Materials Letters 189 (2017) 1–4

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Tuning the biocompatibility of aluminum nitride

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ARTICLE INFO

Keywords:
Semiconductor
Cell adhesion
PC 12 cells
Topography
Surface functionalization
Peptides
Aluminum nitride

ABSTRACT

High-quality, electronic-grade, aluminum nitride thin films grown by reactive sputtering were studied in vitro. The semiconductor material showed high degree of stability in cell culture with very little Al leaching over time. Unlike other III-nitride materials, clean AlN does not promote the adhesion of cells to its surface. The work demonstrates that functionalization with peptides can be used to reverse this behavior. The presence of AlN in cell culture does not have any adverse effects on neurotypic cell behavior as confirmed by cell viability and reactive oxygen species assays.

1. Introduction

Nitride materials have shown great promise for biological applications because of their unique electronic properties coupled with their biocompatibility. Gallium nitride and boron nitride have been investigated. [1] Another nitride material with relevance to electronic/microelectromechanical devices and possessing the needed piezoelectric properties for biosensors [2] is aluminum nitride (AlN). Studies have assessed cell viability and behavior on AlGaN wafers and noted that increasing aluminum concentration resulted in a decreased cell viability. [3] It is important to properly quantify the amount of metal ions. Published reports state that the total allowable concentration of aluminum salts in drinking water is between 9 mg/L [4] and 50–200 μg/L [5] in the human body before negative, toxic effects occur. Other factors that affect cell viability and behavior include the morphology of the cell-surface interface as well as the surface chemical composition which can be altered via covalent and non-covalent functionalization. In this work, high-quality, AlN thin films grown by reactive sputtering with specific surface features were used for an in vitro study with widely used neurotypic PC12 cells. [6] PC12 cells provide a way to assess the utility of materials for bioelectronics platforms. The AlN films were functionalized with peptides. We examined the stability and cell behavior on the AlN surface before and after functionalization.

2. Materials and methods

The AlN layers were deposited on sapphire by low energy ion-assisted reactive DC magnetron sputtering in an ultra-high vacuum system, using pure N_2 (99.999999%) as the working gas and elemental Al target of high purity. The deposition temperature was kept constant at 1000 °C, ensuring a growth rate of 0.05 nm/s. Three AlN samples with thickness of 20, 50 and 100 nm were used. All the layers were determined as single crystalline with (0001) surface orientation with different degree of strain. [7] The pristine AlN films possess uniform facetted surface, Fig. 1**A**.

3. Results and discussion

One strategy to transform inorganic materials into biomaterials is to perform chemical functionalization. We utilized the IKVAV peptide sequence for the variations of our surface functionalization approach. [8] We modified the surface with CIKVAV where the terminal cysteine formed a thioether with chlorines. The AlN was chlorinated with a commercially available precursor, 2-Chloroethylphosphonic acid. [9] The IKVAV motif was used in conjunction with a peptide recognition sequence, SVSVGMKPSPRP-NH_2 (P1). The last modification involved the reverse recognition sequence, PRPSPKMGVSVS- NH_2, RP1 (see Supporting information). AFM measurements compared the surface roughness, Fig. 1**B**. Functionalization with all peptides led to a slight increase in roughness. Aggregates of peptide can be seen on topographical images after covalent attachment of CIKVAV and a fairly uniform coverage. Samples terminated with RP1 and IKVAV-P1 showed similar aggregates.

Seven-day cell culture studies were performed. After 18 h, nerve growth factor (NGF) was added to promote neuronal differentiation.

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http://dx.doi.org/10.1016/j.matlet.2016.11.041
Received 8 September 2016; Received in revised form 7 November 2016; Accepted 11 November 2016
Available online 17 November 2016

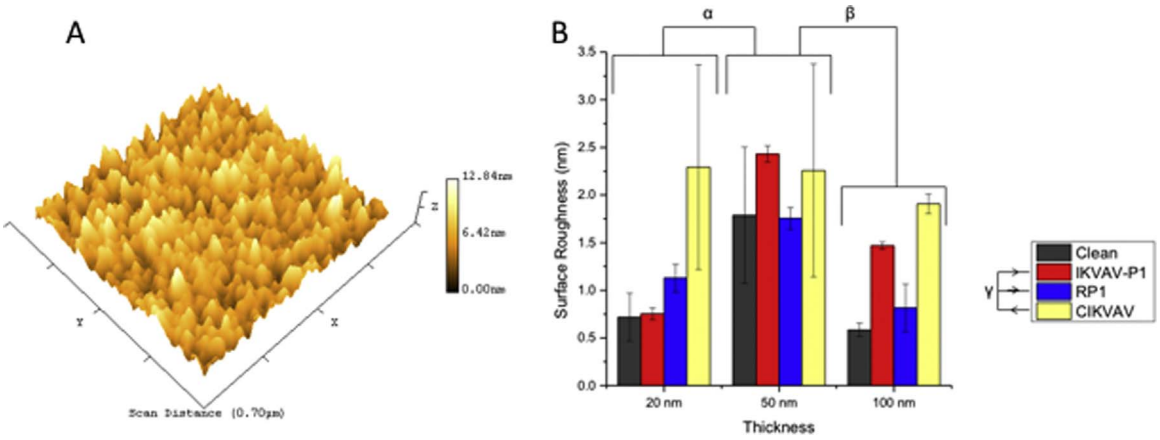

Fig. 1. A) Atomic force microscopy image of the surface morphology; B) Surface roughness (RMS) of samples before and after functionalization.

