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Immobilization of Bioactive Peptides on Benzocyclobutene (BCB) Surface Grafted- Dextran for Neural Implant Applications

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Abstract—In *vitro* cell adhesion and neurite growth on dextran-coated Benzocyclobutene (BCB) films, covalently grafted with bioactive peptides are investigated. For cell/tissue adhesive surfaces, synthetic peptide GRGDSP was employed. GRADSP was used as an inactive control for nonspecific peptide-induced cell adhesion. For surface coatings with neurite growth-promoting activity, the laminin-based peptide SRARKQAASIKVAVSADR was utilized. Three cell lines were utilized in these studies. The cell cultures investigated in this study were 3T3 fibroblasts, neuronal-like PC12 cells, and a glial-like (glioblastoma) T98-G cell line. Chemical composition of all modified surfaces was verified by X-ray photoelectron spectroscopy (XPS). Our non-toxic aqueous methods to graft cell adhesion peptides on dextran monolayer surfaces, effectively limited non-specific cell adhesion and neurite growth in the presence of cultured cells. Microscopic visualization and imaging of these surfaces showed that dextran coatings promoted essentially no adhesion of all cell lines tested. Surface-grafted cell adhesion RGD peptides stimulated fibroblast and glial cell adhesion with minimal neuronal PC12_cell attachment and spreading *in vitro*. In contrast, surface-grafted inactive RAD peptide sequences did not promote significant cell interaction of all cell types indicating that peptide-grafted surfaces did not promote non-specific cell adhesion. Surface-grafted high affinity IKVAV peptides promoted cell type-dependent interactions. The IKVAV-grafted surfaces also promoted neurite growth on all substrates.

Keywords: Biomaterials, Benzocyclobutene, Cell Adhesion, **neurite growth**, Immobilization, bioactive peptide(s), grafted dextran, neural implant

I. Introduction

A permanent, multi-site interface between specific neural populations in cerebral cortex or spinal cord and the external world is a critical enabling technology for fundamental advances in neurological science and medicine. While a variety of complex neural implants have been developed over the past two decades, only singular examples of long-term functional performance have been reported. Thus, despite the enormous gains in technology over this period, the delivery of a reliable, high-capacity, extended-duration interface with the CNS remains largely unfulfilled.

One common failure mode of chronic neural implants is an adverse tissue reaction at the neuron-electrode interface. One outcome of this tissue reaction is injury-activated astrocytes synthesize abnormal amounts of extracellular matrix molecules and deposit them into scar tissue (glial scarring). In functional terms, the reactive astrocytes are sealing off areas of injury and preventing further propagation damage to adjacent healthy tissues (1). The downside to this injury response is that the glial scar is an obstacle or barrier to neurite growth and axonal regeneration (1). This lack of communication at the CNS tissue/implant interface results in failure of the implant to record or stimulate neurons (2).

One approach for stabilizing neuron/implant communication at the CNS tissue/implant interface is to design an implant biomaterial surface that provides a provisional matrix for the promotion of maximal tissue cell adhesion and axon regeneration/neuron ingrowth with minimal inflammatory cell adhesion and minimal glial scarring. In our previous study, processes were developed to effectively integrate bioactive molecules with the surface structures of implantable electrode arrays. In this studies dextran-based bioactive coating technology was extended and developed for Benzocyclobutene (BCB), the base materials utilized to fabricate our new prototype neural implants.

II. Methodology

A biocompatible (5), implantable microeletrode base material, Benzocyclobutene (BCB) was prepared in the cleanroom utilizing fabrication methods. Surface amination chemistries were developed for this material. Dextran and peptides were immobilized the aminated material surface using previously described methods (3, 4). Four synthetic peptides were utilized in these studies. For cell/tissue adhesive surfaces, the peptide GRGDSP (Gly-Arg-Gly-Asp-Ser-Pro) (Life Technologies) was employed. GRADSP (Gly-Arg-Ala-Asp-Ser-Pro) (Life Technologies) was used as an inactive control for nonspecific peptide-induced cell adhesion. For surface coatings with neurite growth-promoting activity, the laminin-based peptide SRARKQAASIKVAVSADR (Bachem) was utilized.

Three cell lines were utilized in these studies. The cell cultures investigated in this study were 3T3 fibroblasts (ATCC #CRL-6476), neuronal-like PC12 cells (ATCC, cat# 30-2004), and a glial-like (glioblastoma) T98-G cell line (ATCC #CRL-1690). Cell adhesion and spreading was assessed using reported methods (3, 4).

