Improved Interactions of Primary Neurons with RFGDT Pyrolytic Carbon and Photocatalytic Titanium for Electrode Implants

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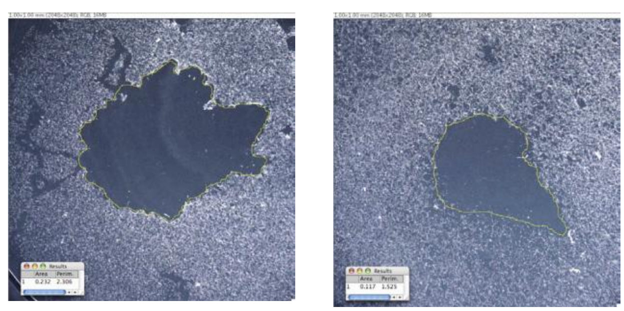
Statement of Purpose: Scar capsule formation around nerve tissue signal-collecting or stimulating electrodes reduces transduced signal fidelity and strength and decreases battery life. The goal of this work is to produce higher quality transduced signals and lower power operations, while extending battery lifetime. The approach is based on our prior observations of Radio Frequency Glow Discharge Treatment (RFGDT)-enhanced cell spreading and attachment with concurrent suppression of free radical generation, to minimize the foreign body reaction (1). The objective is to achieve tissue integration at the bio/material interface, similar to the osseointegration already achieved for dental implants (2). Applications include improved cochlear implants, pain control devices, and intra-retinal implants.

Methods: The materials investigated were small disks of Pyrolytic Carbon (PyC) and commercially pure Titanium (photocatalytic cpTi). Replicate samples were autoclaved (5-6 disks of each material) or RFGDT for 3 minutes (Harrick 32G unit, 0.1 torr, air) (5-6 disks of each material). Additional sterile replicates of each material were characterized by comprehensive contact angle measurements, scanning electron microscopy, energy-dispersive X-ray analysis, X-ray photoelectron spectroscopy, and surface profilometry. Cochlea were removed from post-natal (P3-P4) Sprague Dawley Rat pups (Charles River Laboratories, Inc.) and placed in cold HBSS. While in HBSS, the spiral ganglion neurons [SGN] were extracted from the cochlear basilar membranes and subsequently transferred into SGN culture medium. SGN were further isolated by enzymatic treatment, mechanical pipetting, and centrifugation. Concentrated neuronal micro-tissue layers developed on the test substrata after 48 h (37C, 5%CO_2). A water-jet impingement technique was used to examine attachment strengths of the cultured cells. The cultured cells were authenticated as neurons by staining with primary antibody against neurofilament, mono-beta-tubulin TUJ1 (Covance, LN#14924801). The % area coverage of the sample disks by the stained neurofilaments was determined by image analysis (Image J). The use of animals in this research was approved by University at Buffalo's Institutional Animal Care & Use Committee.

Results: Autoclaved PyC had the lowest critical surface tension of the materials studied and supported a high coverage of prominent neurons, but the cells had the lowest attachment strengths of the 4 test conditions. RFGDT-cpTi supported fewer, better-spread neurons (determined from surface coverage of the stained neurofilaments) than the other 3 test conditions, but the cells had one of the highest average attachment strengths as indicated by the jet impingement method.

Conclusions: Radio Frequency Glow Discharge Treatment (RFGDT) of otherwise biocompatible, implantable, conductive PyC beneficially increases the material's critical surface tension and composite surface free energy for applications requiring intimate bonding of primary neuronal cells to functional electrodes. Improved cell-substratum interaction qualities included increased cell adhesion strength from less than 70 dynes/cm^2 (autoclaved PyC) to greater than 100 dynes/cm^2 (RFGDT PyC). Average adhesive strengths of cells on cpTi did not differ between the two surface treatments. Average areas covered by stained neurofilaments were less for both RFGDT materials, but closer microscopic inspection indicated greater cytoplasmic spreading and membrane contact with the RFGDT materials. Based on prior findings of scar-capsule-free connective tissue integration with RFGDT implants of CoCrMo alloy and semiconductor germanium (3), these results encourage further explorations of RFGDT as a surface modification technique to minimize or eliminate functional neuroelectrode encapsulation by insulating scar layers. Plans for future experiments include evaluations of the signal-recording and stimulation functions of RFGDT electrode materials in an animal model.

Condition	CST [mN/m]	Avg % Area Covered by Neurofilaments	Avg Adhesive Strength [dynes/cm^2]
PyC-autoclaved	35	37%	67
PyC-rfgdt	>72	25%	102
cpTi-autoclaved	36	28%	116
cpTi-rfgdt	>72	19%	106



Cells on PyC after Jet Impingement. Sample area in each image: 1sq.mm. L: Autoclaved PyC, R: RFGDT PyC

References: (1) Baier R, et al. J. Adhesion 2000; 74:79-101; (2) Baier RE, et al. in Tissue Integration in Oral, Orthopedic, and Maxillofacial Reconstruction, (WR Laney, DE Tolman, eds), 1990, Quintessence Publishing Co. Inc, Chicago, pp 240- 249. (3) Baier RE, et al. J Biomed Mater Res 1984; 18:337-355.

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