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Accurate characterization of benign and cancerous breast tissues: Aspecific patient studies using piezoresistive microcantilevers

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ABSTRACT

Breast cancer is the largest detected cancer amongst women in the US. In this work, our team reports on the development of piezoresistive microcantilevers (PMCs) to investigate their potential use in the accurate detection and characterization of benign and diseased breast tissues by performing indentations on the micro-scale tissue specimens. The PMCs used in these experiments have been fabricated using laboratory-made silicon-on-insulator (SOI) substrate, which significantly reduces the fabrication costs. The PMCs are 260 μm long, 35 μm wide and 2 μm thick with resistivity of order $1.316 \times 10^{-3}\ \Omega$ cm obtained by using boron diffusion technique. For indenting the tissue, we utilized 8 μm thick cylindrical SU-8 tip. The PMC was calibrated against a known AFM probe. Breast tissue cores from seven different specimens were indented using PMC to identify benign and cancerous tissue cores. Furthermore, field emission scanning electron microscopy (FE-SEM) of benign and cancerous specimens showed marked differences in the tissue morphology, which further validates our observed experimental data with the PMCs. While these patient aspecific feasibility studies clearly demonstrate the ability to discriminate between benign and cancerous breast tissues, further investigation is necessary to perform automated mechano-phenotyping (classification) of breast cancer: from onset to disease progression.

1. Introduction

According to American Cancer Society, breast cancer continues to be the second leading cause of cancer-related female deaths in the USA, with 232,340 new cases and 39,620 estimated deaths (American Cancer Society, 2013). Future progress in several key areas of cancer research and drug discovery will rely upon the capacity of investigators to reliably detect, characterize and track subtle changes that occur in terms of biomarker and morphologic signatures in the tumor environment during the transformation from the benign to cancerous state. Early detection and treatment of breast cancer can not only prolong the life of the individual, but also lead to an improved quality of life. The importance of early cancer detection and accurate staging of disease for improved treatment has prompted considerable research interest in quantifying the state and progression of cancer. Mechanical phenotyping has been demonstrated as an effective quantitative biomarker for characterizing the state of malignancy in cells (Kim et al., 2009) and tissue (Roy et al., 2010). The conventional AFM technique was used to study nanomechanical properties associated inherent to metastatic adenocarcinoma cells obtained from body cavity fluid samples (Cross et al., 2007). The results revealed that for the similar shapes cells, the mechanical analysis can differentiate normal and cancer cells. Plodinec et al. (2012) studied the change in stiffness of the breast tissue with progression of cancer using AFM cantilever. Suresh (2007) published a detailed study on mechanistic discussions of the connections among alterations to subcellular structures, attendant changes in cell deformability, cytoadherence, migration, invasion and tumor metastasis, and various quantitative mechanical and physical assays to extract the elastic and viscoelastic deformability of cancer cells. A proteomics analyses was carried out to understand the role of tissue stiffness and stress on nucleoskeletal protein lamin-A (Swift et al., 2013). Advances in cancer biomechanics research has been supplemented by a surge in recent development of mechanical property measurement techniques at the micro- and nano-scale (Alessandrini and Facci, 2009). Capacitive force sensors have previously been reported as a reliable means for investigating

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cellular mechanics (Seonghwan et al., 2009). However, these methods have considerable microfabrication challenges. Silicon-on-insulator (SOI) wafers are typically employed for microfabrication of the capacitive sensors as the structure needs to be completely isolated between two electrodes (Sarajlic et al., 2004). The complete isolated structure is difficult to obtain as it is challenging to control the etching area. Moreover, the capacitive method requires complicated electronics for sensor readout. Another promising technique for characterizing tissue samples is Atomic Force Microscopy (AFM), which has already gained wide acceptance in quantifying the material properties of biomaterials (Roy et al., 2013). The AFM system consists of a microcantilever, which is piezoelectrically controlled to indent specimens. The microcantilever deflection is optically sensed, which is related to sample stiffness probed by the AFM (Alessandrini and Facci, 2009). While optical detection is highly accurate in measuring small displacements, it requires precise alignment of the optical system. Furthermore, the optical (laser) measurement of cantilever deflection (Gimzewski et al., 1994; Thundat et al., 1994; Mukhopadhyay et al., 2005; Ghatkesar et al., 2008; Backmann et al., 2005; Berger et al., 1997; Fritz et al., 2000; Lang et al., 1999) has several practical problems such as complex electronics, bulky optics, and inability to be used in opaque liquids. In addition, the AFM is also limited by its low throughput. Commercial AFMs typically use a single cantilever to probe discrete locations on the sample surface, which becomes cumbersome for large specimens such as histopathological tissue. Conventional AFM stages provide a limited range of travel, which necessitates the use of manual positioning systems to align the AFM probe and specimens. As such, piezoresistive sensing mechanisms offer an attractive alternative to the aforementioned techniques.

