

Tensile forces drive a reversible fibroblast-to-myofibroblast transition during tissue growth in engineered clefts

Abstract

Myofibroblasts orchestrate wound healing processes, and if they remain activated, they drive disease progression such as fibrosis and cancer. Besides growth factor signaling, the local extracellular matrix (ECM) and its mechanical properties are central regulators of these processes. It remains unknown whether transforming growth factor- β (TGF- β) and tensile forces work synergistically in up-regulating the transition of fibroblasts into myofibroblasts and whether myofibroblasts undergo apoptosis or become deactivated by other means once tissue homeostasis is reached. We used three-dimensional microtissues grown in vitro from fibroblasts in macroscopically engineered clefts for several weeks and found that fibroblasts transitioned into myofibroblasts at the highly tensed growth front as the microtissue progressively closed the cleft, in analogy to closing a wound site. Proliferation was up-regulated at the growth front, and new highly stretched fibronectin fibers were deposited, as revealed by fibronectin fluorescence resonance energy transfer probes. As the tissue was growing, the ECM underneath matured into a collagen-rich tissue containing mostly fibroblasts instead of myofibroblasts, and the fibronectin fibers were under reduced tension. This correlated with a progressive rounding of cells from the growth front inward, with decreased α -smooth muscle actin expression, YAP nuclear translocation, and cell proliferation. Together, this suggests that the myofibroblast phenotype is stabilized at the growth front by tensile forces, even in the absence of endogenously supplemented TGF- β , and reverts into a quiescent fibroblast phenotype already 10 mm behind the growth front, thus giving rise to a myofibroblast-to-fibroblast transition. This is the hallmark of reaching prohealing homeostasis.

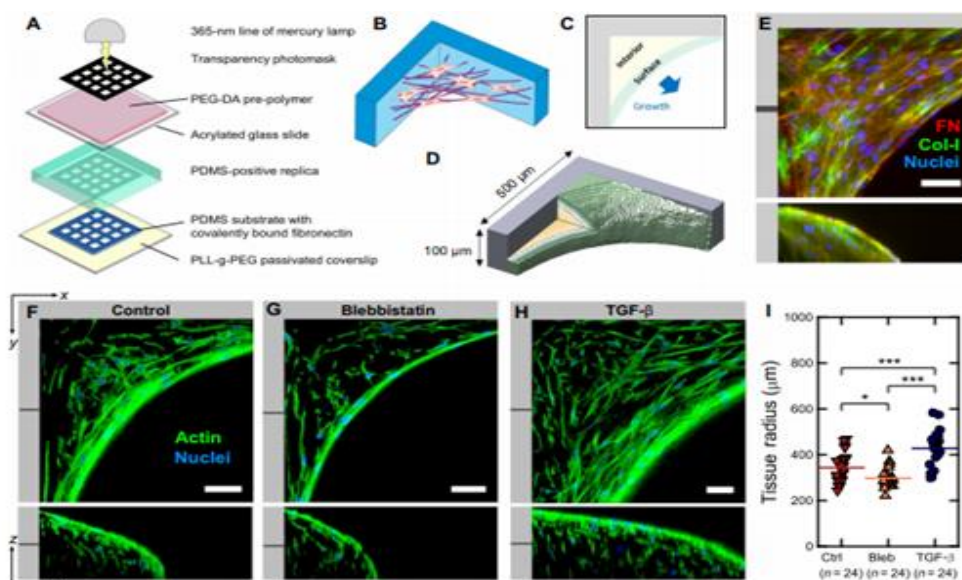


Fig. 1. De novo microtissue growth rate increased with cytoskeletal tension. (A) Schematic of the microfabrication process used to produce thick 3D PDMS substrates with controlled macroscopic geometry. Detailed illustrations of the fabrication process are shown in fig. S5. (B) Illustration of 3D tissue growing in the corners of rectangular substrate pores. (C and D) Illustration of surface and interior regions and tissue dimensions. (E) Maximum intensity projection of the resulting 3D tissue containing cells (blue) and ECM (red, FN; green, Col-I; blue, nuclei). The tissue is rich in both FN and Col-I fibers. (F to H) Single slices through tissues (green, actin; blue, nuclei) at the indicated depth in xy and xz directions, under normal conditions (left) and under conditions of inhibited (middle) and elevated (right) cytoskeletal tension in the presence of 10 μM blebbistatin or TGF- β 1 (1 ng/ml), respectively. The individual channels and an animated z fly-through are shown in fig. S1 and movie S1, respectively. (I) Quantification of tissue radius (distance between the scaffold corner and the growth front at 40 μm of z depth) after 19 days of growth under normal conditions and in the presence of 10 μM blebbistatin or TGF- β 1 (1 ng/ml), respectively. Increased cytoskeletal tension results in increased tissue volume. Scale bars, 50 μm . Data points indicate the tissue radius for individual tissues.