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EFFECTS OF INCREASING NANO-SCALE COATING THICKNESS OF TITANIUM ON THE INTERACTION OF SILICON WITH CANCER CELLS

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ABSTRACT

The effect of increasing nano-scale coating thickness of titanium on the interaction of silicon with cancer cells is investigated. Titanium coating of varying nano-scale thickness was deposited on silicon surface. Surface characterizations of the modified and unmodified samples were carried out using Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Human *osteosarcoma* cells (HOS) were cultured on the surfaces to study the effects of the modification on its cellular response. Optical microscopy and SEM were used to study the morphology of the cells on the different cultured samples. The quality of cells adhesion was also studied using shear assay techniques. 50 nm coating of titanium on silicon surfaces was found to reduce its surface roughness from 1.36 nm to 1.19 nm. This enhances the interaction of the silicon through a rapid spread of the cells. It was also observed that increasing the titanium coating thickness from 50 nm to 100 nm resulted in a further drop in the surface roughness value to 1.12 nm with the consequent increase in its cellular response. The application of increasing nano-scale thickness of titanium on silicon substrate was found to enhance the quantity and quality off cell adsorption, adhesion and proliferation.

Keywords: *titanium, silicon, nano-scale, coating thickness, cellular response*

1. INTRODUCTION

The salient properties of silicon, as well as the ease of its fabrication by micro fabrication techniques make it a suitable candidate material for implantable bioMEM (Petersen, 1982). However, the use of silicon is associated with a high immunological response, which results into fibrous encapsulation of the implant (Mwenifumbo, 2004). This consequently results in the isolation of the devices from the physiological system, thus impairing its effectiveness. Modification of silicon surface is therefore paramount to enhance its cellular interaction and subsequently its biocompatibility and biofunctionality in service.

Several attempts have been made to study the effect of surface modification on silicon cell surface integration (Bhalerao *et al.*, 2004, Milburn and King, 2004, Mwenifumbo, 2004). One of these is the use of biocompatible coatings such as titanium, to enhance the cellular response of silicon based devices. Previous work carried out by the group focused on the general effect of nano-scale titanium coating on silicon biocompatibility (Milburn, 2004, Milburn, *et al.*, 2009, Milburn and King, 2004, Mwenifumbo, 2004). This work, hopes to continue from here by attempting to study the effects of increasing nanolayer coating thickness of titanium on the interaction of silicon with cancer cells.

1.1. Surface Modification

Most often materials that are considered suitable for a particular physiological application based on their bulk properties are found not to be biocompatible with that environment. For example, silicon, which is often considered for the fabrication of biomedical devices such as BioMEMS and biosensors have been found to elicit a high immunological response (Milburn, 2004, Milburn and King, 2004). Some biomedical devices have also been found to result in the denaturing of proteins adsorbed on its surface when implanted in a physiological system. These often result into complications such as inflammation, cyto-toxicity, blood clotting etc., which eventually results into failure, or rejection of such implant in service (Woodward and Salthouse 1986, Williams, 1987, Dickinson and Bisno, 1989, Gristina *et al.*, 1994, George, 2000, Overchin and DeBeer, 2000, Kumari *et al.*, 2002 and Nayab *et al.*, 2003).

It is generally accepted that the interaction of materials with biological molecules such as protein, blood cells, osteoblast cells, osteo sarcoma cells etc is initiated at the surface of the implant materials. This interaction is however dependent on surface attributes such as

wettability, hydrdrophillicity, hydrophobicity, surface free energy, surface charge, surface texture, surface roughness, waviness, porosity, charge distribution, chemical composition, etc. These therefore determine the biocompatibility, biofunctionality, biosafety and biostability of the materials in service (Lee *et. al*., 2002, Lim *et. al*., 2004, Chou *et al*., 1995, Elbert and Habbell, 1996, Richards, 1996, Georgi *et al*., 1996, Brunette, 1998, Brunette and Cheroudi, 1999, Matsuzaka *et. al*., 2003, Kiewetter *et al*., 1996, Hallah *et al*., 2001, Price *et.al*., 2003, Miller *et. al*., 2002, Recknor *et. al*., 2004 and Leventon, 2001). Hence, for enhanced performance, it is often necessary to modify these attributes to induce a specific biological response, or inhibit a potentially adverse interaction, while the bulk properties of the materials are preserved.

