Novel Nano-biosensors for Life Science Systems and their Applications in Early, Accurate, and Non-invasive Melanoma and Other Types of Cancer Detection

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Abstract—**Melanoma (the 5th and 6th most common cancer in Caucasian males and females, respectively), is the most severe form of skin cancer, which is often fatal if recognized in its advanced stage. Melanoma is the tumor that originates from melanocytes (the cells that make the pigment melanin), and may develop from a nevus (commonly named "mole"). Clinically, it is very difficult to correctly differentiate nevi with atypical features or dysplastic nevi, and nevi of special sites from melanoma. Clearly, new, more powerful, less invasive, time consuming and expensive tools are needed for an early and accurate detection of melanoma. In order to address this need, we propose a development of a new set of tools, namely, carbon-nanotube-based biosensors for the early and accurate detection of melanoma. Once successful, we will modify and apply this new technology to early and accurately detect other types of cancer.**

I. INTRODUCTION

Scope of the problem

Nearly 35 years after President Nixon declared war on cancer, the disease remains a most elusive and destructive foe. Researchers know much more today than they did three decades ago about the 200 or more unique diseases known collectively as cancer. They are beginning to pin down each of the steps in the complex pathway that causes the body's own cells to mutiny and begin multiplying in a way that strays drastically and disastrously from the norm. But the war is far from over.

One of the deadly cancers, **melanoma**, often underestimated in its danger, is a form of skin cancer. Melanoma incidence rates are increasing every year. Currently, the melanoma is the 5th and 6th most common tumor in Caucasian males and females, respectively. According to the American Cancer Society, approximately 62,190 men and women (34,260 men and 27,930 women) were diagnosed with melanoma of the skin in 2006, and about 7,910 men and women died for melanoma in the same year.

This is very significant. Yet, many underestimate the importance of early and accurate detection of this disease, assuming that the human eye of the dermatology doctor will catch a problem just in time. However, this is often not the case. It is often very difficult to correctly diagnose a melanoma and differentiate it from an indolent nevus lesion that would never acquire malignant potential. Thus, unnecessary surgeries, which are both expensive and traumatic, are often conducted to avoid missing a potential deadly form of cancer.

A large epidemiological study published a few years ago in JAMA, aimed at quantifying melanoma risk associated with nevi, found that risk for melanoma was strongly related to number of small nevi, large non-dysplastic nevi, and clinically dysplastic nevi. In the absence of dysplastic nevi, increased numbers of small nevi was associated with an approximately 2-fold risk, and increased numbers of both small and large non-dysplastic nevi was associated with a 4-fold risk. One clinically dysplastic nevus was associated with a 2-fold risk (95% confidence interval, 1.4-3.6), while 10 or more conferred a 12-fold increased risk (95% confidence interval, 4.4-31)[1]. Interestingly, similar results were observed in Mediterranean populations, typically considered at lower risk of melanoma, suggesting that these risk factors are important across geographical regions[2]. Nevi and dysplastic nevi are common in Caucasians, particularly in melanoma-prone families, where it is not infrequent to detect hundreds of nevi per person.

The most difficult problem

The biggest problem is the **difficulty in detecting a nevus with malignant potential…**

The sequential progression model for melanocytic tumors from common nevus to malignant melanoma was proposed by Clark almost 30 years ago. The "dysplastic nevus" has frequently been considered a logical offspring of this concept and as a direct precursor of melanoma, analogous to the epithelia - dysplasia - carcinoma sequence. Despite the use of modern molecular methods, there is no consensus as to if the dysplastic nevus represents a true precursor lesion of melanoma, a separate distinct type of nevus, or a diagnostic dilemma.

Currently, the concept of melanocytic dysplasia remains subject to confusing definitions at all levels of the diagnostic process, i.e. clinical appearance, dermatohistopathology, and molecular biology. Nevi fulfilling Clark and Elder' sclassic histological criteria mostly represent "endpoints" of nevocytic evolution, whereas a minority of "dysplastic nevi" represents true melanoma precursors[3]. The unsolved dilemma is that neither clinical, histopathological nor molecular or dermatoscopy criteria exist to make a distinction between dysplastic nevi and early melanomas.

