Fabrication of and Cell Growth on 'Silicon Membranes' with High Density TSVs for Bio-sensing Applications

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***Abstract*—A silicon membrane acting as an interface layer between live cells and the sensing electronics enabling low-cost, high-throughput bio-sensing is proposed; the interface is capable of supporting high pixel density allowing accurate image mapping. Cell attachment and growth was carried out on five different silicon based surfaces and compared to the standard Tissue Culture Polystyrene (TCPS) surface in order to optimize the selection process of the interface material; cell imaging is performed after 48 hours to study the effect of different surface variations (different materials on silicon, presence of metal, increased surface roughness due to CMP, etc.) on cell adhesion and growth. The results show that cells successfully adhere and grow onto the sensing electrodes for all the surfaces under test after the first 48 hours. Key enabling technologies for high pixel density membranes are also presented.**

***Index Terms*— Cell growth, silicon membranes, bio-sensing platform, TSVs, system integration.**

INTRODUCTION

CMOS chips are being increasingly used for bio-sensing applications [1-8]. Integration of bio-sensors in CMOS chips allows for the exploitation of electrical-optical sensing modalities at a low cost. There is a growing interest to relate cellular-based physiological events to changes in current or voltage that can then be sensed using integrated electrodes [1-9]. The electrical response could be induced by the administration of a stimulating agent on electro-active samples (cardiac cells, neurons, etc.). Chemical or biological stimuli in the form of drugs or pathogens can be introduced and any resulting changes in the sensed signal can then be studied to determine the drug efficacy or pathogen mechanistic effects [7, 9].

These cell-based sensing techniques require the growth of cells onto the chip surface; both the successful adhesion and growth of these cells onto the chip is crucial for meaningful sensing. The surface profile has been shown to be an important factor in determining the cell attachment and subsequent growth. In particular, the type of material and the surface roughness play a vital role in determining cell adhesion and growth [10-16]. Growth of human cells on a multi-modality CMOS bio-sensing chip has been shown in [17]. However, growing cells directly onto the chip is tedious due to the need for surface treatments and configuration optimization to enhance bio-compatibility and culture cells. Additionally, culturing cells directly onto the CMOS chip may be expensive because it is difficult to re-use as it must undergo a proper cleansing process or disposed of to avoid contamination [7, 18]. Moreover, even after cleaning and sterilizing the CMOS surface, it is uncertain if the chip is reusable if the introduced biochemical stimulus to be tested is different or if the cell type, from which we wish to observe physiological changes, is different. Contamination poses a risk to the continual proper functionality of the on-CMOS bio-sensor. Furthermore, any electrical connections to the board (e.g. wire bonds in [17]) and culture medium sealing (e.g. PDMS sealing in [17]) need to be removed if a new sample is to be tested during which there is a high chance of damaging the CMOS chip.

Due to these limitations and requirements, there has been some prior research investment focusing on developing interface layers that are disposable and low cost. As these layers primarily provide simple electrical connections, the cost of replacing such interfaces is much lower than replacing complex CMOS chips. Several studies have [19, 20] described such disposable interface microsystems. However, unlike these cases, the disposable interface microsystem proposed in this paper exploits conventional fabrication methods in developing a silicon based interface on which cell adhesion and growth occurs.

In this paper, silicon membranes with gold sensing electrodes and through-silicon-vias (TSVs) are presented as a viable interface layer for low cost cell-based sensing (Fig. 1). Growth of human stem cells onto different silicon based surfaces under test are reported and benchmarked to standard TCPS. Moreover, key enabling technologies for developing these interface layers are also discussed. Specifically, interface layers with high density TSVs for high resolution sensing are shown.

