The Effect of Micromachining Process to the Biocompatibility of 3C-SiC Membranes

N.F. Mohd Nasir, E. Gan, R. Shukla, T. Istivan, E. Pirogova, P. Tanner and A.S. Holland

Abstract— 3C-Silicon Carbide (SiC) has been identified as a potential biomaterial for implantable devices. The prospect of 3C-SiC membrane as the working component for BioMEMS in in-vitro blood pressure sensing could be an excellent challenge for biomedical engineers. Although, 3C-SiC is known to be biocompatible, but the micromachining process normally used in semiconductor industry might alter the biocompatibility of 3C-SiC. In this study, we investigate the biocompatibility of 3C-SiC which had been subjected to Potassium Hydroxide (KOH) wet etching and reactive ion etching (RIE) using tetrafluoromethane (CF4). The Chinese Hamster Ovary (CHO) cells were directly cultured onto the prepared samples of 3C-SiC, Si and controls to investigate 3C-SiC biocompatibility. The cells were detached from the substrates and grown further in 6 well plates for another 24 hours at 37°C with 5% CO2 and 95% relative humidity for further testing. Cell viability percentage was determined by Trypan Blue exclusion technique which revealed the preference of the cells to grow and to proliferate on the treated SiC samples. However, the results could be inaccurate due to the nature of the testing which compromises the further ability of the cells to proliferate. Thus, a comparison was made by using Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay, a typical cytotoxicity assay, and also PrestoBlue™ reagent (which is the state-of-the-art assay for biocompatibility determination) to confirm the cell viability on the treated and untreated SiC. Thus, the results showed that the cells proliferated better on surface of the 3C-SiC treated with halogenated plasma which indicate the enhancement of biocompatibility of the semiconductor material due to the RIE.

I. INTRODUCTION

Microelectromechanical (MEMS) devices with high biocompatibility can be used for new and effective medical devices in human disease prevention and detection systems. The best condition for human physiological measurements is to avoid disruption of a patient’s normal activity and this can be achieved by implantable biomedical microdevices [1]. Apart from the electronic design and fabrication issues, the material that should be chosen for these types of applications should firstly be shown to be biocompatible and ideally be suitable for use in conventional Silicon (Si) microfabrication technology [2, 3].

SiC is also currently getting significant attention as a biomedical material of interest. Vigorous biocompatibility studies were performed on the devices fabricated from hexagonal crystal variants of SiC, either 6H-SiC or 4H-SiC [4-7]. For example, the application of amorphous SiC (a-SiC) as a coronary stent coating shows contrary findings [8-10]. a-SiC was found to reduce coronary events in patients, but, in another study, restenosis was shown in both patients implanted with bare stents and SiC coated stents after 6 months of surgery [9,10]. This proposes a further investigation on the biocompatibility and suitability of SiC as a material for implants.

Biocompatibility could be investigated by using assays commonly used in life science studies such as Trypan Blue exclusion assay, MTT assay and PrestoBlue assay. Trypan Blue determines cell viability by utilizing the ability of living cell membranes to exclude Trypan Blue dye, as opposed to a dead cell which has no such ability [11].

Yellow tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Invitrogen) assay is a conventional assay that is widely used to detect cell viability and cell cytotoxicity on the basis of detecting the conversion of yellow tetrazolium salt of the reagent by the viable cell into insoluble purple formazan [12-15]. In order to solubilize the formazan, dimethyl sulfoxide (DMSO) or isopropan-2-ol (IPA) is added to determine the absorbance of the solutions spectrophotometrically [12,13]. PrestoBlue™ reagent is a resazurin based solution used to quantitatively measure cell viability by determining the level of cellular metabolic activity of living cells [16-18]. It is a state-of-the-art cell viability reagent which has less protocol, much rapid than MTT assay and is the most important aspect, it has limited side effect on the tested cells [19]. PrestoBlue™ is a new type of cell viability assay, which has been introduced recently for cell viability quantitative measurement.

Related studies on the effect of the fabrication process in semiconductor such as potassium hydroxide (KOH) etching of silicon substrate and reactive ion etching (RIE) on the biocompatibility of AlGaN/GaN [20] and polyimide [21] have been reported. The authors of these studies concluded that these processes posed little effect on the materials biocompatibility and cytotoxicity. Thus, the effect of typical micromachining steps used in semiconductor
devices fabrication on cell viability nature of 3C-SiC is established and included in this study.

