Modulating cell adhesion and viability with nanorod coated surfaces

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Introduction

Nanostructured surfaces are promising materials for modulating cell behavior. One class of nanostructures that has received recent attention in the literature is a surface covered with upright slender cylinders, variously referred to as nanoposts, nanorods and nanocolumns. [1-3] In this study, we investigated the use of nanorods for modulating the adhesion and viability of NIH 3T3 fibroblasts and umbilical vein endothelial cells. Our results indicate that nanorods strongly influence cell adhesion and viability. Our results indicate the promise of using anti-fouling nanorod coatings on the surface of biomedical devices.

Methods

Zinc oxide (ZnO) nanorods were made by a solution-based hydrothermal growth method. [4] SiO_2 was coated on ZnO nanorods with plasma enhanced chemical vapor deposition at 50 ˚C (thickness controllable from 5 to 20 nm). For control substrate, we used 22 mm square glass cover slips (Corning, Inc., Lowell, MA). Cells of two different types were seeded on FN-coated substrates. NIH 3T3 fibroblasts were cultured in DMEM (Mediatech, Inc., Herndon, VA) supplemented with 10 % donor bovine serum (DBS) (Invitrogen, Eugene, OR). Human umbilical cord vein endothelial cells (HUVECs) were cultured in EBM-2 Basal Medium and EGM-2 SingleQuot Kit (Lonza, Walkersville, MD). The live/dead viability/cytotoxicity kit for mammalian cells (Invitrogen, Eugene, OR) was used for quantifying adherent cell viability on each substrate. Cells were incubated at 30-45 minutes with calcein AM and ethidium homodimer-1 (EthD-1). Epifluorescence images of five to twelve random fields were collected on a Nikon TE 2000 inverted microscope using a 10 X lens. The average number of cells adherent on each substrate, the number of adherent live cells (stained green with calcein AM) and adherent dead cells (stained red with EthD-1) were quantified from these images using the NIS-Elements program (Nikon). Three independent experiments were performed.

Results

Cell spreading area was greatly reduced, and focal adhesions and stress fibers were not visible in cells cultured on ZnO nanorods [5]. The average area of cell spreading was decreased significantly on nanorods compared with flat substrates (a reduction of 60-70 %). These trends were observed in three different cell types. Scanning electron microscopy (SEM) studies indicated that cells were not able to assemble lamellipodia on nanorods. This was consistent with the result of time-lapse phase contrast imaging which showed that cells initially adherent to nanorods are unable to spread because of the lack of lamellipodia formation.

As ZnO nanorods could potentially create differences in matrix protein adsorption, or cause local solution toxicity, we coated the nanorods with SiO_2.The number of attached and live cells was quantified on 50 Å SiO_2 coated nanorods, 100 Å SiO_2 coated nanorods, and glass at 24 hours of culture. The total number of adherent cells and of adherent live cells at the end of 24 hours was greatly decreased on nanorods compared to glass. Because cells were seeded at equal cell densities on the substrates, the ratio of the number of attached cells on the nanorods to that on glass represents the effect of topography on cell survival (Figure 1). The fact that the fraction of attached live cells decreased on the nanorods in endothelial cell (HUVEC) and fibroblast (NIH 3T3), are consistent with previous observations that topological cues at the nanoscale can profoundly modulate cell behavior [1-3].

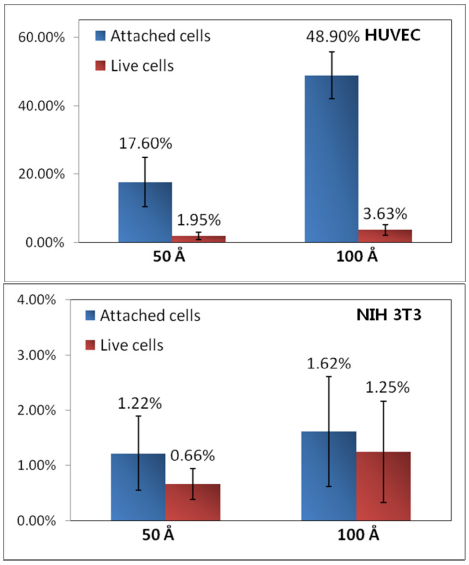

Figure 1. The number of attached and live cells on 50 Å, 100 Å SiO_2 SiO_2 deposited nanorods normalized by the number of attached and live cells on glass respectively. Bars indicate standard error of the mean.

Conclusions

Our results indicate that the nanorods can be used as an adhesion-resistant biomaterial capable of inducing death in anchorage-dependent cells. A better understanding of the mechanisms for the observed effects will be a key for designing optimal nanorod based substrates for minimizing cell adhesion and survival.

References

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