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Cytotoxicity Evaluation of Microsystems Materials Using Human Cells

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Cytotoxicity of silicon, silicon dioxide (SiO_2), and silicon nitride (Si_3N_4) is evaluated *in vitro* on the human lung (WI-38) cell line. The biocompatibility evaluation is performed via protocols based on an ISO 10993 standard, which should provide a comparison with other biomaterials. The microsystem materials are extracted in minimal essential media (MEM). Extracts from natural black rubber and polypropylene served as the positive and negative controls, respectively. The extracts are added to 80–90% confluent cell monolayers, which are subsequently incubated for 48 h. The cell monolayers are examined using light microscopy and scored on a relative scale of 0–4 based on the degree of cellular destruction. All microsystem materials scored 0 while the negative and positive controls scored 0 and 4, respectively.

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1. Introduction

There is considerable interest in the development of microsystems (microelectromechanical systems or MEMS) for biomedical applications. Numerous research and commercialization efforts are underway to create sophisticated biological research tools and high-performance clinical devices by exploiting the potential for size miniaturization and integration with microelectronics afforded by microfabrication and micromachining techniques.[3,5–7] Most efforts have generally focused on the development of device designs and associated fabrication protocols with the intent of achieving specific, often mechanical and short-term, functionality. Although this approach may be suitable for the development of microsystems for certain biomedical research applications, requirements for long-term and/or clinical performance necessitate an examination of biocompatibility.[6] This issue is especially crucial for microsystems for implantable applications. The body's natural defense mechanism that begins with inflammation coupled with the corrosive effects of biofluids can disrupt the functionality, or even, destroy microsystems.

The deployment of implantable microsystems based on silicon and related microelectronics materials has generally relied on protective packaging approaches to isolate the microsystem from the hostile body environment. For example, micromachined pressure sensors are encapsulated with biocompatible silicone gels to isolate the piezoresistive sensor from body fluids. The protective packaging approaches can result in two primary drawbacks: attenuation of signal/stimulus that must be communicated between the physiological environment and microsystem; and, increased size that detracts from the benefits of miniaturization particularly when working in constrained spaces or at the cellular level. Consequently, we are investigating alternate approaches to enhancing the biocompatibility of microsystems. However, there is a paucity of data on the biocompatibility of microsystem materials. Previous efforts on biocompatibility testing of microsystem materials were usually limited to specific applications, and often, relied on nonstandard protocols.[1,2] For example, the pre-testing procedures employed in many studies did not correspond to acceptable sterilization protocols for a clinical device. Although such efforts are valuable in shedding insight into microsystem performance *in vivo* within a research context, the regulatory scrutiny associated with medical devices will require biocompatibility evaluation of microsystems based on standardized tests. The first-ever evaluation of common microsystem materials using the ISO 10993 battery of tests was recently reported.[4] Evaluation of material cytotoxicity using the ISO 10993–5 standard: "Test for Cytotoxicity – *In Vitro* Methods," was identified as an essential step towards certifying the biocompatibility of implantable devices. This paper reports the *in vitro* evaluation of cytotoxicity of silicon, silicon dioxide, (SiO_2), and silicon nitride (Si_3N_4) based on the ISO standard using human embryonic lung cells. The preparation of microsystem materials is described and followed by a brief description of the cytotoxicity evaluation procedures. Results of the cytotoxicity evaluation are presented, with the response of the microsystem materials normalized to standard controls.

2. Materials and Methods

2.1 *Specimen preparation*

The materials selected for the evaluation were semiconductor-grade single crystal silicon (Si), SiO_2, and Si_3N_4. This choice of materials was motivated by their ubiquitous utility and ready availability within the microsystems community. One lot of nine virgin, prime grade, single side polished, 100-mm-diameter, 500-μm-thick, <100>-oriented, *n*-type, Si wafers were cleaned using a conventional piranha cleaning procedure, which involved a 20 min immersion in 3:1 H_2SO_4/H_2O_2 mixture, followed by thorough rinsing in deionized (DI) water, and drying with nitrogen gas. Afterwards, three wafers were set aside while the other six were prepared for furnace growth and deposition of SiO_2 and Si_3N_4, respectively. Prior to placement in the furnaces, the wafers were further cleaned using a dual-stage RCA cleaning procedure, which removed any residual surface organic and inorganic contaminants. Thermal oxidation at 1100°C and atmospheric pressure was conducted on three wafers to grow a 1.5-μm-thick SiO_2 film, while a 1200 Å-thick Si_3N_4 layer was deposited on the remaining wafers using the low pressure chemical vapor deposition (LPCVD) process. All nine wafers were then individually packaged in sealed sterilization pouches. Finally, steam sterilization was performed on the packaged wafers using an autoclave in the gravity mode with an exposure time of 30 min at 132°C.

