Transition Metal Dichalcogenides as Cell Culture Platforms

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ABSTRACT

Transition metal dichalcogenides (TMDs) are emerging two-dimensional (2D) nanomaterials with unique material properties. While the role of TMDs in biomedical applications has been investigated, TMDs as engineered biological substrates have yet to be systematically studied. In this work, the design and fabrication of TMDs (WS_2 and MoS_2) yield cell culture platforms for cell adhesion with downstream analysis of cellular morphometric features (i.e., cell area and eccentricity). It is observed that the presence of TMDs improves cell adhesion and viability on cytotoxic SiO_2. Furthermore, cell morphology is determined to be more elongated on MoS_2 than WS_2. Though there is no significant difference in the average cell area between MoS_2 and WS_2, a larger distribution of cell areas is observed for the WS_2 sample.

Keywords: transition metal dichalcogenides, fibroblast cells, cell-substrate interactions, cell adhesion, morphometric features

1 INTRODUCTION

A key determinant of cell function and tissue organization is the cell-adhesive interactions, which link cells to an underlying substrate. Cell adhesion influences various aspects of cellular physiology, including survival, proliferation, migration, and differentiation [1]. Thus, understanding of cell-substrate interactions is essential in the fabrication of engineered substrates with design parameters that yield desirable cellular responses in biological applications.

Transition metal dichalcogenides (TMDs), such as WS_2 and MoS_2, have been widely explored for their utilization as two-dimensional (2D) semiconducting nanomaterials with unique electronic, mechanical, and catalytic properties [2]–[5]. Since its recent emergence, much research has been done to integrate these unique properties of TMDs into various biomedical applications, including drug delivery, therapeutics, biosensors and bio-imaging [6]–[9]. Although cytotoxicity studies have been performed on TMDs [10]–[15], cell-substrate interactions of TMDs are not well reported in literature. Therefore, further study is needed to illuminate the influence of TMDs on the adhesive interactions of biological cells and the subsequent incorporation of these nanomaterials for potential biological applications.

Here we utilize WS_2 and MoS_2 as cell culture platforms to probe cell-substrate interactions via analysis of cellular morphometric features. WS_2 and MoS_2 are grown via chemical vapor deposition (CVD) on SiO_2 substrates and seeded with human fibroblast cells. TMD-free SiO_2 substrates as control samples are implemented to test the effect of the TMDs on fibroblast cell adhesion. After a 24-hour culture, the cell-substrate interactions are probed using a methyl violet staining. Optical microscopy (OM) yields images from which cell morphometric features (cell area, eccentricity) are extracted and measured.

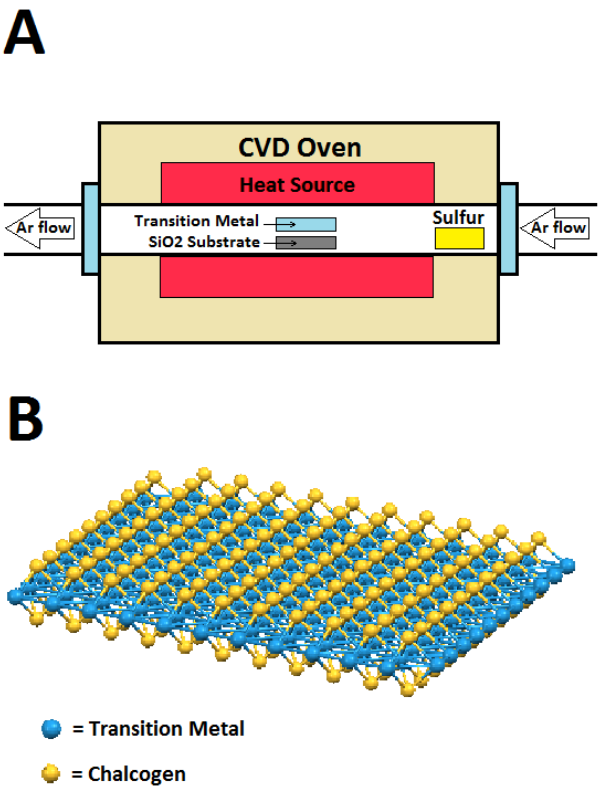

Figure 1: (a) Schematic of the CVD growth setup for TMD sample fabrication, (b) 3D model of a TMD monolayer