Various surface modification techniques for the enhancement of the efficiency, durability and biocompatibility of biomedical devices abounds. These are classified into either physiochemical or biomolecular surface modification (Puleo and Bizis, 1992, Bunshah, 1992, Mrksich et. al., 2003, Hubler and Hirvonen, 1994, Khor *et al*., 1998, Ingber and Jamieson, 1993, Jethanandani, 1997, Wolgeuth, 2000, Leventon, 2001, Davis *et al*., 2002 and Ratner *et al*., 2004). Physiochemical techniques involved the physical alteration of atoms or molecules at the surface. This is usually achieved using laser irradiation, plasma, mechanical blasting, spraying, etching and chemical modification. Physiochemical modification may also be carried out by coating, grafting, sintered beads and thin film deposition of other material on substrate to enhance its biocompatibility (Pilliar, 1990, Bunshah, 1992, Khor *et al*., 1998, Wolgeuth, 2000, Leventon, 2001). Bimolecular surface modification technique on the other hand entails the surface immobilization of biomolecules on the material, which render the materials receptive to the biomolecules such as cells, tissues and proteins in the environment (Davis *et al*., 2002). This work is based on the modification of surface attributes using surface coating technique.

1.2. Surface Coating

Researches focused on developing coating systems for biomedical applications abounds. These have been applied through a range of technique, which entails the use of varieties of materials and molecules such as ceramics, polymers, metals, and bioactive molecules (Bunshah, 1992, Hubler and Hirvonen, 1994, Smith, 1994, Tay *et al*., 1995, Komu *et al*., 1996, Jethanandani, 1997, Khor *et al*., 1998, Wolgeuth, 2000, Leventon, 2001, Davis *et al*., 2002, Mwenifumbo, 2004, Milburn and King, 2004). Some of the surface coating processes often utilized in biomedical application include; ion-beam assisted deposition (IBAD), vapour deposition process, polymer coating, porous ceramic coating and porous metallic coatings (Pilliar, 1990, Bobyn, 1982, Hubler and Hirvonen, 1994, Bunshah, 1992, Khor, *et al*., 1998, Leventon, 2001).

Most often surface coating is applied to biomedical devices and implants for diverse reasons. In orthopaedic for example, the deposition of porous metal or ceramic coating on implant has been found to facilitate both implant fixation and bone ingrowths (Khor *et al*., 1998 and Anselme, 2000). Similarly, the use of porous metal coatings have been found to minimize the prominent problem of loosening of implant with time in cemented joint -implant (Pilliar, 1990, Khor *et al*., 1998). Thin film deposits of other materials on implants have also been shown to enhance the biocompatibility, biofunctionality and biostability of implants (Pilliar, 1990, Khor *et al*., 1998, Mwenifumbo, 2004, Milburn and King, 2004, Milburn *et al*., 2009). Thin film deposition of titanium on silicon, for example, has been shown to improve the cellular response of silicon, subsequently enhancing its biocompatibility (Mwenifumbo, 2004, Milburn and King, 2004, Milburn *et al*., 2009). This work however attempted to study the effect of increasing the coating thickness of nano-layer deposit of titanium on the interactions of silicon with cancer cells.

2. METHODOLOGY

2.1. Silicon Pre Experiment Surface Treatment

The silicon, utilized in this experiment, was of n-type, four-inch single crystal Si (100) wafers with 375 mm thickness. The wafers were cut into appropriate dimensions using a diamond scribe. Samples to be used for the cell culture were cut into 5mm × 5mm, for cell spreading experiment and 35mm × 35mm for shear assay experiments. The samples were initially ultrasonically cleaned in acetone for 30 minutes. They were then cleaned according to the Standard Clean Procedure for Si, SC. This entails boiling of the wafer samples in 1:3 H_2SO_4: H_2O_2 for 45 minutes, to remove organic contaminants from the wafers' surfaces, which was followed by an extensive rinse in double distilled water (dd H_2O). The samples were further boiled in a 1:1 HCl: H_2O_2 solution to remove metallic contaminants. The silicon samples were then given a final rinse in dd H_2O after which they were sterilized in ethanol.

