

# **Modulation of Cellular Behavior by Nanoscale Topographic Cues**

**Christopher J. Murphy DVM, PhD**

*Professor*

*Department of Surgical Sciences*

*School of Veterinary Medicine*

A quick thanks for the invitation. Milan in particular gave a great introduction but all of the speakers have sort of set the stage for me to talk today. I do want to acknowledge Paul Nealey, because we are doing a project that is really at the intersection of material science, chemical engineering, and cell and molecular biology. And frankly, none of us are just smart enough to do all of it by ourselves and I do want to recognize Paul, because without his input into the design of the substrates that I'm gonna be talking about, none of the cell biology I am going to be showing you today would be possible. The question that we are trying to answer is where does nanoscale topography fit into cuing on cells. We all know that culture media soluble factors, cell-cell contacts, temperature, pH, a variety of factors impact on a how a cell behaves. The question that we are trying to answer is, is nanoscale topography a fundamental cue in modulation of cell behaviors. Things such as adhesion, shape, differentiation, proliferation, and here is an example of a human corneal epithelial cell that is sitting on a flat surface the typical tissue culture polystyrene and if you want to ask the question, yes it really is flat. And here is a human corneal epithelial cell that is sitting on seventy nanometer ridges. You can see that it markedly effects cell alignment and elongation. The overview of our research plan is that we spent several years actually describing the native topography that the cells interface with and was specifically focused on the basement membranes. We then used it as a rational starting point for the design of synthetic matrices that expand from the nanoscale size up to the microscale size. Microscale is important because it is where the bulk of the literature exists in topographic cuing. Many laboratories around the world that have focused on this, and due until recently the constraints in fabrication most of the data was generated on microscale. And then the nanoscale of course gives us the biomimetic length scale. Then we wanted to determine the phenotypic impact of the topography we specifically looked at orientation, morphology, adhesion, migration, proliferation, and differentiation, and then of course once you know what the cells are doing, we can go after the signaling pathways. When we first started this project, we often had people ask us what's the mechanism? We didn't even know what the cells were gonna do on it yet, and people wanted us to give them the molecular mechanisms. For those of you familiar with the cornea, you know that it's composed mostly of extracellular matrix. It is one of the few structures in the body that we never want to have vascularized unless you're a manatee, who normally have a vascularized cornea, in order to remain transparent. Here is an example of a corneal surface where we partially digested the cells away using dispase and EDTA. Here is the anterior corneal epithelial cells. Here is the basement membrane. And here is what that side of the basement membrane looks like in ultra high resolution SEM. You can see that the bulk of the material that we're looking at here is not flat. My

degree initially was in anatomy and I thought that the basement membrane was planar structures forever because of the TM images. You can see that it is really a felt like arrangement of fibers, pores, and elevations that span generally between twenty and two hundred nanometers. The average pore size for example is seventy nanometers, the average elevation is one hundred and fifty nanometers, and the average fiber diameter is about thirty nanometers. If you looked at a single basal corneal epithelial cell and looked at a very small section of it and blew it up, this is what that part of the single cell is interfacing with. What this means is that each cell is in contact with thousands of topographic features. I want to acknowledge Dr. George Abrams, who did the bulk of this work during the course of her PhD thesis and who is now doing a post doctoral fellowship in our laboratory. Well you can use this as a rational starting design and working with Paul Nealey in the nano fabrication facilities available at the University of Wisconsin. You can make nanobumps, nanopores, nanofibers, and these fibers are ridges and grooves. And I do want to introduce the term pitch, because I didn't know what it was when the engineers first started talking to me about it. Pitch refers to the ridge width plus the groove width together. It's a cycle. It's a period. It's like a wavelength. Well the engineers were really happy to start making, through standard lithographic procedures, somewhat we affectionately refer to as the six pack in the lab. These things are two millimeters in diameter and we span again from the microscale up here two millimeters. And we've also shown that the interaction with the substrate depends upon not just integrins but also syndecans and we have used some peptide sequences that are isolated that are unique to the syndecans and some that are unique to the integrins and we've shown that indeed when you put them both together you get a normal morphology. When you put just one, you get a very different morphology when the cell is in contact with the nanoscale topography. You can effect local adhesion formation. You can actually change the orientation of the focal adhesions. You can change the orientation of the stress fiber to the focal adhesion by changing the scale of the topography. Curiously, when you get down to the smallest scale features, you have fewer stress fibers and if you work on cells *in vivo* you know you don't often see stress fibers. You see lot's of stress fibers when they are on planar surfaces. This is just showing that rho is upregulated at twelve hours. Rac and CDC42 are downregulated. Here's the flat control. So in summary, and there's lots of other data that I could share with you but due to the time constraints I'm not going to, I'll spare you. We're also looking at vascular endothelial cells, urothelial cells, and skin cells currently and we're looking at the effect on cancer cells. But here you can see that it effects migration, adhesion, it affects proliferation and differentiation. Who cares? It has immediate relevance to in-vitro studies. Real cells never see flat and I think we have to question the experiments that we're doing, since we've shown that the soluble factors can their effect can be changed by topography. We have to start questioning ourselves about doing things the way we normally do it on standard flat plastic ware and if you put an AFM probe on tissue culture polystyrene it is

flat. It need partially explain why in-vitro findings may not translate to the in-vivo condition. It has immediate relevancy to emerging strategies in tissue in stem cell engineering and of course the development of prosthetics. And topography can be tailored to promote or inhibit specific cell behaviors to benefit performance. I'd like to acknowledge the large number of collaborators that I've had over the years on this project, number of postdocs and again I want to specifically acknowledge Paul Nealey who is my partner in crime in this entire work. Thank you for your attention.