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A relatively common, recently recognized, and growing group of nevi may simulate both dysplastic nevi and melanomas. These lesions have been termed, generically, ' nevi of special sites' . These lesions may show architectural features overlapping with melanomas and may be over diagnosed as such. If not interpreted as a melanoma, they may be classified as dysplastic nevi. However, many of these lesions appear to be isolated phenomena, not associated with measurably increased melanoma risk. At the same time, authentic dysplastic nevi may occur in these same ' special sites' . It is just as important to recognize truly dysplastic nevi as such, as it is to avoid over diagnosis of nevi of special sites as dysplastic nevi, or as melanomas. In addition to dysplastic nevi and nevi of special sites, other skin lesions, such as Spitz nevi, may resemble melanoma. Moreover, melanoma may develop in areas not frequently examined, such as the vagina, or develop without typical melanin component (acromic melanomas) making the diagnosis of such disease very challenging even to an expert and skilled dermatologist.

II. PROPOSED POTENTIAL SOLUTIONS

In order to address this need, we propose a development of a new set of tools, namely, carbon-nanotube-based biosensors for the early and accurate detection of the melanoma.

Recently, we have witnessed significant interest in biological and medical applications of novel inorganic nano-materials such as nanowires and nanotubes for creation of new types of analytical tools for biotechnology, life sciences and medical diagnostics. Planar semiconductors can serve as the basis for chemical and biological sensors in which detection can be monitored electrically and/or optically. For example, a planar field effect transistor (FET) can be configured as a sensor by modifying the gate oxide (without gate electrode) with molecular receptors or a selective membrane for the analyte of interest; binding of a charged species then results in depletion or accumulation of carriers within the transistor structure. An attractive feature of such chemically sensitive FETs is that binding can be monitored by a direct change in conductance or related electrical property, although the sensitivity and potential for integration are limited. The physical properties limiting sensor devices fabricated in planar semiconductors can be readily overcome by exploiting nano-scale FETs. Such FETs have now been realized from both carbon nanotubes[4,5] and semiconductor nanowires (for a recent review see ref. 6). Indeed, their unique properties have generated a wealth of research on nano-electronic devices and nanosensors. First, binding to the surface of a nanowire (NW) or nanotube (NT) can lead to depletion or accumulation of carriers in the "bulk" of the nanometer diameter structure (versus only the surface region of a planar device) and increase sensitivity to the point that single-molecule detection is possible. Second, the small size of NW and NT building blocks and recent advances in assembly indicates that dense arrays of sensors could be prepared.

Because semiconducting SWNTs have a very high mobility and all their atoms are located at the surface, they are the ideal material for ultra small sensors. Kong *et al.*[7] were the first to build a SWNT chemical sensor for the detection of NO_2 and NH_3 gas. Calculations suggested that direct binding of electron withdrawing NO_2 or electron-donating NH_3 gas molecules to the NT surface chemically gated these devices. Following this initial success, many research groups started to construct SWNT chemical and biological nanosensors[8-15]. Recently, our group at Caltech has developed nanotube sensors incorporated into microfluidic channels to detect ionic and fluidic transport[16]. An individual nanotube is operated as an electrochemically gated transistor, as shown in Fig. 1. Establishing a changing fluid flow rate shifts the transistor threshold by an amount proportional to the flow rate because of the induced streaming potential. This setup is readily adapted for the proposed cancer detection experiments, with the addition of suitable chemical functionalization of the oxide or the nanotube itself. The docking of the analyte with the complementary target will alter the charge environment around the nanotube and shift the transistor threshold, enabling detection of the chosen markers (schematic diagram shown in Fig. 2).

