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Surface topography and chemistry shape cellular behavior on wide band-gap semiconductors

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ABSTRACT

The chemical stability and electrical properties of gallium nitride make it a promising material for the development of biocompatible electronics, a range of devices including biosensors as well as interfaces for probing and controlling cellular growth and signaling. To improve the interface formed between the probe material and the cell or biosystem, surface topography and chemistry can be applied to modify the ways in which the device interacts with its environment. PC12 cells are cultured on as-grown planar, unidirectionally polished, etched nanoporous and nanowire GaN surfaces with and without a physisorbed peptide sequence that promotes cell adhesion. While cells demonstrate preferential adhesion to roughened surfaces over as-grown flat surfaces, the topography of that roughness also influences the morphology of cellular adhesion and differentiation in neurotypic cells. Addition of the peptide sequence generally contributes further to cellular adhesion and promotes development of stereotypic long, thin neurite outgrowths over alternate morphologies. The dependence of cell behavior on both the topographic morphology and surface chemistry is thus demonstrated, providing further evidence for the importance of surface modification for modulating bio-inorganic interfaces.

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1. Introduction

The preferential adhesion of cells to topographically roughened surfaces is a well-documented phenomenon [1–4], and can be considered in the context of the wide range of physical structures presented by the extracellular matrix (ECM) during development. These structures differ based on the functional role of the tissue under consideration, and the "ideal" surface for motivating maximal cell growth and viability generally bears structural and mechanical similarity to the tissue from which that cell line is derived [5–7]. Recent advancements in tissue engineering and implant design have taken advantage of this observation, with the preparation of more biomimetic surfaces involving addition of a micro- or nanotexture to the substrate or device surface [8]. Cells are exquisitely sensitive to a wide range of stimuli provided by their surroundings; in addition to this topographic sensitivity [9–11], cells are known to respond to mechanical forces [12–15], electric fields [16–18] and – perhaps the most thoroughly investigated – chemical cues [19–21]. These factors have been extensively studied in isolation; however, there are fewer resources documenting the combined effect of multiple factors in a specific, well-defined manner. Given the complex roles of these multiple stimuli in controlling cellular adhesion and differentiation, performing such combinatorial studies is integral for elucidating the mechanisms involved in cell development.

The conventional cell culture technique involves the seeding of cells on a flat planar surface, typically either glass or plastic. As the literature on and understanding of mechanobiology has expanded, however, the importance of micro- and nanoscale surface topographies for promoting specific cellular behaviors has been established. Identification of the features that drive said behaviors is a factor of importance for tissue engineering; as an example, stem cell differentiation can be guided solely by changing the roughness and elasticity of a surface, with cells taking on characteristics of the tissues with comparable properties [5,12,22]. In addition to

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straightforward variation in roughness, neural cell growth is demonstrably different on ordered vs. disordered arrays of electrospun poly(ε-caprolactone) (PCL) nanofibers generated under otherwise identical conditions [23]. While the exact change in behavior due to topography depends on cell type, this represents a path through which surfaces of comparable surface roughness but variable topography can be used to influence cell growth.

Neural cells are of particular interest in considering the applications of semiconductor materials for interfacing biosystems [24]. The axonal growth cone is particularly sensitive to external stimuli, and the prominent neurite extensions provide a distinct means of assessing changes to cell response [25]. Rat pheochromocytoma (PC12) cells are a well-established model system for neurons that differentiate upon exposure to nerve growth factor (NGF) [26–28]. PC12 cell sensitivity to nanoscale structures has been confirmed on nanoscale ridges between 70 and 250 nm in width [29], and previous studies have also assessed the role of a laminin-derived peptide motif in improving PC12 cell adhesion to a semiconductor surface [30,31]. The semiconductor used in these studies, gallium nitride (GaN), has emerged as a compelling semiconductor material for interfacing with cells. Its biocompatibility [31–33], chemical stability [34] and electrical performance in ionic solutions [35] all point to the potential of GaN for developing functional devices for probing cells and biosystems. GaN has been extensively studied by the photonics community, and processes for generating nanopores [36,37] or nanowires [38,39] have been developed to improve emission characteristics for optoelectronic applications. Chemical modification schemes featuring both covalent functionalization [40,41] and identification of recognition peptides that demonstrate specific adhesion to the GaN surface [34,42] have also been established. The existence of topographic and chemical modification schemes, in addition to the known favorable electrical and optical properties of GaN, provide compelling reasons to study the potential of GaN for interfacing with biological tissues and systems.

