethiols. The ellipsometry measurements have indicated that the T11 layer is 0.55-nm thick, which suggests that the T11 chain tilts approximately 70° from the surface normal. This is more than the 57° reported for the octadecylthiol deposited on (001) GaAs [24]. A possible reason for this tilt difference could be related to the more hydrophilic nature of the T11 amino terminal group. Deposition of biotin followed by that of avidin led to the further enhancement of the PL signal. The amplitude of this enhancement was comparable to that from the same event on the GaAs–T2 interface. Although the GaAs–T11 interface facilitates the attachment of a higher density of surface amino groups and therefore a higher concentration of biotin molecules, these biotin headgroups are closely packed and only part of them are accessible for avidin to form biotin–avidin complexes. To create better binding between avidin and biotin headgroups, the concentration of biotin headgroups on the surface of GaAs should be diluted [30].

A deposition of biotinylated thiol (TB) on (001) GaAs was carried out from the mixture with another thiol (TD), which had a similar chemical structure but no biotin headgroup (cases d and e in Fig. 3). Because TD was synthesized following the approach to synthesize peptides in the solid phase, there is a carboxylic acid group ($-CO_2H$) near the $-HS$ (as shown in Fig. 1). The hydrophilic carboxylic acid group induces the base section of this molecule ($HSCH_2(CO_2H)CH-$) to closely contact the GaAs surface, while the rest of its branch remains tilted to the surface normal. Following the results suggesting that maximum avidin uptake occurs at a biotin composition of 50–60 [30], we applied a 50/50 TB–TD solution in our study. The GaAs–(TB + TD) interface was found to be stable at an ambient environment for 3–4 weeks, as judged by the almost unchanged intensity of the PL signal. Likely due to the relatively low surface density of sulfur atoms, such treatment induced only a 50% PL enhancement over the bare GaAs wafer. A strong efficiency of the avidin uptake on such a surface, however, has been confirmed by a significant increase (about 3.5 times) of the PL signal in comparison to that from an etched and unprotected GaAs surface. A similar amplitude PL enhancement was observed for the sample coated with the fluorescein-stained avidin deposited on the TB surface (case e in Fig. 3). It should be underlined that qualitatively similar results concerning the PL enhancement from the thiol-coated GaAs surface have been previously observed by us [19, 20]. The long methylene chain thiols have systematically induced a stronger PL signal from GaAs than those with short chains, as observed with low-temperature PL measurements. Of particular importance for practical applications is that the PL results reported in this paper have been obtained at room temperature.

The PL spectroscopy results and the expected stability of the GaAs–thiol–biotin–avidin interface have been corroborated by the measurements of the fluorescence from stained avidin that was deposited directly on GaAs or on



a b

FIGURE 4 Fluorescence microscope images of stained avidin (A*) directly deposited on GaAs surface (**a**) and stained avidin immobilized on GaAs via biotinylated thiol (TB) (**b**)

the GaAs surface coated either with T11–B or biotinylated thiol. Figure 4a shows a faint visible green fluorescence observed from the surface of GaAs that was directly coated with stained avidin. The scattered bright spots are likely due to the agglomeration of avidin in the vicinity of physical defects on the GaAs surface. After washing this wafer with detergent, the green emission disappeared entirely, which indicates that the avidin was not efficiently attached to this surface. Detergent washing also removed stained avidin from the GaAs–T11–B surface (picture not shown). In contrast, the fluorescence from stained avidin immobilized on the GaAs–TB surface was relatively easy to detect as illustrated in Fig. 4b. Quantitative analysis of the fluorescence intensity from an area of 80 μm × 80 μm has indicated that the image in Fig. 4b is 2.4 times more intense than that in Fig. 4a. The large bright spots in this figure may originate from dust contamination or from the defect-mediated agglomeration of avidin. Qualitatively similar intensity fluorescence was observed from this sample following its washing with a standard detergent. We argue that this is strong evidence of the efficient attachment of avidin molecules to the surface of (001) GaAs via a specific biotin–avidin interaction. Such an interface is expected to be very stable and, consequently, could offer an advanced platform for developing GaAs-based biodetectors. It is not clear whether the fluorescence which survived detergent washing on the TB-coated sample can be ascribed to the specific biotin–avidin binding originating from TB, or whether it is due to the enhanced formation of the avidin–biotin interface in a TD diluted environment. Further investigations are required to clarify this issue.

4 Conclusions

In conclusion, a variety of GaAs–thiol interfaces have been investigated aiming to immobilize avidin on a (001) GaAs surface. Directly deposited avidin on a GaAs surface via physical adsorption does not survive detergent washing. Amino group terminated short chain alkanethiols are poorly organized on the surface and they cannot anchor a high concentration of biotin headgroups; therefore, they have a low efficiency of avidin intake. Long-chain alkanethiols with amino terminal groups form highly packed films on the surface, which significantly increase the PL signal from GaAs (measured in the band gap emission region). It appears however that due to the high density of biotin headgroups anchored on this surface, avidin cannot access all of them and only a small fraction of biotin–avidin binding occurs. The highest efficiency of avidin immobilization was observed on the surface of GaAs coated with a biotinylated thiol which was diluted with 2-(11-acetylamino-undecanoylamino)-3-mercapto-propionic acid. Most of the avidin molecules that were immobilized on the surface survived the detergent washing procedure as confirmed by the fluorescence microscopy measurements. We believe that these results represent important progress towards the development of a GaAs (bulk or quantum dot) based biodetector.

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