Indeed, such a setup based on nanotubes or nanowires has already been recognized as a very promising system for biosensors. In a first step, Chen et al.[13] immobilized proteins on the sidewall of carbon nanotubes through a linking molecule, but they did not report the electrical characteristics. Proteins carry pH-dependent charged groups that can electrostatically gate a semiconducting SWNT, creating the possibility to construct a nano-size protein and/or pH sensor. Even more interesting, redox enzymes go through a catalytic reaction cycle where groups in the enzyme temporarily change their charge state and conformational changes occur in the enzyme. This enzymatic activity can potentially be monitored with a nanotube sensor.

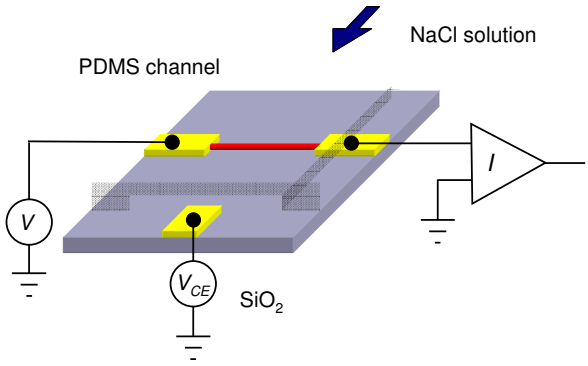

Fig. 1: Experimental setup for nanotube flow sensor. An ionic solution is passed through a microfluidic channel that has an integrated individual single-walled nanotube transistor. The voltage V_{CE} applied to the counter-electrode acts as a gate voltage, and the conductance is measured through the attached source and drain electrodes.

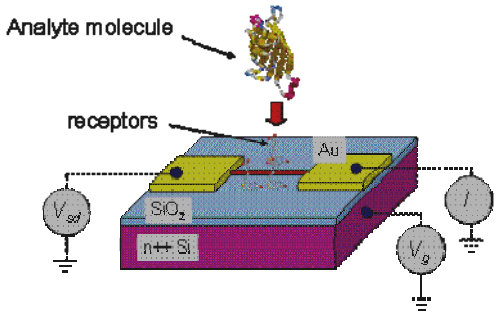

Fig. 2. Schematic of nanosensor field-effect transistor (FET) based on nanowire or nanotube (shown in red).

Nanotubes have potentially an advantage over the nanowires, since they have a diameter that is 1 order of magnitude smaller than the nanowires, therefore there might be an increased sensitivity to be discovered in detecting small molecules. In addition, their surface is directly exposed to the environment, circumventing any issues that may arise from the presence of a surface oxide. In our work, we plan to use the network nanotube array devices (NTNFETs), among other nanotube-based sensors, to be most effective in this investigation as label free detection devices. An electron microscope image of a NTNFET is shown in Fig. 3, taken from the work of Alexander Star, *et al.*[15] Label-free detection has several advantages including cost, time, and simplicity. Hand-held field-ready devices as opposed to laboratory methods using labor-intensive labeling and sophisticated optical equipment will be enabled by this approach.

III. SECONDARY AIM

Since it is necessary to sense a set of cancer markers for melanoma simultaneously, we need to design a sensing platform for detection of a set of well-established cancer markers used and proposed to be used for diagnosis now. These include MART-1 (MelanA). This antibody stains all melanocytes, benign and malignant. It is helpful in diagnosing melanoma in situ *vs.* a dysplastic nevus; and Ki-67, a proliferation marker. Other newly proposed markers of invasiveness and metastatic dissemination of melanoma will be also tested, not only for early detection of melanoma, but potentially also for prognosis. Examples of additional markers that could be tested are listed in Table 1. [17]

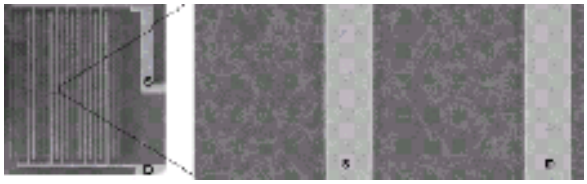

Fig. 3. Scanning electron microscope image of a nanotube network FET. The distance between the source (S) and drain (D) electrodes is 10 μm. Image taken from ref. 15.