2.2. Surface Coating

Nano-scale coating thicknesses of 50nm and 100nm layer titanium were deposited on some of the previously cleaned silicon samples by electron beam evaporation technique. The choice of an initial coating thickness of 50nm was based on previous work which established that a minimum of 50nm coating thickness is required for complete surface coverage using the electron beam evaporation process (Mwenifumbo, 2004). The samples were then ultrasonically cleaned in a solution of dd H_2O and detergent for 20 minutes. This was followed by a rinse in dd H_2O and further sonication in acetone for 20 minutes. The samples were then given a final rinse in dd H_2O and passivated in 30% nitric acid for 15 minutes. The passivated samples were afterwards rinsed with dd H_2O, and dried with nitrogen gas. Surface characterization of the silicon sample were carried out before and after the surface modification process using scanning electron microscopy (SEM) and atomic force microscopy (AFM).

2.3. Cell Culture Experiment

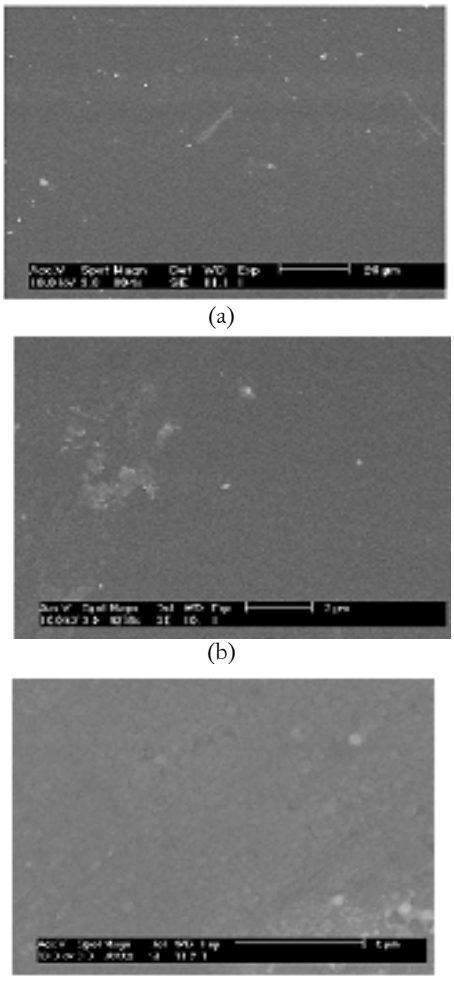
Human Osteosarcoma cells (HOS), which was obtained from American Type Culture Collection in Manassas, VA, was utilized in this experiment. Before the experiment the HOS cells were maintained in 25 cm^2 cell culture flasks (Franklin Lakes, NJ) using Dulbecco's Modified Eagle's Medium (DMEM) that was obtained from Quality Biological, Gaithersburg, MD. The DMEM was modified using 10 % Fetal Bovine Serum, 5 % Amphotericin and 5 % Penicillin/Streptomycin, all of which were obtained from Quality Biological, Gaithersburg, MD. The cell culture was kept in an incubator, at an incubation temperature of 37°C, regulated with 5 % CO_2, 95 % air, and a saturated humidity. A cell suspension was prepared from the HOS cells. The concentration of the cells in the suspension was determined by carrying out a hemocytometer count of the cells in the suspension and it was found to be approximately 50,000 cells /mL.

In order to equilibrate the silicon samples with the cell culture environment, the samples were placed in cell culture wells containing 1.5 mL of serum-free DMEM and allowed to incubate for fifteen minutes at 37°C. One hundred micro litres of the cell suspension was pipette onto each of the samples' surface. They were then incubated at 37°C for their allotted culture time i.e. 15 minutes, 30 minutes, 60 minutes and 24 hours. These were utilized to study the attachment of cells to the various surfaces at the different culture time. Samples for the adhesion test were cultured for 48 hours incubation times prior to shear assay experiment. Optical microscopy was used to study and record the quality of cells attachment to the various surfaces at the different culture time while shear assay experiment was used to study the adhesion of cells on the different substrate (Anselme *et al*., 2000, Mwenifumbo, 2004).