II. MATERIALS AND METHODS

A. Sample Preparations

A layer of n-type 3C-SiC were grown epitaxially on 650 μm thick, <100> Si substrates by chemical vapor deposition (CVD) as described by Wang et al. [22]. The preparation of the 3C-SiC samples using reactive ion etching of 3C-SiC using CF$_4$ gas and wet etching of Si using KOH up were described elsewhere by the authors [23, 24]. Si chip was used as a comparison. For control, the rectangular Thermaxx™ coverslips (pretreated for tissue culture application) as positive control and for the negative control, the coverslips were treated with Bovine Serum Albumin (BSA) was prepared.

Before any treatment, all the samples were rinsed with acetone, IPA and deionized (DI) water and later dipped in hydrofluoric (HF) acid for 30 seconds to remove natural oxide. Si and 3C-SiC samples were diced into 10.5mm ×22mm similar to Thermaxx™ coverslips shape and size. The samples were then sterilized by double autoclaved (121°C) and treated with ultraviolet light (UV) radiation for 15-20 minutes prior to work.

B. Cell Culture and Trypan Blue Exclusion Technique

The Chinese Hamster Ovary (CHO) cells were subcultured on the sample materials (Thermaxx™ slips, Si and 3C-SiC) placed in 6 well cell culture plates (Greiner, # 657 185). Approximately 2 ml of stock solution of media (Dulbecco’s Modified Eagle Medium (Gibco®)) together with 1.0 ×10$^5$ cells/ml were seeded to each well containing the materials. The seeded cell culture plates were then incubated for 4 hours at 37°C and 5% CO$_2$ overnight. Two 96 well culture plates were prepared for MTT assay test and PrestoBlue™ Cell Viability reagent (Invitrogen, USA) test.

C. MTT Assay

The plated cells were added with 20 μl of MTT assay. The plate was covered with the aluminum foil to protect MTT from light and it was agitated with the plate shaker for 5 minutes to ensure the MTT mixed uniformly with the cells mixture. Subsequently, it was incubated for 4 hours at 37°C and 5% CO$_2$ and later, 25 μl of the mixtures were removed from the plated cells. Insoluble purple formazan were formed and then dissolved by adding 50 μl of DMSO on each plated cell.

After that cells were incubated at 37°C and 5% CO$_2$ for 10 minutes. Experiments were performed in triplicates with each experiment repeated three times.

Cellular viability can be measured by:

$$\frac{(OD_{952\text{Sample}} - OD_{952\text{Background}})}{(OD_{952\text{Control Sample}} - OD_{952\text{Background}})} \times 100 \quad (1)$$

D. PrestoBlue™ Reagent Test

PrestoBlue™ Cell Viability reagent (Invitrogen, USA) assay (10 μl) as supplied were added to the mixture and uniformly mixed by using the plate shaker for 5 minutes. The plate was incubated for 2 hours at 37°C and 5% CO$_2$ before measuring the absorbance at 595 nm. Each experiment was made in triplicates and the experiments were repeated three times. Cellular viability was measured by expression (1).

III. RESULT AND DISCUSSION

A. Trypan Blue Exclusion Technique

Trypan Blue stain exclusion technique is a very powerful tool since it is not only able to provide information on the amount of cells (living or dead) but also capable in providing preliminary result about cell viability. Here, the cell viability percentage is assessed indirectly based on the CHO cell membrane integrity [25].

The cell viability result (Fig. 1) shows the highest percentage of viable cell was from the RIE and KOH treated 3C-SiC/Si samples and the untreated 3C-SiC/Si samples. All the 3C-SiC/Si based samples showed a higher cell viability (in percentage) compared to Si samples.

The highest percentage of cell viability is for the KOH and RIE treated samples which is approximately 88%. This is due to the high ratio of the amount of the living cells to the total cells of the RIE and KOH treated 3C-SiC samples and the difference of this ratio is only about 10%.
Meanwhile, the differences for other samples (for the positive control, negative control, Si, untreated 3C-SiC and KOH only treated samples) ranges from 25-33% which explained lower cell viability percentage obtained for these samples.

Although this technique is able to measure cell membranes integrity but on the other hand, this technique compromised the ability of cells to continue their proliferation [25]. Thus, other types of assays capable of measuring cell viability are required for accurate verification the cell viability on the materials of interest.

