The Effect of Nanopatterning Poly(methyl methacrylate) on Glial Cell Activation and Proliferation

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Statement of Purpose: Neural implants have the potential to make positive impacts on numerous patients suffering from a multitude of CNS and PNS diseases. However, the cellular response to these implants is the formation of a glial scar tissue, which therefore prevents the implant from performing its function. The cells responsible for the glial scar formation, the astrocytes, make up approximately 50% of the glial cells in the CNS. Current research to optimize the long term function of these implants has focused on modifying biomaterial to inhibit glial activation. Studies on nanopatterning of the implant surface demonstrate improved biocompatibility (1). Furthermore, poly(methyl methacrylate), (PMMA), has been clinically used for several implant strategies(2). Thus we have employed the process of hot embossing to create nanopatterns on PMMA. Moreover, we examined the effect on astrocyte activation, which is characterized by an increased proliferation and up regulation of glial fibrillary acid protein (GFAP) (3). Overall, we expect that nanopatterning will inhibit glial cell activation.

Methods: Surfaces of 1cm^2 PMMA wafers were hot-embossed in order to either generate a pattern of 3600 grooves/mm or an even non-patterned surface. In addition, glass wafers were also tested as a material control surface. C6 rat astrocytoma cells were seeded at a density of 2000 cells/wafer 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 on the wafers at days 3 and 7. Calcein AM (Invitrogen, Carlsbad, CA) fluorescence microscopy was utilized to image the alignment of the cells on the different surfaces.

Results: When assessing the MTT assays, we determined on day 7 that the glass wafers contained the largest number of cells, followed by non-patterned surface. More interesting, the nanopatterned surface contained a notable decrease in cell number as compared to both the control surfaces (Figure 1). Moreover, the RT-PCR data correlated directly with that of the MTT. The non-patterned surface had an increase in GFAP transcripts, with at least twice as many at the end of day 7 as compared to the nanopatterned surface (Figure 2). When examining the morphology of the glial cells on the surfaces, we found that the cells aligned in a monolayer according to the direction of the patterning, whereas the cells seeded on the non-patterned surface were randomly clustered in congregates (Figure 3).

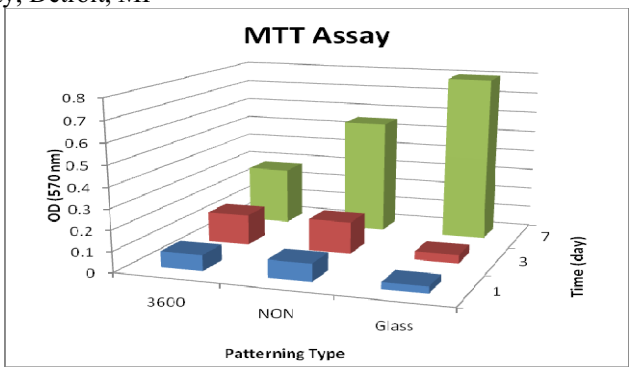

Figure 1. MTT assays: non-patterned surface has 2X the amount of cells at the end of day 7 as nanopatterned surface.

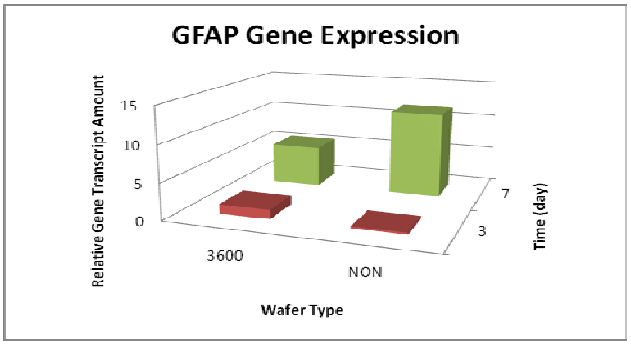

Figure 2. RT-PCR: Non-patterned surface has 2X as much GFAP gene expression as nanopatterned surface.

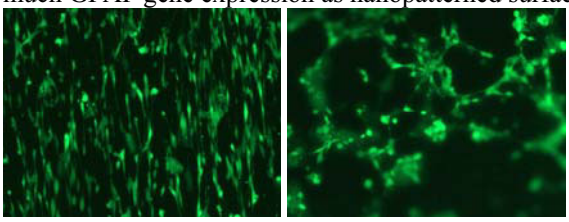

Figure 3. C6 cells on patterned PMMA wafer (left) and non-patterned wafer (right) on day 7.

Conclusions: Increased number of astrocytes and increased proliferation rate is found on both the glass and non-patterned surfaces. The patterning appears to inhibit the rate of proliferation, number of cells on the surface and the organization of the cells. A sudden increase in the number of proliferating astrocytes with the non-patterned surface is an indicator of reactive astrocytosis which is known to be a major component of glial scar tissue formation. Consequently, the nanopatterning of the PMMA surface appears to inhibit glial activation and may ultimately reduce the glial scar formation in vivo.

References:

(1) Kriparamanan R et al. J Nanosci Nanotechnol. 2006 Jul;6(7):1905-19. (2) Frazer RQ et al. J Long Term Eff Med Implants. 2005;15(6):629-39. (3) Polikov VS et al J Neurisci Methods 2005;148:1-18.