3. RESULTS AND DISCUSSION

3.1. Results

The Scanning electron microscopy (SEM) and atomic force microscopy (AFM) micrographs of the control and titanium coated samples are presented in Figs 1 and 2. The AFM surface roughness parameter obtained for both the silicon and the titanium coated silicon samples are also presented in Table 1. The attachment and time-dependent spreading of HOS cells cultured on the silicon and the modified silicon samples are shown in the optical images of the cultured samples, Figs 3 to 7. After 15 minutes, cells were observed to have attached on the surface of the samples having a spherical shape, Fig..3. Although the adsorption, attachment and spreading of cells could be observed on both the uncoated and coated silicon samples, it can however be noted that the 100 nm titanium coated samples had more cells attached on it at 15mins culture time, which is followed by the 50 nm coated samples (Fig. 3b and 3c). The appearances of filopodia, caused by the outward motion of the cytoplasm of the cells were observed in titanium coated samples and it can also be noted that this was enhanced with increased coating thickness (Figs 3b and 3c). Spreading of the cells to the more flattened morphology was noticed as the cells form focal adhesions with adsorbed proteins on the surface of the substrate at increased culture time, Fig. 4 to 7. The proliferation and spreading of the attached cells was also observed to have increased with increased titanium layer coating thickness (Figs 3-7). Furthermore the shear assay result shows that the quality of attachment of the adsorbed cells was enhanced with increased titanium coating thickness, Table 2.



(a)

(b)

(c)

Fig. 1: SEM images of surfaces of silicon and titanium coated silicon samples: (a) Silicon(b) Silicon + 50nm Ti Coating and (c) Silicon + 100nm Ti Coating

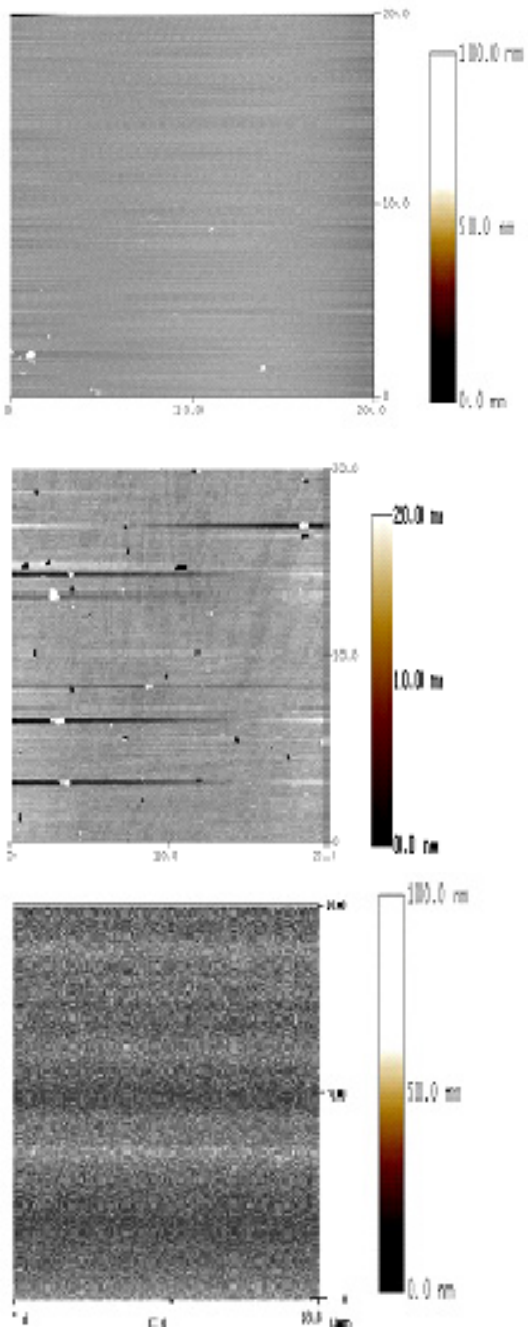


Fig. 2: AFM Images of surfaces of silicon and titanium coated silicon samples: (a) Silicon (b) Si + 50nm Ti Coating and (c) Si + 100nm Ti Coating

Table 1: AFM results of biomaterials surfaces

Biomaterial surface	Scan height (nm)	RMS Surface roughness (nm)
Silicon	100.0	1.36 ± 0.48
50nmTi Coated Silicon	20.0	1.19 ± 0.18
100nmTi Coated Silicon	100.0	1.06 ± 0.06.