2.2 *Test protocol*

The microsystem materials were evaluated using the MEM (minimal essential media) elution test, which is designed to determine the cytotoxicity of extractable substances. In this test, extracts of the material sample are added to cell monolayers and incubated. Sample extracts for each microsystem material were generated by immersion of 154 cm^2 sections of the sterile wafers in 1X MEM with 5% calf serum for 25 h at 37°C. In addition, extracts were also generated from natural black rubber and polypropylene to serve as the positive and negative controls, respectively.

Human lung (WI-38) cells were seeded onto six well cell culture plates and incubated until 80–90% confluent. The cell culture media was then removed from the culture plates and replaced with sample extracts. Specifically, test extracts and corresponding 1:2, 1:4, and 1:8 dilutions were prepared and filtered. 6 mL of each of the different extracts were added to three wells of cells, which were then incubated at 37°C with 5%CO_2 for 48 h.

2.3 *Evaluation*

The cells were examined after 48 h of incubation using a light microscope. Each well was examined carefully to discern any changes in cell morphology including rounding and lysis. The degree of cellular destruction was estimated from the morphological changes relative to the negative and positive controls, which were given scores of 0 and 4, respectively (Fig. 1). The cytotoxicity test is considered valid if all negative control wells score 0 and all positive control wells score 3 or higher.

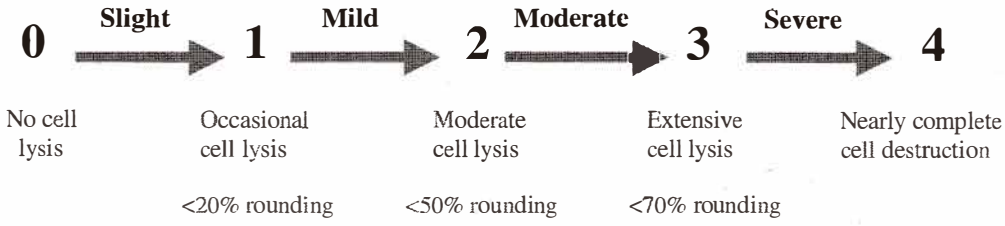

Fig. 1. Scoring scheme for cytotoxicity evaluation based on morphological changes. Negative and positive controls score 0 and 4, respectively.

3. Results and Discussion

Microscopic examination of cells exposed to extracts of the positive and negative controls revealed the range of cellular destruction that might be expected due to cytotoxicity. The cells exposed to the extract of the negative control still spread to confluence on the well surface after 48 h of incubation and exhibited intracytoplasmic granules (Fig. 2). In contrast, wells exposed to extracts of the positive control exhibited extensive lysis (Fig. 3). The few (<1%) cells that were still intact displayed rounded bodies.

Table 1 presents the cytotoxicity scores of the each well relative to those of the positive and negative controls for the Si extract. In all three wells, the positive control scored 4 while the corresponding wells for the negative control scored 0. The media control category refers to cells cultured with a blank media extract (no test material). The 0 score for the media control signifies that the MEM did not stimulate or suppress the cytotoxic effect of the test material. Table 2 summarizes the resulting average scores of all materials evaluated for cytotoxicity. All microsystem materials scored 0, which was consistent with the negative and media controls.