2 METHODS

2.1 TMD Growth

Figure 1a shows the schematic of the CVD growth setup for TMD sample fabrication. A clean 4-inch Si wafer with 90 nm thick SiO_2 is deposited with a 5 nm thick MoO_3 or WO_3 film from pellets (Sigma-Aldrich) via electron beam

evaporator (Denton Explorer) and cut into 1x1 cm areas, designated the transition metal source chips; separately, another clean 4-inch Si wafer with 90 nm thick SiO_2 is cut into 1x1 cm areas, designated the growth chips, as the substrate in which TMD monolayers (Figure 1b) are deposited upon. A transition metal source chip is placed face to face atop of a growth chip, and it is loaded together into the center of a 3" diameter quartz tube within an MTI 1200X 1-zone furnace. An aluminum crucible containing 0.9 g of powdered sulfur (chalcogen source) is placed upstream for sublimation into gaseous sulfur, and a mass flow controller (Aalborg) delivers Ar at 30 sccm as an inert gas carrier from 300°C, with a base pressure of 200 mTorr. Using the temperature controller, the TMD growth program is setup according to the following sequence: (1) maintain room temperature for 5 min to get a high vacuum degree via pumping, (2) ramp at 18°C/min to 550°C, (3) ramp at 6.0°C/min to 750°C, (4) maintain 750°C for 25 min, (5) cool down via natural heat dissipation to 450°C, at which point the Ar gas is turned off and furnace opened for rapid cooling.

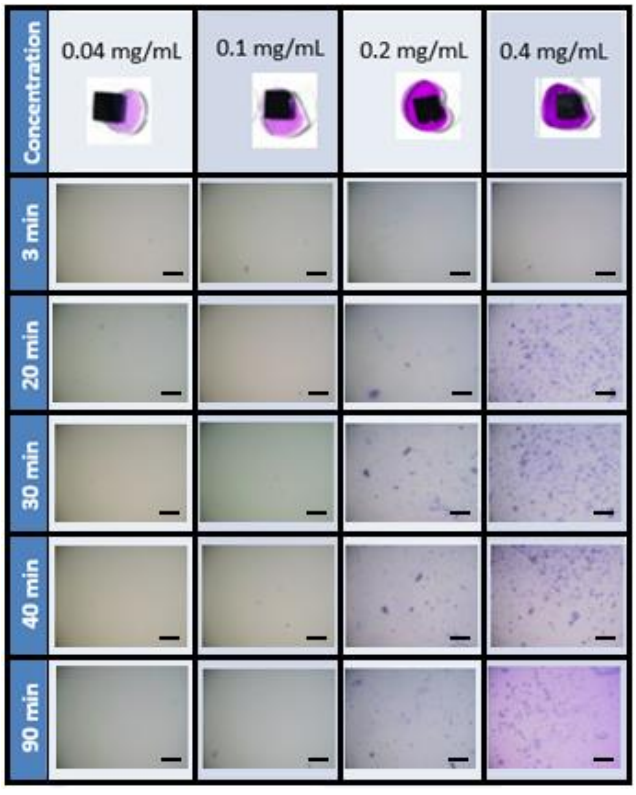

Figure 2: Methyl violet stain concentration diagram of OM images at 3, 20, 30, 40, and 90 min, using concentrations of 0.04, 0.1, 0.2, 0.4 mg/mL; scale bar = 50 um

2.2 Cell Culture

As-grown TMDs on SiO_2 substrates were cultured with neonatal human dermal fibroblasts (NHDFs) in high-glucose Dulbecco's modified Eagle's medium (DMEM), containing 1% penicillin/streptomycin and 10% fetal bovine serum in basal media. SiO_2 substrates without TMDs were also used for comparison as control samples, and all samples were seeded with NHDFs at the P7 generation. NHDFs were cultured on each substrate for 24 hr at standard incubating conditions (37°C, 5% CO_2). Cell seeding densities were optimized at 15,000 cells/cm^2 to achieve sufficient sample sizes and enable single cell morphology observation. Prior to seeding, the SiO_2 control and TMD substrates were each coated with sterile filtered poly-L-lysine (0.01%) to promote cell attachment.