Piezoresistive sensors can be used in opaque liquids and do not require complex readout electronics. The design of piezoresistive sensors by changing the parameters like doping concentration, piezoresistor dimensions and using different manufacturing techniques for conventional diaphragm shapes, square, and circular shapes have been studied by several groups (Merlos et al., 2000; Bae et al., 2004; Pramanik et al., 2006; Harley and Kenny, 1999). Piezoresistive sensors are widely employed as sensing elements in pressure sensor (Boisen and Thundat, 2009; Gautsch et al., 2002; Hierlemann et al., 2000; Kanda and Yasukawa, 1997), chemical sensors (Bae et al., 2004; Pramanik et al., 2006; Cho et al., 2008), force sensors (Yang et al., 2003) and stress sensors (Loui et al., 2008). In addition, multiple piezoresistive cantilevers can be microfabricated in an array-format (Seonghwan et al., 2009), which considerably improves the sensing throughput and offers a cost-effective approach for quantifying biomaterial mechanical properties.

To our knowledge, there is no existing study utilizing piezoresistive microcantilever force sensors for detecting cancer progression in tissue. In this paper, we report on the fabrication and testing of MEMS-based piezoresistive microcantilevers with an SU-8 tip for detecting benign and cancerous breast tissue by indenting designated tissue regions inside breast tissue cores obtained from seven different specimens. In the Materials and Methods section, we discuss the steps involved in fabricating the microcantilever and the AFM experimental setup used to assess the sensor performance. In Section 3, we discuss the sensitivity and performance of the sensor on breast tissue specimens which also include our future research goals in this area.

2. Experimental work

2.1. AFM experimental setup

The AFM experimental setup used in this study is shown in Fig. 1. The AFM system is comprised of the AFM scanning head and the controller (MFP-3D-BIOTM, Asylum Research, Inc.) coupled to an inverted microscope (Model: TE2000U, Nikon, Inc.) such that the AFM head rests on the microscope. The whole setup is enclosed within an acoustic hood to isolate it from external noise. A CCD camera (QImaging Inc, Model: Retiga 2000R) is mounted to the microscope.

The range of the *X* and *Y*-axes of the piezoactuated stage is 90 μm and the customized range for the *Z*-axis is 40 μm. Situated at the base of the microscope is a motorized MP-285 micromanipulator (manufactured by Sutter Instruments, Novato, CA), to which is attached a custom-made end-effector. The fabricated microcantilever is attached to an angled slide holder mounted on the end effector. The MP-285 has a step resolution of 40 nm and a range of 2.54 cm along the *X*- and *Y*-axes. The micromanipulator, microscope and the AFM head are placed on a vibration isolation table (manufactured by Herzan) to eliminate base vibrations. A detailed explanation of AFM probe–sample interactions and tissue microarray preparation protocol is presented in our earlier work (Roy et al., 2010). The AFM setup was specifically used for measuring the spring constant of the fabricated cantilever. The

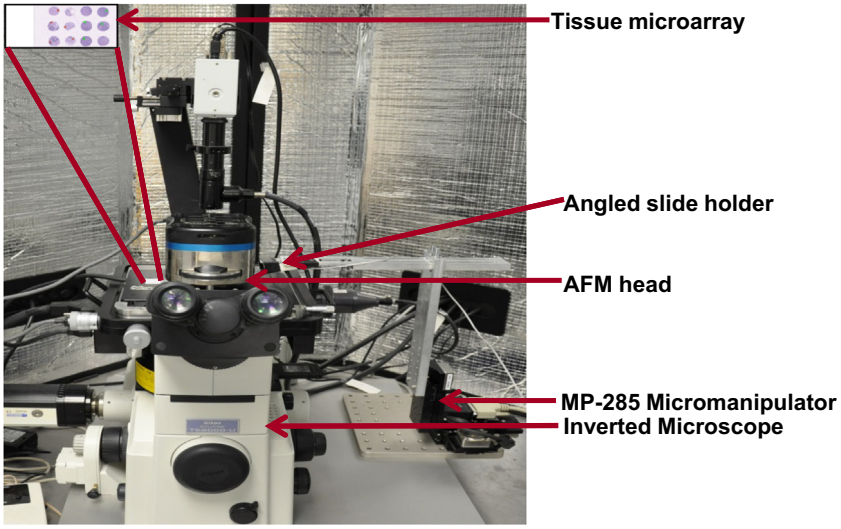

Fig. 1. AFM experimental setup integrated with piezoresistive sensor used for nanoindentation.