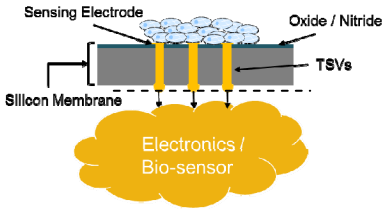

Fig. 1. Envisioned silicon membrane interface

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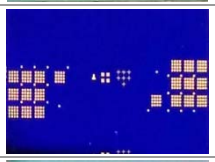
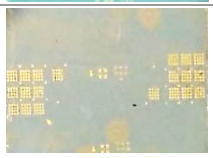
SILICON MEMBRANES' FABRICATION

Five different surface topologies were studied to characterize the surface biocompatibility in terms of cell attachment and growth; specifically the effect, if any, of surface profile and the presence of metal electrodes and TSVs were studied. The different membrane surfaces are summarized in TABLE I.

TABLE I
SURFACES UNDER TEST FOR CELL SEEDING AND GROWTH

Surface	Image
Nitride on Silicon	
Oxide on Silicon	
Nitride on Silicon with Sensing Electrodes	
Oxide on Silicon with Sensing Electrodes	
Oxide on Silicon with TSVs and Sensing Electrodes	

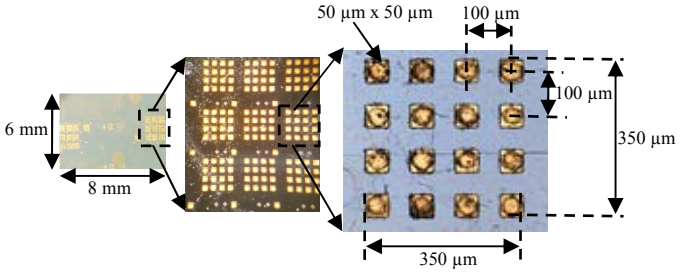


Fig. 2. Pixel group layout for silicon membrane

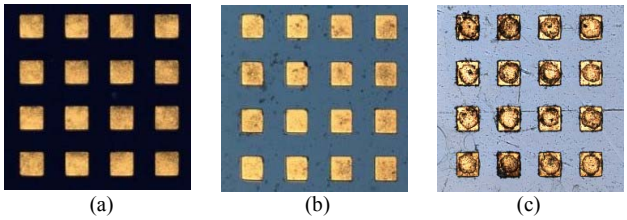


Fig. 3. Pixel groups with array of sensing electrodes on (a) nitride, (b) oxide, and (c) silicon membrane with TSVs with oxide surface

Fig. 2 shows the pixel group layout on the fabricated silicon membrane, which, by design, corresponds to the pixel group on the CMOS chip in [17]. Fig. 3 (a) and 3 (b) show the pixel group layout without TSVs on nitride and oxide, respectively, whereas Fig. 3 (c) shows the silicon membrane with TSVs and sensing electrodes with oxide on silicon; the TSVs were fabricated using the mesh process outlined in [21]. The sensing electrodes, for all of the above mentioned samples, were formed by evaporating metal followed by a lift-off process. Ti/Cu/Au with thicknesses of 500/1500/300 nm were deposited to form the sensing electrodes. The fabricated sensing electrodes have a pitch of 100 µm with each electrode having a dimension of 50 µm x 50 µm, as shown in Fig. 2. The fabrication process for the silicon membrane with TSVs is outlined in Fig. 4. X-ray images of a pixel group in the fabricated membrane is shown in Fig. 5. The X-ray image shows void free vias, which are essential in obtaining accurate images of the cells on the membrane surface. The profilometer scan data for the fabricated sensing electrodes with and without TSVs is shown in Fig. 6. The profile for the silicon membrane with TSVs shows large surface variations; these are primarily introduced by dishing and erosion during the CMP process.

