**Comparative Assessment of Iridium Oxide and Platinum Wires Using an *in vitro* Glial Scar Assay**

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**Statement of Purpose:** Neural electrodes with safe stimulation are able to deliver a charge to the interface and tissue without inducing chemical reactions on the electrodes [1]. Moreover, good electrode materials have low impedance and high capacitance [2]. Higher charge injection capacity is thought to lower the potential required for stimulation, thus reducing the injury at the stimulation site [3]. Platinum and iridium oxide are the two most commonly used materials in practical medicine [1]. Platinum is resistant to corrosion and has charge storage capacities in the range of 300-350μC $cm^{-2}$ [1]. Iridium oxide has reversible redox reactions that significantly increase the charge injection capacity to the range of 2-3 mC $cm^{-2}$. Studies have shown iridium to have lower impedance when compared to platinum [2]. Although, both platinum and iridium oxide have been identified as biocompatible, corrosion-resistive candidates for electrical stimulation and implantation, there lacks research in the assessment of glial scarring around these materials[1,3]. Recently, Polikov et al have adapted an *in vitro* glial scar assay in order to assess the inflammatory response on a molecular level [4-5]. We utilized this method to observe the cellular reactivity to platinum and iridium oxide wires through real-time PCR to determine the relative gene expression of glial fibrillary acidic protein (GFAP), a cell activation indicator, and immunofluorescent imaging.

**Methods:** Neuron-glia cultures were prepared from the cortex tissues of Sprague-Dawley embryonic day 17 rats. Dissociated cells were seeded at a concentration of $7x10^5$cells/well into poly-D-lysine-coated glass cover slips in 12-well plates. Cells were incubated in culture media containing, 56% MEM, 20% DMEM, 10% fetal bovine serum (FBS), 10% horse serum (HS), 4% penicillin/streptomycin. After ten-days, cultures were used for treatment. Platinum wires (20 um dia) were purchased from a manufacturer. $IrO_2$ was sputter coated onto the platinum wires. The thickness of the $IrO_2$ thin film was measured 200 nm using profilometer. Controls were the cells growing on the poly-d-lysine coated glass wafers without wires. Sterilized wires were cut into 5mm pieces, of which one wire was placed randomly into each well over the cultured cells. Real-time PCR (RTPCR) was performed to observe the gene expression of GFAP at days 1, 4 and 7. Cells were fixed with 4% paraformaldehyde at days 4 and 7. Astrocyte specific antibody, GFAP (Millipore, Billerica, MA) was used to stain astrocytes, Milli-Mark Pan Neuronal Marker (Millipore, Billerica, MA) was used to stain neurons, and all cell nuclei were attained with DAPI.

**Results:** RTPCR results revealed that there was a decrease of GFAP expression on both materials at day 4, but a rise by day 7. Immunofluorescent imaging showed an even distribution of cell types around both types of wires, with less than 2 neurons around the wires. The thickness of the glial scar grew over time; from 6.09±0.30 μm to 11.27±1.23μm on one side of the $IrO_2$ wire and 4.22±0.45 μm to 6.76±0.45 μm on one side of the Pt wire.

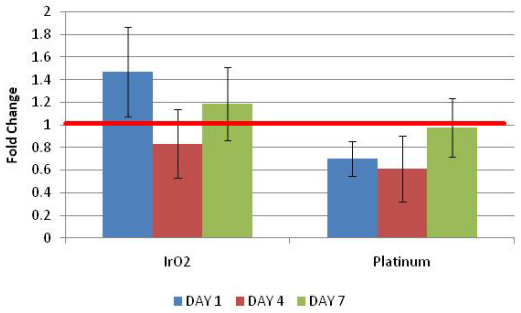


**Figure 1.** RTPCR relative GFAP gene expression. Red horizontal line is the control.

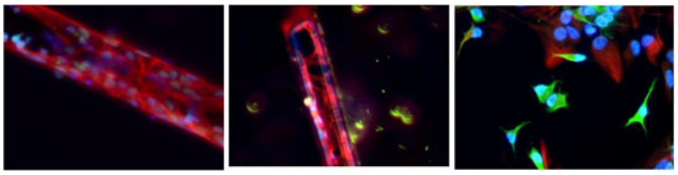

**Figure 2.** Immunofluorescent images of (from left to right) platinum wire, $IrO_2$ wire, and control. Astrocytes are red, neurons are green, and cell nuclei are blue.

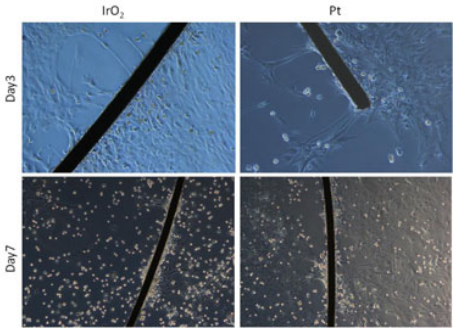


**Figure 3.** Phase contrast images displaying the growth of the glial scar over time around the platinum and $IrO_2$ wires.

**Conclusions:** Results indicate a slightly less of a reactive response to platinum than to $IrO_2$ wires. Cells appeared to migrate around the platinum wires more than the $IrO_2$ wires. Future research in the functionality of the neurons is required.

**References:**

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