Astrocyte Response to Various Biomaterials

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Statement of Purpose: The need to find biocompatible surfaces for medical implants, such as, neural electrodes, is imperative. It is crucial for medical devices intended for long-term in vivo purposes to go through meticulous investigation of biocompatibility requirements. Materials such as SU-8, platinum and poly methyl methacrylate, (PMMA) have been employed for the production of microelectro-mechanical systems for medical applications (bioMEMS) (1). Besides its application as a near UV photoresist in high-aspect-ratio MEMS, SU-8 was used for many of its useful material properties including, its dielectric properties, transparency to visible light, a low Young's modulus, cost, and most importantly its biocompatibility (2, 3). PMMA is a polymer that has been often used in not only numerous medical devices, but specifically bioMEMS/bioNEMS devices (4, 5). Platinum is used as stimulating/recording electrodes for it excellent conductivity (6). The objective of this study is to investigate biocompatibility of these materials in vitro with central nervous system cells. Here, we used real-time PCR to determine the biocompatibility of three different surfaces, using glass as a control. We examined the gene expression of MAP2K1 (proliferation indicator) and GFAP (cell activation indicator). In addition, we utilized a more standard method to determine cellular growth rate, MTT assays.

Methods: SU-8 samples were fabricated by spincoating SU-8 photoresist (2015) onto 1cm^2 silicon wafers. An E-beam evaporator was utilized for the production of the platinum samples; a thin film of Pt (200nm) was deposited on 1cm^2 silicon wafers. PMMA sheets were cut into 1cm^2 pieces. Glass wafers were employed as a control surface. C6 rat astrocytoma cells were seeded at a density of 2000 cells/cm^2 and incubated in F-12K medium containing 10% horse serum, 2.5% FBS, and 1% antibiotic-antimycotic. MTT assays were performed at three time intervals (days 1, 3, and 7 post seeding), in order to evaluate cell proliferation. Real-time PCR was done to measure the amount of GFAP and MAP2K1 on the wafers at days 3 and 7.

Results: Examination of the MTT assays showed that the SU-8 surface had the fewest cell number on day 7. Furthermore, the RT-PCR data revealed that not only did SU-8 exhibit little increase in the amount of MAP2K1transcripts between days 3 and 7, but a decrease in the level of GFAP transcripts by day 7, indicating that the cells were stable on the SU-8 surface. A sudden increase in cell number on the Pt, PMMA, and glass surfaces was observed on day 7 of the MTT assay. This observation is dually noted with the results of the RT-PCR; Pt had a sudden increase in MAP2K1 transcripts at day 7 and PMMA had a large increase in GFAP transcripts on day 7 as well.

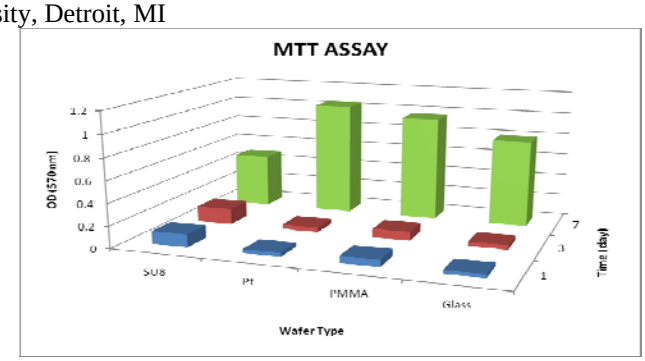

Figure 1. MTT assays: SU-8 has the lowest number of cells at the end of day 7.

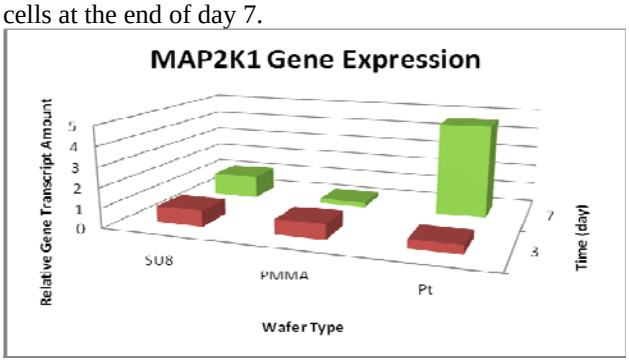

Figure 2. RT-PCR (MAP2K1): SU-8 has no change in MAP2K1 transcript levels between day 3 and 7.

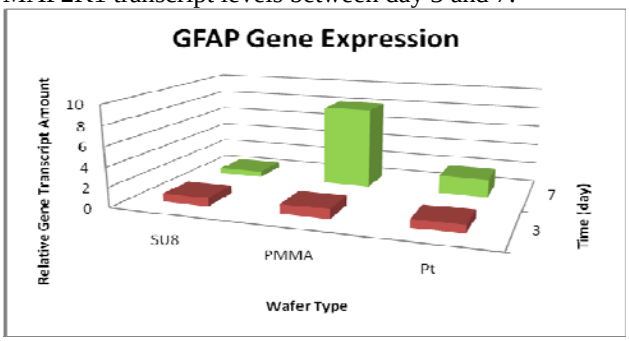

Figure 3. RT-PCR (GFAP): SU-8 has a decrease in GRAP transcript amount.

Conclusions: The low number of cells on the SU-8 surface, decrease in GFAP, and no change in MAP2K1 gene expression by day 7 indicate that SU-8 is a biocompatible material. Given that a sudden increase in the number of proliferating astrocytes, the case of the Pt and PMMA surfaces, is an indicator of astrocytosis which is known to be a major component of glial scar tissue (6).

References: (1) Kotzar G et al. Biomaterials 23(2002) 2737-2750. (2)Hopcroft M et al. Fatigue & fracture of engineering materials & structures (8756-758X), 28 (8), p. 735. (3) Voskerician G et al. Biomaterials 24 (2003) 1959–1967 (4) Bhushan B. Microelectronic Engineering 84(2007) 387-412. (5) Frazer RQ et al. J Long Term Eff Med Implants. 2005;15(6):629-39. (6) Polikov VS et al J Neurisci Methods 2005;148:1-18.