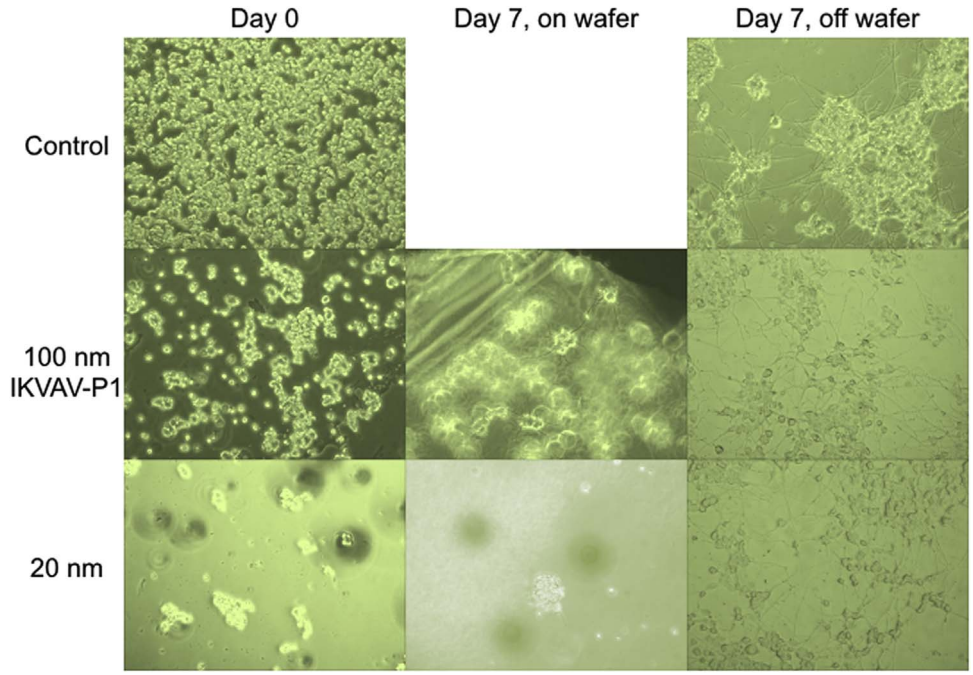

Fig. 2. Representative optical microscopy images of cells on and around AlN samples.

[10] The cell behavior on and off the AlN surfaces was monitored for 7 days, Fig. 2. The control shows cells were heavily populated and grew long and interconnected neurites over 7 days. The 100 nm IKVAV-P1 sample represents healthy cell growth on the sample. Cells did initially adhere to the sample (Day 0), but few cells were present on the sample after 7 days. The cells that were present on the sample formed clumps and extensions. The 20 nm sample represents the least healthy cell growth on the sample. There was some initial cell adhesion to the surface but the cells are clumped. After 7 days, there were very few cells present on the surface and they were very clumped and only formed a few short extensions. We observed healthier cells on the 100 nm IKVAV-P1 sample – a chemically modified, rougher sample – than the 20 nm sample. Healthy cells with long and interconnected neurites were present in the wells around the AlN. Table 1 summarizes cell behavior. All untreated samples had cells present on the samples after 7 days but the cells were clumped and exhibited little to no neurite outgrowth. Since all samples had healthy cells in the surrounding well we conclude that the presence of these types of AlN films does not cause any adverse effects.

The cell viability in each cell culture well was assessed with an alamar blue assay, Fig. 3**A**. The values from day 0 are significantly different from all other days, and the values from day 1 are significantly different than days 3 and 7. This shows an increase in cell viability/cells present in each well after day 0. The only statistically different treatment is the 50 nm RP1, which shows that the treatments had little effect on the cell viability. A reactive oxygen species (ROS) assay was used for a relative comparison over time, Fig. 3**B**. There was a statistical difference between day 1 and day 3 and between day 3 and day 7. With respect to effect of surface modification, the only statistical difference was recorded between the clean 20 nm and CIKVAV functionalized ones. The ROS data confirms that peptide modification of the AlN surfaces does not adversely affect the PC12 cells.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to measure the amount of aluminum leached, Fig. 4. There is already aluminum in the media prior to the addition of the AlN samples. There is a statistically different concentration of aluminum in solution from day 0 to day 1 and 3, and from day 1 and 3 to day 7. The leaching concentrations appear to decrease after day 0 which supports the notion that there is an initial leaching timeframe followed by a period of relatively higher stability. The increase from day 3 to day 7 can be attributed to a longer soaking period (4 days versus 1 or 2 days for all other measurements). The EPA standard for Al in drinking water is