Table 2: Cellular adhesion stress for silicon and coated silicon samples

Materials	Cellular adhesion stress (Pa)
Silicon	64 ± 3
Silicon + 50nm Ti	75 ± 7
Silicon + 100nm Ti	104 ± 3

3.2. Discussion of Results

The SEM and AFM micrographs, Fig. 1, show the topography of the surfaces before the cell culture experiment. These show that the surface of the uncoated silicon samples has a higher surface roughness when compared to the titanium coated samples, Fig. 2. These results corroborate with the measured surface roughness value, Table 1, as it was observed that the silicon samples had a higher surface roughness than the titanium coated samples. Furthermore it was noted that further decrease in surface roughness was observed with increasing coating thickness (Table 1). The increase in quantity and quality of cells attached to the titanium coated samples (Figs 3- 7) may be attributed to the decrease in surface roughness of the samples with titanium layer coating thickness observed in Fig. 1 and Table 1. A further increase in the coating thickness of the titanium led to a further decrease in the surface roughness parameter with the consequent enhancement of the cellular interaction of the substrate with the cells (Figs 3- 7).

Changes in the surface chemistry and surface energy, which may have resulted from the titanium nano-layer coating on the silicon sample, may also be responsible for the increased quantity of cells attached on the coated samples. The atoms within the silicon are held together by covalent bond while that of the coating deposited on it is bonded by a comparatively stronger ionic bond. Subsequently, the silicon has a lower surface energy when compared to the titanium deposited on it. Hence the titanium coated surface, which have a higher surface energy and is subsequently more hydrophilic, will have a higher tendency for the adsorption, adhesion and proliferation of cells when compared to the uncoated silicon surface. Increasing the coating thickness may have resulted in an increase in the tendency of the coating to behave more like the bulk, titanium material. Hence more cells were noticed to have adsorbed on the 100 nm layer titanium coated samples when compared to the 50 nm layer coated surface.

In the same vein, the spreading and proliferation of cells on the titanium coated samples were observed to be better than that of those found on the uncoated samples (Figs 2 to 7). By 15 minutes, some of the cells cultured on the nano layered, titanium coated samples were already showing an outward extension of its cytoplasm, which also resulted in the formation of filopodia (Figs 2b and 2c). The cells on the uncoated sample were however still in their spherical form, Fig. 2a. The spreading of cells therefore tends to take place more rapidly in the titanium coated silicon substrate, when compared to the uncoated samples Figs 2 to 7. This enhanced cellular interaction may be ascribed to the biocompatibility of titanium, which has been attributed to the chemical stability and integrity of its surface oxide film (Feng *et al*, 2002, Feng *et al*., 2003). Increasing titanium layer coating thickness subsequently resulted in rapid attachment and spreading of HOS cells observed on the 100 nm coated samples (Figs 2 to 7).

From the shear assay result (Table 2) the cellular adhesion stress was found to be higher for HOS cells cultured on the titanium coated surface when compared to those cultured on the uncoated silicon surface. The adhesion of cancer cells can was also found to increase with increasing thickness of the titanium layer coating. This shows that the application of increasing nano-scale titanium coating on silicon improves the adhesion of cancer cells, consequently, its biocompatibility.

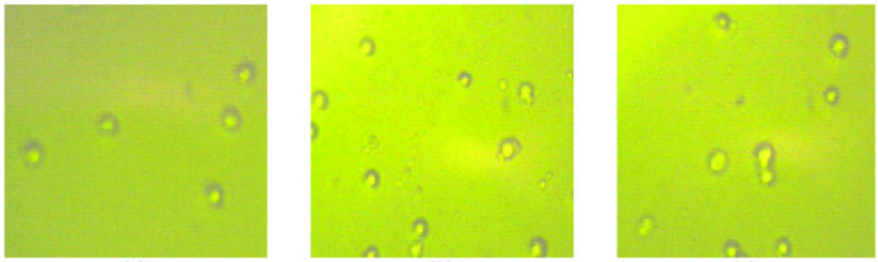


Fig. 3: Optical Images of Cells Cultured for 15mins. on (a) Silicon (b) Silicon with 50nm Titanium Coating and (c) Silicon with 100nm Titanium Coating