III. Results

Results of the cell adhesion studies (Fig 1) can be summarized as follows: 1) Dextran coatings promoted essentially no adhesion of all cell lines tested indicating that this coating has the potential to minimize inflammatory responses *in vivo*; 2) RAD peptide-grafted surfaces did not support adhesion of all cell types indicating that peptide-grafted surfaces did not promote non-specific cell adhesion; 3) RGD peptide-grafted surfaces promoted substantial fibroblast and glial cell adhesion with minimal neuronal cell adhesion indicating a substrate that selects against neuron adhesion; and 4) IKVAV peptide-grafted surfaces promoted substantial neuron cell adhesion and minimal fibroblast and glial cell adhesion indicating a substrate that is selective for

neuron adhesion and selective against fibroblast and glial cell adhesion.

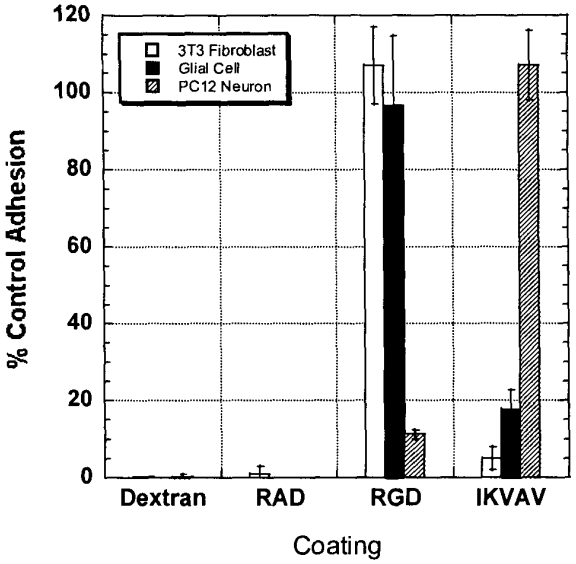

Figure 1. 3T3 fibroblast, T98-G glial cell, and PC12 neuronal cell adhesion on surface-modified BCB-coated silicon wafers.

IV. RESULTS AND DISCUSSION

As expected, cell adhesion and spreading was very low in serum-supplemented medium on dextran-coated surfaces for all cell types on all base materials (Fig 1). Inactive, non-adhesion promoting GRADSP peptides immobilized on dextran-coated materials produced adhesion and spreading at levels comparable to (not significantly different than) dextran-coated substrates (Fig 1). These results suggest that peptides (without cell adhesion-promoting activity) grafted to dextran-coated substrates did not alter the nonadhesive properties of dextran. Therefore, the adhesion promoting activity of RGD and SIKVAV peptides grafted to dextran-coated surfaces was intrinsic to the adhesive signals presented by the surface-grafted peptides without contributions from adsorbed serum-derived adhesion proteins.

RGD peptide-grafted surfaces were observed to promote substantial fibroblast and glial cell adhesion with minimal neuronal cell adhesion indicating a substrate that selects against PC12 adhesion (Fig 1). Lower PC12 adhesion may not be representative of neuron cell adhesion to RGD peptide-grafted substrates in general, since PC12 cells have long been reported to be loosely adherent cells in culture, requiring special ECM coatings on cell culture substrates to enhance cell adhesion (6). The RGD peptide-coating technology may be utilized for neural implants where tissue anchorage is desired to limit injurious micromotion at the tissue/implant interface.

IKVAV peptide-grafted surfaces promoted substantial neuron cell adhesion and minimal fibroblast and glial cell adhesion indicating a substrate that is selective for neuron adhesion and selective against fibroblast and glial cell adhesion (Fig 1). The IKVAV-grafted surfaces also promoted neurite growth on all substrates (Data not shown). These results are in agreement with other reports in the literature (7, 8). Applications for the IKVAV peptide-grafted coating technology include developing tracts for guidance of neurite growth on implant surfaces and coating areas around recording/stimulation sites to promote neurite growth near these sites for optimal signal transfer between neurons and implants.

V. CONCLUSION

This study demonstrated that dextran-based surface modifications could be developed for neural implant microfabrication applications and that surface grafting of specific adhesion peptides promoted cell type-selective responses.

Also, with dextran-based surface coatings, it will be possible to develop well-defined surface modifications that promote specific cell interactions and perhaps better performance in long-term biomaterial implants.

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