The Direction of Stretch-Induced Cell and Stress Fiber Orientation Depends on Collagen Matrix Stress

Roland Kaunas, Abhishek Tondon
Department of Biomedical Engineering, Texas A&M University, College Station, TX

Statement of Purpose: Cell structure depends on both matrix strain and stiffness, but their interactive effects are poorly understood. We investigated the interactive roles of matrix properties and stretching patterns on cell structure by uniaxially stretching U2OS cells expressing GFP-actin on silicone rubber sheets supporting either a surface-adsorbed coating or thick hydrogel of type-I collagen. We propose that active orientation of the actin cytoskeleton perpendicular and parallel to direction of stretch on stiff and soft substrates, respectively, are responses that tend to maintain intracellular tension at an optimal level. Further, our results indicate that cells can align along directions of matrix stress without collagen fibril alignment, indicating that matrix stress can directly regulate cell morphology..

Methods: U2OS osteosarcoma cells stably expressing GFP-actin and human mesenchymal stem cells (hMSCs) were cultured on collagen coated silicone rubber stretch chambers (STREX, Japan) or on chambers modified to support 3-D collagen gels. Images of actin stress fibers (SFs) were analyzed using a custom algorithm in MATLAB (the MathWorks, Natick, MA) to quantify the density distribution g(θ) of SFs within each cell. Time-lapse movies of SF realignment was performed with a confocal microscope and stretch device in a custom environmental chamber. Peripheral and central SFs were inhibited with 30μM ML7 and 10μM Y27632 (Calbiochem), respectively.

Results: U2OS (Figs. 1A&B) and hMSCs (Figs. 1C&D) were subjected 10% cyclic uniaxial stretch at 1Hz on stiff collagen-coated chanbers (Figs. 1A&C) and on soft collagen gels (Figs. 1B&D). The histograms summarize SF density distributions obtained from n=60 cells per condition. Confocal reflectance images of collagen fibrils in regions containing a U2OS cell (Fig. 1E) and devoid of cells (Fig. 1F) and circular histograms depicting collagen fibril alignment indicate the cell and SF alignment are not associated with collagen alignment. To determine the dependence on stretch frequency on collagen gels, the extent of cell and SF alignment was quantified in U2OS cells subjected to 3 h of 10% cyclic uniaxial stretch at 0.01, 0.1 and 1 Hz on collagen gels. Decreasing stretch frequency significantly reduced cell and SF alignment parallel to the direction of stretch.

A rapid stretch of 20%/s to a constant magnitude of 10% uniaxial stretch induces alignment of U2OS cells and their SFs parallel to the stretch direction on soft collagen gels, but not on stiff collagen-coated chambers. Further, a slow ramp of 0.2%/s to 10% stretch significantly reduced cell and SF alignment on the soft gels. Previous studies indicate that uniaxial stretching of collagen gels causes anisotropic changes in gel stiffness, with the stiffness increasing in the direction of stretching. Thus, we prestretched the gel uniaxially by 10% before seeding the gel with U2OS cells and observed that the cells and SFs aligned to less of an extent than with the rapid stretch.

The cells on soft gels contained SFs that were mainly confined to the periphery of the cell. Peripheral SFs are sensitive to inhibitors of myosin light chain kinase (ML7), while central SFs are sensitive to inhibitors of Rho kinase. Stretch-induced alignment on collagen gels was blocked by the myosin light-chain kinase inhibitor ML7, but not by the Rho-kinase inhibitor Y27632.

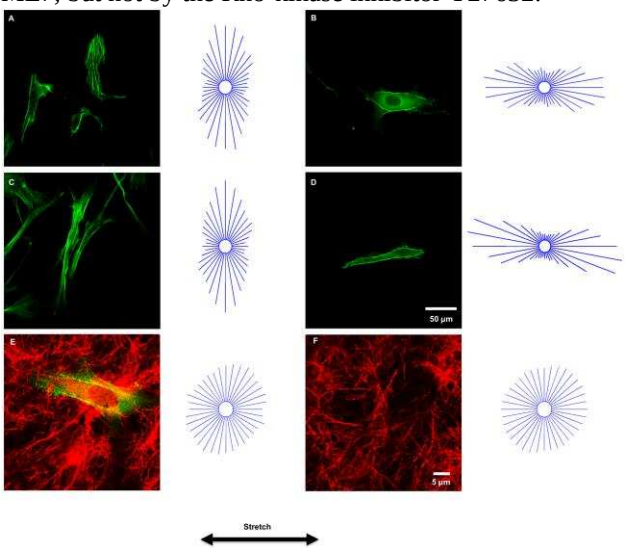

Figure 1. Cyclic stretch-induced SF alignment on soft collagen gels and stiff silicone rubber sheets.

Conclusions: Our results demonstrate that stretch-induced cell and SF alignment are highly dependent on the mechanical properties of the collagen matrix upon which cells are cultured. Cyclic stretch promoted alignment parallel to the direction of stretch in cells with attenuated contractility caused by adhesion to a soft collagen gel, as judged by the relatively few SFs relative to that in the same cell type on collagen-coated silicone rubber. This is consistent with previous studies performed with cells on fibronectin-coated silicone rubber showing that stretching promotes SF alignment parallel to the direction of stretch when cell contractility is attenuated with small molecule inhibitors of Rho-kinase or MLCK (Kaunas 2005, Lee 2010). This is in stark contrast to the perpendicular alignment observed when cell contractility is at normal levels for cells on silicone rubber coated with collagen or fibronectin (Kaunas 2005). In the case of a step stretch, cell and SF alignment was only observed on soft collagen gels, but not on silicone rubber coated with collagen. Our results provide evidence that the parallel alignment on soft gels is due to both anisotropic mechanical properties as well as a direct response by the cells to a stretch stimulus.

References: Kaunas R. PNAS 2005 102:15895-900; Lee CF. BBRC 2010 401: 344–349.