Marker	Correlation with tumor thickness	Correlation with pathological stages	Correlation with survival	Independent prognostic marker
p-AKT		×	×	×
Ephrin A1	×		×	
Apaf-1		×		
Bcl-2		×	×	×
β-Catenin	×	×	×	
CEACAM1	×		×	×
P-cadherin	×	×	×	
CXCR4	×	×	×	×
Cox-2		×		
Ets-1		×	–	–
HIF			×	×
MITF		×	×	
MTAP	×	×		
Pleiotrophin		×	×	
PLK-1		×	×	
PUMA		×	×	
SOCS	×	×		
Wnts		×		

Table 1 Examples of additional markers that could be tested.

To meet this objective, we shall design and fabricate NTNFET devices as micro-arrays for high-throughput function determination. We will develop miniaturized assays that accommodate extremely low samples volumes and enable the label-free, rapid, simultaneous processing of vast number of proteins (cancer markers). Because electronic measurement using NTNFET devices involves molecular interactions between protein or DNA molecules and sidewalls of carbon nanotubes, carbon nanotubes themselves function as labels. As a first step we shall try to bind melanoma cancer marker Ki-67 – monoclonal antibody to the oxide surface near the nanotube (later other ones will also be tried). We will use Schiff's base linkage chemistry to immobilize the monoclonal antibodies on the silica substrate (see *e.g.* ref. 18).

Next, we shall address the secondary aim, by multiplexing and detecting several melanoma cancer markers, which will be suggested by our pathologist. Finally, we shall proceed with future aims, including a design and fabrication of a lab on the chip and hand-held device for an early, accurate, and

very sensitive detection of melanoma and possibly other types of cancer.

IV. FUTURE AIMS

Why lab on the chip, for complete diagnostics of melanoma cancer markers in vivo, as a hand-held device for clinical studies? Currently, typical diagnosis is carried out starting with the visit to the dermatologist and his/her eye exam of the skin, possible biopsy, and photographs of the nevi that are followed up in time. In order to diagnose properly, the excision is necessary and it may vary in size, the extent of surgery and overall caused trauma to the patient. The diagnosis may take up to a month long and the markers used to stain the nevi are generally recognized through reading under microscope. If the cancer has already spread throughout the body, it may be too late to treat it in a timely fashion. On the other hand, there is only that much skin that can be excised. For patients from families prone to melanoma, it is virtually impossible to remove or suspect nevi. In addition, many of the removed nevi are examined and diagnosed not to be cancerous. Therefore, we have many unnecessary surgeries and patient's trauma plus significant cost of the operation. This is why new methods for fast, non-invasive, and accurate diagnosis *in vivo* are necessary. We plan to place our sensing platform on the chip, in near future, with additional electronics and photonics parts for *in vivo* imaging/visualization capabilities. We envision this tool to be a hand-held device that would replace the current clinical diagnostic methods by giving us fast and accurate response on change of electrical conductance, as the specific cancer marker detection, and visualization of the affected area at very high resolution. Once successfully tested in clinical trials, these tools could be applied to detect other types of cancer.

V. ADVANTAGES OF A PROPOSED METHOD OVER CURRENT DIAGNOSTIC METHODS

Clearly, the advantages are many and very critical for the survival rate of patients potentially diagnosed with melanoma. This project, as the first ever launched in this area, would open many avenues to alternative diagnostic methods for detecting melanoma almost instantly in a non-evasive fashion. This would help the public to save time, money, unnecessary trauma connected with surgery, a long wait-time for results, costly surgery expenses, and most importantly, to save their lives.

VI. CONCLUSIONS

Since this an innovative start-up study in this area, we believe that the advantages of the anticipated results and further research would lead to break-through tools for fast, accurate, and inexpensive in-vivo diagnosis of melanoma, and other cancers, saving thousands of lives every year.

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