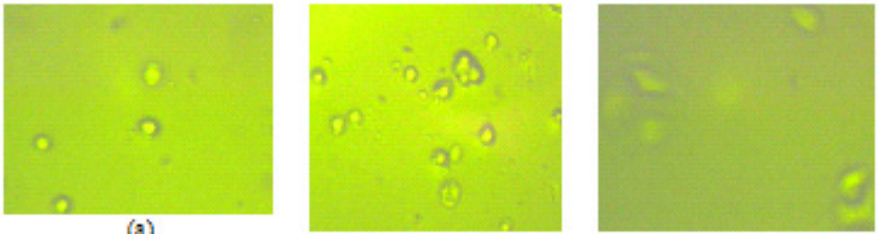


Fig. 4: Optical Images of Cells Cultured for 30mins. on (a) Silicon (b) Silicon with 50nm Titanium Coating and (c) Silicon with 100nm Titanium Coating

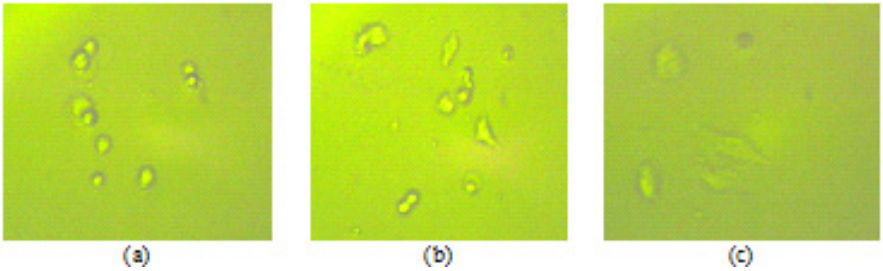


Fig. 5: Optical Images of Cells Cultured for 45mins. on (a) Silicon (b) Silicon with 50nm Titanium Coating and (c) Silicon with 100nm Titanium Coating

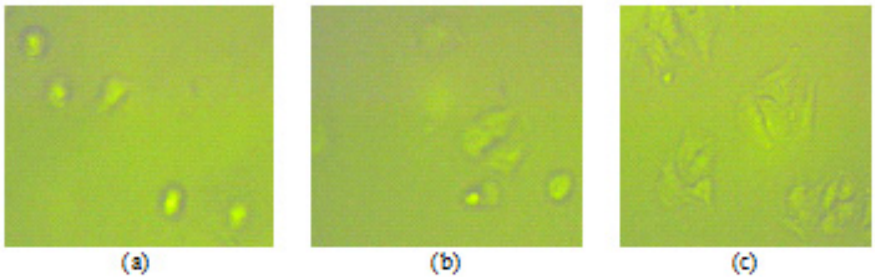


Fig. 6: Optical Images of Cells Cultured for 60mins. on (a) Silicon (b) Silicon with 50nm Titanium Coating and (c) Silicon with 100nm Titanium Coating

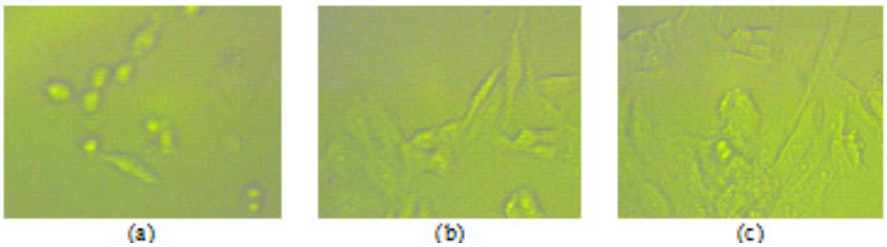


Fig. 7: Optical Images of Cells Cultured for 24hrs on (a) Silicon (b) Silicon with 50nm Titanium Coating and (c) Silicon with 100nm Titanium Coating

4. CONCLUSIONS

Application of increasing coating thickness of biocompatible titanium on silicon had resulted in a corresponding decrease in surface roughness, increased surface energy, and subsequently an increase in the quantity and quality of cells attached on the modified surface. This, in addition to the bioactive properties of the titanium coating, which became more pronounced with increased coating thickness, had resulted in a much improved cellar response. Hence increased titanium coating thickness has been shown to improve silicon interaction with HOS cells. This will enhance its use in the design and fabrication of biosensors and other biomedical devices for cancer diagnosis and treatment.

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