The evaluation of cytotoxicity microsystem materials using a test protocol based on the ISO 10993 standard allows for a direct comparison with other reference biomaterials such as polypropylene. To our knowledge, this study is the first to report on the cytotoxic effect of microsystem materials on human cells. Like the negative control, specimens of Si, SiO_2, and Si_3N_4 did not exhibit discernable cytotoxicity. However, it should be noted that the *in vitro* assay has limitations that must be considered when designing implantable biomedical microsystems. For example, cytotoxicity might be exhibited over a longer period of exposure. Also, the reaction of cells to the actual material surface might be different from the response elicited by test extracts. Nevertheless, our results support the notion that the evaluated materials are suitable candidates for further development of implantable biomedical microsystems. The data should be used as a guide to material selection; various devices and tools will still require additional cytotoxicity and biocompatibility testing, which will be determined by requirements of the specific application.

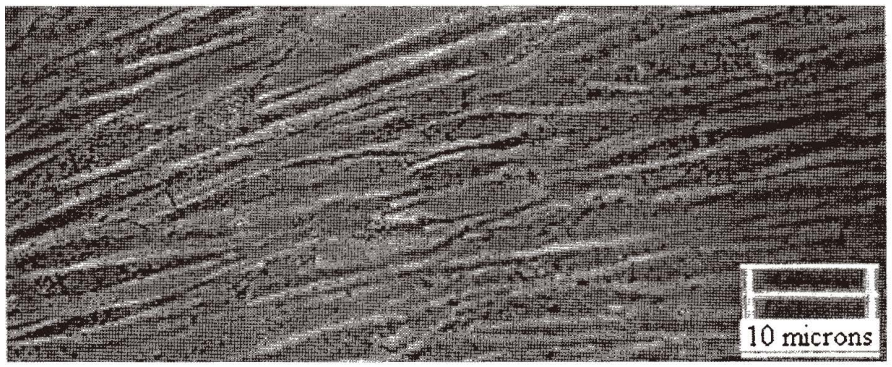

Fig. 2. Optical micrograph of WI-38 human cells exposed to extract of negative control (polypropylene) for 48 h. Cells have spread on the surface of the culture well with no rounding and intracytoplasmic granules are visible.

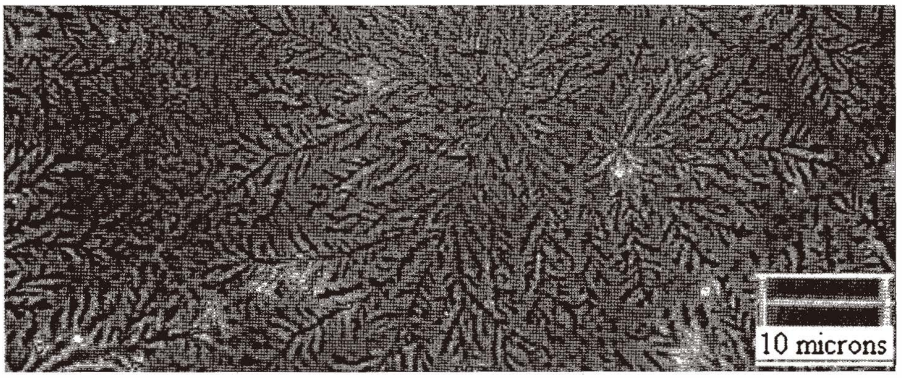

Fig. 3. Optical micrograph of WI-38 human cells exposed to extract of positive control (natural black rubber) for 48 h. Nearly complete cell destruction is evident.

Table 1
Cytotoxicity scores of wells exposed to extracts of Si and controls.

Material Extract	Well 1	Well 2	Well 3
Negative control	0	0	0
Media control	0	0	0
Positive control	4	4	4
Sample 1:1	0	0	0
Sample 1:2	0	0	0
Sample 1:4	0	0	0
Sample 1:8	0	0	0

Table 2
Cytotoxicity score summary.

Material	Reactivity	Score
Polypropylene (Negative Control)	None	0
Natural Black Rubber (Positive Control)	Severe	4
Si	None	0
SiO_2	None	0
Si_3N_4	None	0

4. Conclusions

Human lung cells (WI-38) have been used to evaluate the cytotoxicity of Si, SiO_2, and Si_3N_4. The evaluation was conducted an *in vitro* assay based on an ISO 10993 standard, which should allow for direct comparison with reference biomaterials. None of the extracts of specimens of microsystem materials exhibited cytotoxicity. These results support the notion that Si, SiO_2, and Si_3N_4 are suitable candidates for further development of implantable biomedical microsystems.

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