Astrocyte Reactivity to Neural Implant with Porous Silicon Backbone Support

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Statement of Purpose: Requirements for chronic neural implant include physical durability for implantation and long-term biocompatibility [1-3]. While silicon based implant affords enough mechanical strength for surgical implantation, the stiff silicon in the brain causes a shear force between the organism and implant due to physiological micromotions of soft brain tissues. This damage of neural elements and implants leads to a foreign body reaction, resulting in the proliferation of a glial scar thus preventing neural fibers to be recorded or stimulated. Therefore, it is important to develop a hybrid implant that is stiff enough for surgical implantation and afterwards becomes flexible in the brain.

Porous silicon (pSi), where nanopores are fabricated by electrochemical etching, has been recently used to improve the recording capability and the tissue compatibility of neural electrodes [1,2,4]. In addition, porous silicon has been shown biodegradable in-vivo and degradation products (silicic acid) is safe [5]. This study takes advantage of porous silicon's previous usage and utilizes the material in a novel way. We goal to use pSi as a backbone support for the thin-film electrodes encapsulated in polymers during implantation, thereafter, the porous silicon will degrade and dissolve into physiological solutions, thus rendering a flexible neural electrode for the longevity of the implant. The current study observes astrocyte proliferation, viability, and nucleus morphology to examine the biocompatibility of the biodegradable pSi.

Methods: Porous silicon (pSi) was manufactured by electrochemical etching. Several samples were allowed to dissolve completely, of which the remaining particles were collected and quantified using inductively coupled plasma (ICP). C6 rat astrocytoma cells were seeded at a density of $5x10^2$cells/well in a 96-well culture plate (for MTT assay) and $3.5x10^3$cells/well in a 12-well cell culture plate (for cell staining) and incubated in F-12K medium containing 10% horse serum, 2.5% FBS, and 1% antibiotic-antimycotic. One day post cell seeding, the pSi particles were serially diluted in the 96-well plate for MTT assay. Whole wafers were placed on top of the seeded cells in the 12 well plates for cell staining. Cells grown in culture plates served as control. MTT assays were performed at three time intervals (days 2, 5, and 7 post seeding), in order to evaluate cell proliferation. Live/Dead (Invitrogen, Carlsbad, CA) fluorescence microscopy was utilized to image cell viability at days 5,7 and 9. Cells were fixed in 4% paraformaldehyde at days 2, 5, and 7. Cells were then stained with rhodamine phalloidin (Invitrogen, Carlsbad, CA) flourescense stain to image the cells' cytoskeleton and double stained with DAPI (Sigma-Aldrich, St. Louis, MO) to image the cell nucleus.

Results: Astrocyte proliferation decreased as the pSi concentration increased at all three time-points. The percent of viable cells growing with the pSi wafer decreased at day 7compared to the amount on day 2, however went up to over 90% by day 9. The projected area of the cells' nucleus with the porous silicon wafer were consistently lower than the cells' grown in the culture plate without the wafer for the first 7 days, however, became more spread out by day 9.

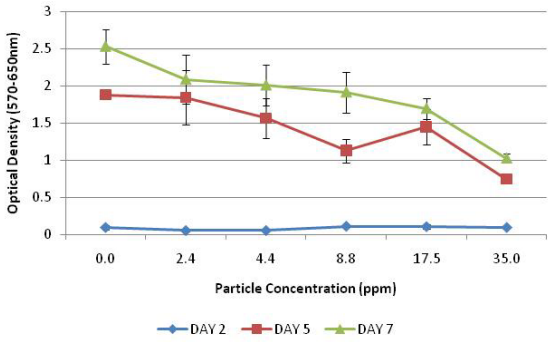

Figure 1. MTT Assay results.

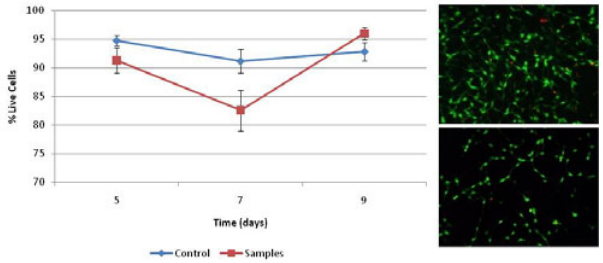

Figure 2. (A)Viability staining results. Live/Dead stain (images taken at 20X) (B) Control sample at day 5 (C) pSi sample at day 5.

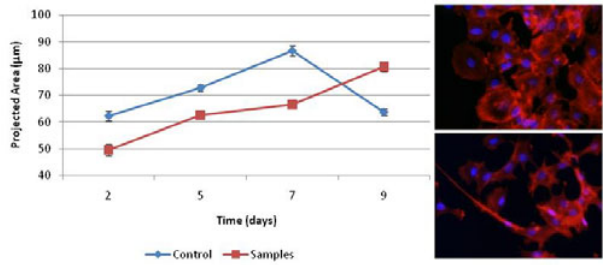

Figure 3. (A) Projected area of cells' nucleus. Rhodamine phalloidin and DAPI stain (images taken at 40X) (B) control sample at day 5 (C) pSi sample at day 5

Conclusions: Porous silicon demonstrates biocompatibility at less than 4.4ppm with astrocytes. Moreover, after initial cell reaction to the material, astrocytes demonstrate viability and nuclear morphology similar to control samples by day 9 in vitro, thus suggesting possible long term biocompatibility.

References:

1. Johansson F. Acta Biomater. 2009 Jul;5(6):2230-7
2. Persson J. J Biomater Sci Polym Ed. 2007;18(10):1301-8.
3. Marin C. Front Neuroengineering. 2010 May 28;3:8.
4. Moxon KA. J Biomater Sci Polym Ed. 2007;18(10):1263-81.
5.Park JH. Nature Nano 2009 8:33-336