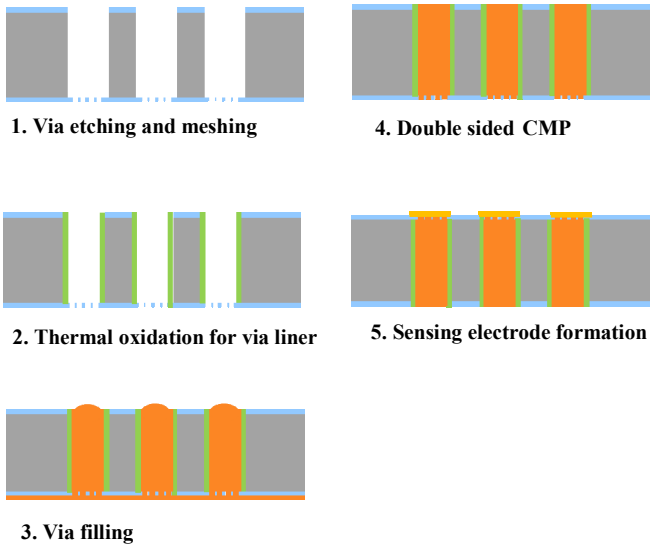


Fig. 4. Fabrication flow for the silicon membrane with TSVs

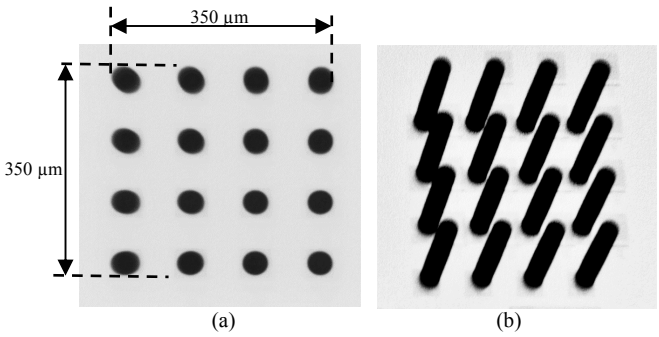
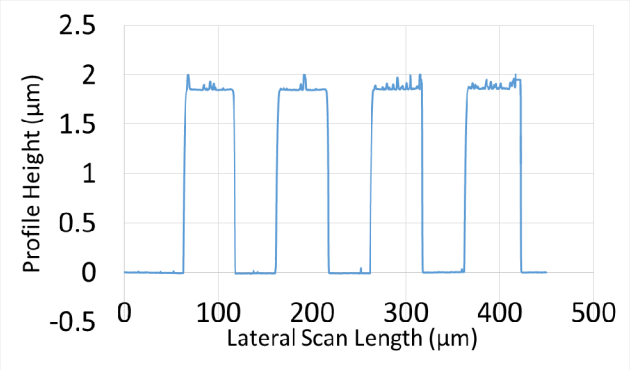
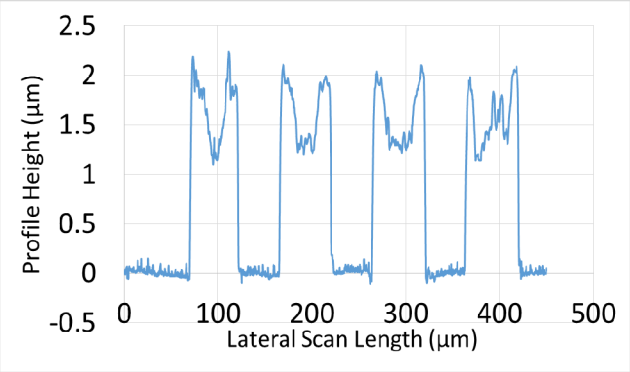


Fig. 5. X-ray image of pixel group in the fabricated silicon membrane (a) top view, (b) angled view showing void free vias



(a)



(b)

