Collagen Thin Films for Improved Nanoscale Mechanical Properties in Cellular Measurement Assays

NIST scientists are developing cell culture systems utilizing thin films of extra-cellular matrix (ECM) proteins such as collagen, for robust, reproducible, and analytically tractable biomimetic cell growth matrices. Standardized ECM culture conditions for cell-based assay are expected to result in more physiologically-relevant in vitro cellular phenotype and molecular measurements. This work has potential significance in drug discovery and development research, as well as understanding physiological processes such as wound healing and tumor growth.

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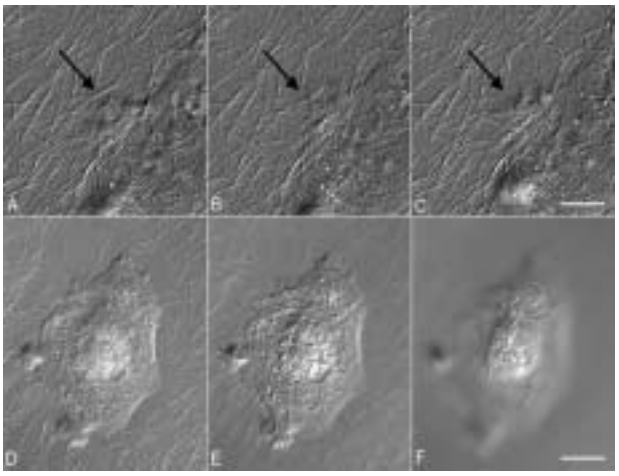
Cells recognize their environment through specific receptor molecules, and these molecular interactions result in intracellular signaling pathways that influence cell response to other environmental entities such as pharmaceuticals. Despite this knowledge, most laboratory culture cells in plastic dishes instead of on a matrix of physiologically relevant extracellular matrix proteins because commercial and laboratory preparations of matrix proteins are notoriously irreproducible and poorly characterized. Thin films of matrix proteins such as collagen can provide robust, reproducible, and analytically tractable biomimetic cell growth matrices. These thin films can also provide new insight into the environmental parameters, such as mechanics, that direct cell response.

Cells are used in the testing of therapies and drugs, in the diagnosis of disease, and occasionally as therapies themselves. Cells are also critical research tools as surrogates for even more complex intact organisms. An important determinant of cell response is the extracellular matrix (ECM) proteins that cells adhere to. However, there is a great deal of variability in laboratory preparations of matrix proteins. As a result, comparison of data from different labs is problematic.

Cellular assay systems employing well-controlled ECMs are expected to provide researchers with better estimates of how cells function in the body, and enable development of safer drugs, medical devices and cell-based therapeutics.

Using thin film technology to self assemble Type 1 collagen into fibrils at alkanethiol-coated surfaces, we have demonstrated that thin films of the ECM protein collagen can be robust, analytically tractable, and mimic *in vivo* conditions. Thin films of collagen appear to be identical to thick gels of collagen with respect to a number of phenotypic parameters, and result in quantifiable phenotypic responses in cells that are highly reproducible.

Much is yet to be learned about what environmental cues elicit cellular responses. Not all of the parameters of the ECM that are important in determining cell response have identified. In addition to chemical recognition, we have found that cells are very sensitive to the mechanical nature of collagen fibrils. While it has been known that cell respond differently to bulk polymers with different compliance, we have shown for the first time that cells are very sensitive to mechanical properties of their matrix at the nanoscale.



Smooth muscle cells can be observed to move collagen fibrils in thin films of collagen (Panels A-C) as they extend and retract their cell processes. Cells manipulate the fibrils by pulling them up over the top of them, thus creating a 3-D environment for themselves; this is observed by examining cells at different focal planes (D-F).

We identified the nanomechanical effects on cells by comparing thin films of fibrillar collagen that remained hydrated with thin films that were allowed to dry extensively, such as might occur during storage or shipping.

Our results show that when collagen fibrils are kept fully hydrated, they remain flexible. Cells on flexible collagen fibrils are in a senescent, non-growth state, which is appropriate for most normal cells in the body. However, when collagen fibrils are allowed to dry, they become stiffer. Cells appear not to be able to move dried fibrils, and cells on dried fibrils are observed to spread more, change their gene expression and cytoskeleton arrangement, and grow and divide approximately 4 times as rapidly.

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