2.3 Cell Stain

Immediately following the 24 hr culturing, cell morphology is observed via optical microscope (Horiba Xplora), utilizing methyl violet (Sigma Aldrich) as a cell stain. As shown in Figure 2, concentrations of methyl violet in DI water (i.e., 0.04, 0.1, 0.2, and 0.4 mg/mL) are first tested on SiO_2 control samples (without cells). OM images were taken at the same locations after 3, 20, 30, 40, 90 min. Higher concentrations result in supersaturation of methyl violet overtime due to solvent evaporation, as observed overtime by the violet conglomerate formations at higher concentrations. Therefore, it was determined that 0.1 mg/mL was an adequate concentration for the cell stain, as no metyhl violet conglomerate formation is observed after 90 minutes. Furthermore, the time of exposure of cells to cell stain is tested as shown in Figure 3. SiO_2 control substrates are cultured as described above and exposed to the methyl violet cell stain concentration of 0.1 mg/mL for varying durations (i.e., 30 sec, 60 sec, 90 sec, 120 sec). It is determined that 120 sec of exposure to the cell stain is sufficient for observing cell morphology of the adhered fibroblast cells via optical microscope.

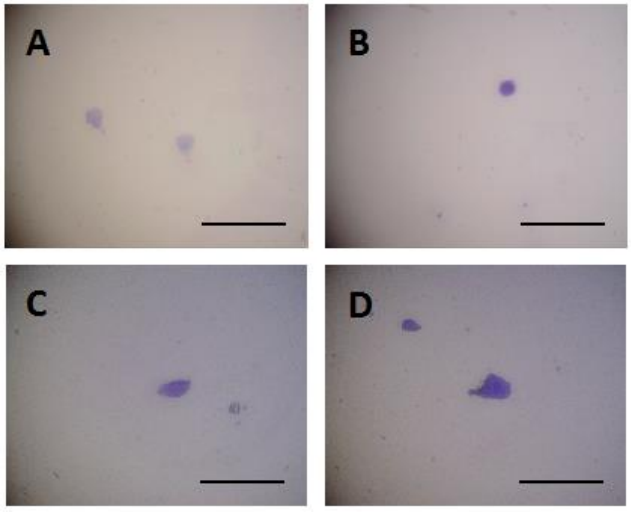

Figure 3: OM images after cell culturing on SiO_2 control samples, including methyl cell stain exposure for (a) 30 sec, (b) 60 sec, (c) 90 sec, and (d) 120 sec; scale bar = 100 um

3 RESULTS AND DISCUSSION

Representative optical microscope images of fibroblast cells cultured on the sample substrates are shown in Figure 4. Visual observation confirms a clear trend of larger surface areas and less eccentricities of cells in the presence of TMDs. In comparison, the morphology observed for the SiO_2 control is indicative of a cytotoxic platform that will prohibit further application. Therefore, TMDs as cell culture platforms enhance the adhesion of fibroblast cells, despite exposure of an underlying substrate that is unfavorable to cell attachment.

Cellular morphometric features (i.e., cell area, eccentricity) confirm the observation that the presence of TMDs improve the cellular adhesion on the cytotoxic SiO_2 substrate. Cells adhering to SiO_2 are significantly smaller and more eccentric than those in the presence of TMDs. Furthermore, cell morphology is observed to be more elongated on MoS_2 than WS_2. Though there is no significant difference observed between the average cell areas between MoS_2 and WS_2, a significantly larger distribution of cell areas is observed for the WS_2 sample. Therefore, the MoS_2 sample results in more consistent cell morphology than WS_2.

The experimental results from the cell-TMD substrate study can be explained by the chemical composition of the TMDs. Since chalcogen atoms compose the outer layers of TMDs as illustrated by the chemical structure in Figure 1b, both MoS_2 and WS_2 samples exposed a vast majority of the sulfur atoms at the basal planes, thereby shielding adhered cells from the underlying SiO_2. This TMD structural similarity is consistent with the expectation that cell adhesion would be more similar between MoS_2 and WS_2 than the SiO_2 control.