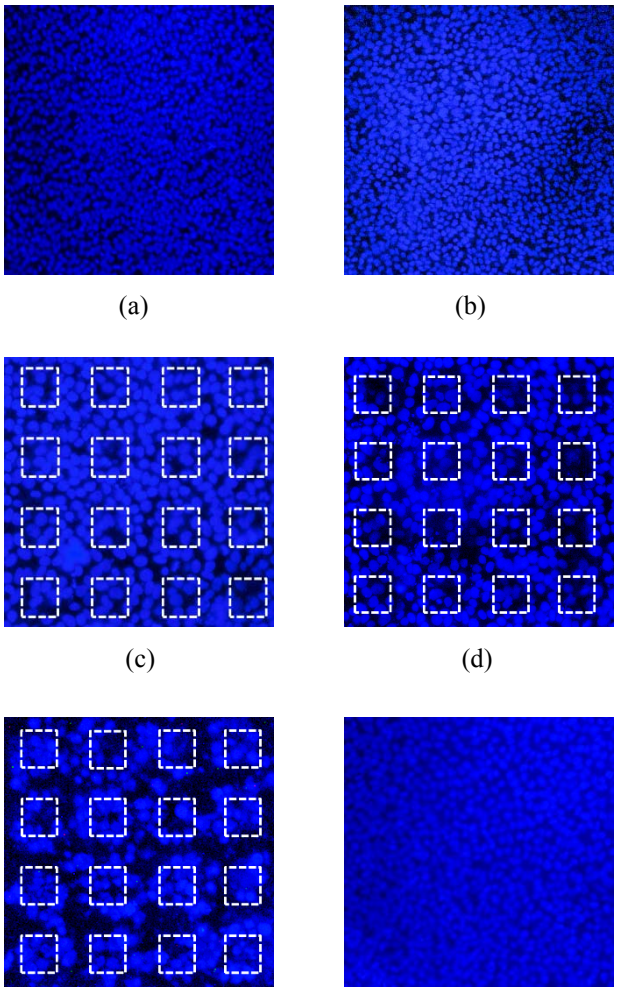
Fig. 6. (a) Profilometer scan data for four adjacent sensing electrodes on silicon membranes (a) without TSVs, (b) with TSVs

CELL GROWTH ON SILICON MEMBRANES

Cell Growth Methodology

Each of the five experimental surfaces (silicon nitride with and without sensing electrodes, silicon oxide with and without sensing electrodes, and silicon oxide with TSVs and sensing electrodes) were sterilized with three washes of ethanol followed by three washes with water. All surfaces were then coated with Matrigel (Corning) for 24 hours at 37°C prior to cell seeding. Human pluripotent stem cells were cultured in serum free media (mTeSR, StemCell Technologies) for several passages prior to seeding experiments. Cells were then dissociated to single cell suspension and seeded onto each of the five silicon surfaces at a concentration of 200,000 cells/cm^2. As a control, cells were similarly seeded onto TCPS, a common material used in standard culture dishes. Cells were cultured for 48 hours prior to fixation and staining with Hoechst nuclear dye. Cell seeding was visualized with an epifluorescent microscope.

Growth Results



(a) (b) (c) (d) (e) (f)

Fig. 7. Cell growth results after 48 hours of seeding on (a) nitride, (b) oxide, (c) nitride with sensing electrodes only, (d) oxide with sensing electrodes only, (e) silicon membrane with oxide surface, sensing electrodes, and TSVs and (f) control with cells on TCPS

Fig. 7 shows the initial attachment and growth of cells 48 hours after seeding. Nitride and oxide on silicon, as shown in Fig. 7 (a) and (b), respectively, show almost identical results to that of the TCPS control (Fig. 7 (f)). For samples with sensing electrodes, the cell attachment appears lower; however, as shown from Fig. 7 (c) through 7 (e), after 48 hours, all the sensing electrodes show some cell adhesion and would be expected to grow and cover the entire surface if left for a longer time period. In these initial cell seeding experiments, human pluripotent stem cells were used as a proof of concept. However, these cells can be later differentiated into cardiac cells or neurons, allowing for more complex studies and integration of the silicon membrane interface for advanced sensing applications including, but not limited to, studying drug effects on cardiac cell beating or neuron activity.

CONCLUSION

A silicon-based membrane interface is proposed for low-cost, high resolution bio-sensing applications. By avoiding cell growth directly onto the CMOS chip, the chips can be reused, thereby dramatically reducing overall cost. Despite large surface variations introduced during the CMP process and the presence of metal sensing electrodes, the silicon membrane is shown to be suitable for growth of human stem cells – all the sensing electrodes in a pixel group show cell attachment and growth 48 hours after cell seeding.

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