GEOMETRIC MODULATION OF ENDOTHELIAL NITRIC OXIDE SIGNALING

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ABSTRACT

NO is a short-lived molecule that rapidly diffuses away from its source making direct measurements of its concentration extremely challenging. The flow conditions needed to study the response to physiological shear stresses further reduces the concentration because of convective transport. Our previous experimental and theoretical studies also suggest that, in addition to the relative levels of expression of signaling molecules involved in NO production, their spatial distribution within the cell is a critical determinant of the resulting signaling behavior. We found that expression levels and distribution of endothelial nitric oxide synthase (eNOS) and its major regulatory protein, caveolin-1 (Cav-1) in cultured endothelial cells can be modulated by controlling cell structure. In particular, elongation and alignment induced by culturing the cells on a substrate with oriented microtopography causes a dramatic redistribution of eNOS such that it becomes colocalized with Cav-1on the cell surface. We believe this represents a phenotype the more closely approximates a normal endothelium in vivo. Furthermore, on some patterns, the surface distribution of Cav-1 and eNOS becomes non-uniform, concentrating in lines corresponding to actin filament bundles aligned with the microgroove pattern. This phenomenon provides an experimental tool to manipulate the spatial relationships between signaling domains that will allow the systematic testing of hypotheses regarding the role of intracellular transport in NO signaling.

KEY WORDS
Molecular and Cellular Engineering, Nitric Oxide, Microtopography, Transport, Cell Signaling

Nitric oxide (NO) is mediates a wide range of behaviors critical to normal vascular function. In addition to its primary role as powerful vasodilator, NO inhibits platelet aggregation, adhesion of leukocytes to endothelium, and vascular smooth muscle cell proliferation. Impairment in NO availability is a characteristic of endothelial dysfunction - a precursor to atherosclerosis and hypertension. Our previous experimental and theoretical studies suggest that, in addition to the relative levels of expression of signaling molecules involved in NO production, their spatial distribution within the cell is a critical determinant of the resulting signaling behavior.[1] In this study, we show that expression levels and distribution of endothelial nitric oxide synthase (eNOS) and its major regulatory protein, caveolin-1 (Cav-1), in cultured endothelial cells can be modulated by controlling cell structure.

Cell culture substrates with microtopography made of PDMS were prepared from microfabricated silicon molds as previously described [2]. We found that confluent monolayers of endothelial cells respond to these patterns by elongating and aligning with the direction of the grooves. For 1 micron deep grooves, 3.5 μm wide and 3.5 μm apart, cells aligned by these topographical cues have dramatically increased expression of cav-1 and eNOS that appear punctuate on the cell surface suggesting caveolar localization. Non-aligned cells grown on flat substrates of the same material have low levels of expression that appears to be compartmentalized within the cell’s interior. On the 5 μm deep grooves (2.65 μm wide, 4.5 μm apart), prominent actin bundles appear aligned with the grooves. In these cells, the spatial distribution of eNOS and Cav-1 appears to be affected by the actin organization. Thus, there are regions of the cell with higher concentration of these signaling molecules separated by regions sparsely populated with them.

Nitric oxide production from endothelial cells grown on patterned substrates was measured using a nitric oxide specific electrode (WPI, Inc.) lowered to within 10 μm of the cell surface as visualized by light microscopy. Cells were stimulated with ATP (435 μM), and nitric oxide concentration was recorded in real time. Cells grown on patterned surfaces had greater peak increases of nitric oxide that were sustained longer than cells on non-patterned surfaces. Cells grown on the 5 μm deep patterned substrates had ATP-induced peak NO transients that were approximately twice the peaks for cells on flat substrates (p < 0.05; Patterned n=6, Non-Patterned n=5).

Conclusion

These results show that it is possible to create cultured endothelial monolayers with the structural and functional characteristics of the natural endothelium. These data also demonstrate that the spatial relationships between
these relationships in pathological conditions may represent a novel mechanism of endothelial dysfunction. The integration of a microfabricated substrate with our NO sensing technique under flow conditions will constitute a novel biomimetic platform for in vitro experimentation. Furthermore, this approach can be extended to a lab-on-a-chip, high throughput experimental system by further integrating microfabricated sensors.

Acknowledgements

This work has been supported in part by NIH/HL068164 and NSF/BES0301446.

References