**Figure 1.** The cell viability percentage was obtained using Countess® Automated Cell Counter (Invitrogen) after stained by Trypan Blue dye.

### B. MTT Assay

KOH treated 3C-SiC/Si and RIE and KOH treated 3C-SiC/Si in comparison to the positive control is expressed in mean ± standard deviation. Here, the result for the RIE and KOH treated 3C-SiC/Si samples are in agreement with the result presented in the previous section where CHO cells showed higher viability level on the plasma etched and KOH treated 3C-SiC/Si samples which is approximately 91%.

Viability percentage of CHO cells on both Si and negative control are better using MTT assay viability test (which are approximately 91%) in comparison to untreated 3C-SiC and KOH only treated samples) contradicted with the Trypan Blue exclusion assay results. This contradiction is not surprising since, MTT assay, despite known as convenient and economic [4], but sometimes provides confusing and inaccurate result [26]. This is indicated in Fig. 2 by high standard deviation error bars where non-living cells are also able to reduce tetrazolium salt into soluble formazan [27]. Thus, as precaution, an extra care is needed while using this assay. In this case, these contradicting results should be accompanied and verified by another independent cell viability assay.

**Figure 2.** CHO cells viability percentage on the materials against control using MTT assay (Invitrogen, USA) assay.

### C. PrestoBlue™ Reagent Test

The result here has confirmed the positive effect of RIE on 3C-SiC. However, PrestoBlue™ indicates that KOH treated 3C-SiC/Si samples were also favored by the cells to rapidly grow. CHO cells were more viable on these types of samples. Therefore, it can be suggested that surface treatment of SiC surface might positively altered for a better cell growth or at least not to be toxic and thus, biocompatible.

As can be seen from Fig. 3, untreated 3C-SiC showed a proliferation rate similar to Si and the negative control. The results obtained were also relatively more reproducible in comparison to the MTT assay based on the small standard deviation error, which indicates the higher accuracy of the used reagent that supports the claim by Newman et al. [19].

**Figure 3.** CHO cells viability percentage on the materials against control using PrestoBlue™ Cell Viability reagent (Invitrogen, USA) assay.

From here, in most results, 3C-SiC has better biocompatibility quality in comparison to Si. This reaffirmed
the biocompatibility of SiC against Si in literatures [6,13,28,29]. Chemical treatment such as immersing 3C-SiC in KOH or HF solution did not alter 3C-SiC biocompatibility. This case was different with plasma treated 3C-SiC. The alteration of 3C-SiC surface had showed impact on its biocompatibility. Thus, based on the viability tests, plasma treatment of the 3C-SiC surface had improved the biocompatibility quality of 3C-SiC. Plasma treatment might deposited C-rich layer on the 3C-SiC surface [30] and it is suggested carbon as a biomedical material showed good electrochemical potential comparable to living cells which lead to better quality of cell attachment [31].

IV. CONCLUSION

The assessment and comparison of the analyzed samples such as: Si, untreated 3C-SiC/Si, KOH treated 3C-SiC/Si and RIE and KOH treated 3C-SiC/Si of their biocompatibility characteristics has reveals important discoveries in this subject. The CHO cells viability was chemically assessed using Trypan Blue exclusion assay, MTT assay and PrestoBlue™ reagents. The quantification of both total and living cells showed interesting results where cell counts for untreated and KOH treated 3C-SiC/Si samples were much higher than the positive control. Two viability tests were conducted to further verify the results. The viability of the cells previously cultured on RIE and KOH treated 3C-SiC/Si was high in both tests. However, lower results were obtained with MTT assay for untreated and KOH treated 3C-SiC, but this discrepancy can be attributed to the accuracy of the assay. KOH treated 3C-SiC also showed high cell viability with PrestoBlue™ test. Here as a final conclusion statement, treating 3C-SiC with plasma gasses such CF$_2$ would improve the biocompatibility of 3C-SiC.

ACKNOWLEDGMENT

The authors would like to thank Mr. Eltaher Elshagmani and Mrs. Layla Mehdi Alhasan for their assistance in performing the cell culture in the School of Applied Science, RMIT University. The authors are also grateful to the support given by the Biomedical Electronic Engineering Programme and the School of Mechatronic Engineering, Universiti Malaysia Perlis for all the support given.

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