However, edge terminations of TMD crystals expose either transition metal or chalcogen atoms, which may contribute to observed statistical significances between the TMD species [4]. Substrate surface has been shown to influence cell adhesion via surface-directed control of integrin binding, as the number and size of focal adhesion proteins are known to correlate with cell adhesion [16], [17]. Thus, variations in surface chemical species and their spatial orientations may likewise influence the underlying interactions with cell surface proteins, resulting in observable differences in morphometric features. Ongoing studies using immunofluorescent staining of subcelluar structure will provide a deeper understanding of the role of TMDs in cell adhesion and guidance.

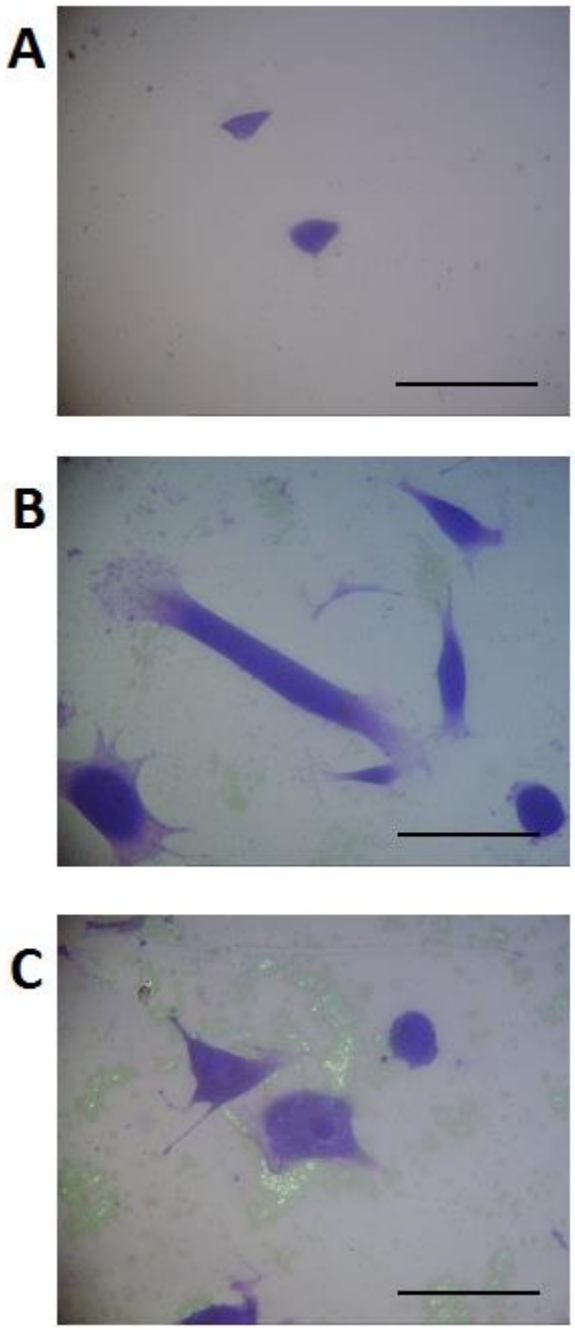

Figure 4: Representative optical microscope images of fibroblast cells cultured on (a) SiO_2, (c) WS_2 on SiO_2, and (e) MoS_2 on SiO_2; scale bar = 100 um

4 CONCLUSION

This paper demonstrates that the presence of TMDs improves cell adhesion and viability on cytotoxic SiO_2. To characterize this effect, cell morphometric features are quantitatively analyzed with statistical significance. Specifically, MoS_2 substrates yield a more elongated cell morphology compared to WS_2 substrates. No significant difference in mean cell area is observed between MoS_2 and

WS_2. Analysis of the variance indicates a significantly larger distribution of cell areas for WS_2 than MoS_2. Thus, MoS_2 results in a more reliable response than WS_2. Therefore, TMDs are promising candidates for cell culture platforms, which may further expand their applications in biomedicine when combined with their unique electronic, mechanical, and catalytic properties. Further work remains concerning the influence of TMD species and spatial orientation to provide a greater understanding on the causal relationship between TMD substrate properties and cell adhesion.

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