An important factor to consider with the recent boom in research coupling engineered nanostructures with biomolecules and biological environments is the dynamics of proteins interacting with the engineered surfaces. Nanoscale topographic modification has become more widespread as researchers continue evaluating the dynamics of cell–nanostructure interactions [43–46]. Semiconductor nanostructures have attracted a great deal of interest as materials for field effect transistor-based biosensors, as the reduction in bulk material allows a significantly smaller quantity of analyte to elicit the same degree of change observed in a planar bulk device [47] With the expanding interest in nanostructure-based devices, it is increasingly important to evaluate the dynamics of interaction between biomolecules and nanostructured or nanotextured materials. Development of nanoscale roughened surface morphologies on GaN can have a significant effect on the water contact angle [48], with potential implications as to the wetting behavior observed when exposed to physiologic solutions and subsequent interaction between soluble proteins and the GaN surface. Local variations in surface curvature have also been demonstrated to influence the orientation of laminin, a protein associated with cell adhesion, adsorbed to the surface [49]. Given the general sensitivity of cell membrane receptors to the structure and conformation of proteins, this could have significant effects on subsequent cell–surface interactions. Contradictory to these results, however, are those suggesting that nanometer scale roughness does not influence the amount or structure of protein on a surface [50] or that the only changes in quantity of adsorbed protein relates to the change in surface area brought on by increasing roughness [51]. Further exploration of the interactions between surface nanostructures and biomolecules will provide settlement to this discrepancy.

This study brings together both the nanoscale topographic modification of a semiconductor material and chemical modification by introduction of the isoleucine–lysine–valine–alanine–valine (IKVAV) motif, a core peptide domain of laminin. The IKVAV motif can play a role in neural adhesion and differentiation, and has been used to treat surfaces in schemes involving both physisorption and covalent modification [30]. By evaluating the combined effect of topography and chemistry, the effects of nanostructured GaN topography on both PC12 cell development and IKVAV peptide physisorption are catalogued.

2. Materials and methods

Chemicals were ordered from Sigma Aldrich unless otherwise specified. Rat pheochromocytoma (PC12) cells were purchased from ATCC (Manassas, VA). Type I Rat Tail Collagen was purchased from BD Biosciences (San Jose, CA). Dulbecco's modified Eagle's medium (DMEM), high-glucose formulation, fetal bovine serum, horse serum, 0.5% trypsin-ethylenediaminetetraacetic acid, murine nerve growth factor and penicillin–streptomycin were purchased from Invitrogen (Grand Island, NY). The peptide sequence (referred to in the text by the primary motif, "IKVAV") was a 19-amino acid sequence, CSRARKQAASIKVAVSADR, purchased from GenScript (Piscataway, NJ).

2.1. GaN template preparation

2.1.1. GaN growth

The gallium nitride template consisted of an unintentionally doped, 1 μm thick, gallium polar gallium nitride film grown on c-plane sapphire by metalorganic chemical vapor deposition and exhibiting a smooth morphology [52]. The film was grown on a high temperature AlN layer grown on a low temperature AlN nucleation layer to control polarity. This structure yielded a gallium nitride film with a dislocation density of 1×10^9 cm^{-2}. The chosen growth conditions, in addition to the low sapphire substrate miscut, yielded a surface morphology dominated by growth spirals arising from screw dislocations intersecting the growing surface.

Polished substrates were generated using a mechanical polishing wheel with 6 μm diamond slurry (Buehler METADI). Porous substrates were generated using an electroless wet chemical etch [36]. Briefly, surfaces were cleaned using HNO_3 prior to sputter coating with Pt islands, 1 mm in diameter. Pt-decorated surfaces were etched in a solution of 1:2:1 38% H_2O_2, 49% HF and methanol for 1 h under a 100 W UV lamp. Multiple rinses in methanol and deionized water were used to remove etch residue.

2.1.2. GaN NW growth

Samples were grown on Si(1 1 1) by plasma assisted molecular beam epitaxy (PAMBE). Prior to loading into the MBE, the Si substrates were cleaned by ultrasonication in acetone, methanol and isopropanol, each for 10 min, and treated with buffer oxide etch. Before growth, the substrates were outgassed at 905 °C. Temperature readings were taken with a pyrometer. The appearance of the 1×1 reflection high energy electron pattern at 905 °C, as well as the 7×7 pattern at temperatures lower than 830 °C, ensured the cleanliness of the samples. The RF plasma power was 405 W with a nitrogen flow rate of 1.3 sccm. The beam equivalent pressure for Ga was 2.9×10^{-8} torr, resulting in a III/V ratio of 0.16. We employed a two-step growth process. The two-step growth process had an initial nucleation temperature of 790 °C for 22.5 min, followed by a growth time of 12 h at 825 °C.

For cell culture experiments, samples were sonicated 15 min each in acetone, ethanol and deionized water. Surface cleaning

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