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The effect of growth factor environment on fibroblast morphological response to substrate stiffness

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ABSTRACT

According to conventional understanding regarding dependence of cell behavior on substrate stiffness, tissue cells typically remain round on soft substrates but spread on stiff substrates. The current studies were carried out to learn if the growth factor environment influenced the foregoing relationship. Using standard methods, we prepared planar (2D) polyacrylamide (PA) gels ranging from 0.5 to 40 kPa and covalently cross-linked with fibronectin and collagen at concentrations ranging from 2.5 to 50 μg/ml. We carried out experiments with fibroblasts varying in their ability to form actin stress fibers and focal adhesions. In fetal bovine serum (FBS) containing medium – the growth factor environment in which most studies on cell spreading and substrate stiffness have been carried out – cell spreading increased with increasing substrate stiffness and adhesion ligand density. However, in platelet-derived growth factor (PDGF) containing medium, cell spreading was relatively independent of substrate stiffness and adhesion ligand density except little cell attachment occurred in the complete absence of cross-linked adhesion ligands. If cell contraction was blocked with blebbistatin, then cell spreading in FBS-containing medium became independent of substrate stiffness. The findings suggest that under growth factor conditions that stimulate global cell contraction (FBS), cell spreading cannot occur unless adhesion ligand density and substrate stiffness result in cell–substrate interactions strong enough to resist and overcome the inward tractional force. Under growth factor conditions that stimulate global cell protrusion (PDGF), such resistance is not required.

1. Introduction

Mechanical interactions between cells and their extracellular environment play key roles in diverse aspects of normal cell physiology including cell migration and proliferation [1–4]. Changes in cell–matrix interactions contribute to the pathological features associated with tumor progression [5–8], scarring [9–11] and aging [12]. One of the key goals of studying biomechanical interactions between cells and matrix has been development of materials for tissue engineering [13–17].

Substrate stiffness is one of the biomechanical properties of particular interest with regard to its influence on tissue cell behavior. On soft 2D substrates (e.g., ~1 kPa polyacrylamide gels), cells spread poorly even at an optimal concentration of covalently cross-linked adhesion ligand. As stiffness increases to 50 kPa, cell spreading increases [18–20]. Also, cells migrating on soft substrates exhibit a property called *durotaxis* meaning that they move preferentially up stiffness gradients and not the reverse [21–23].

Research in our laboratory focuses on the interactions between fibroblasts and three dimensional collagen matrices with a particular interest in motile and mechanical interactions involved in matrix remodeling and cell migration [24]. Important differences exist between the biomechanical behavior of fibroblasts interacting with 3D matrices compared to those described above with soft 2D substrates. For instance, fibroblasts can spread well on soft (5–65 Pa) collagen matrices, much softer than the softest PA gels. The shape of cell spreading varies from dendritic extensions lacking stress fibers to more stellate extensions containing prominent stress fibers depending on collagen matrix density. However, the difference reflects a change in spacing between collagen fibrils rather than a difference in matrix stiffness [25]. Also, fibroblasts migrating in 3D collagen matrices are not limited by durotaxis. In nested collagen matrices, cells can migrate from inner ~15 mg/ml collagen matrices to outer 1.5 mg/ml collagen matrices [26], which means crossing a stiffness gradient from ~600 Pa to ~6 Pa [25]. Keratocytes can cross an even higher stiffness gradient from inner ~133 mg/ml inner collagen matrices to outer 2.5 mg/ml matrices [27].

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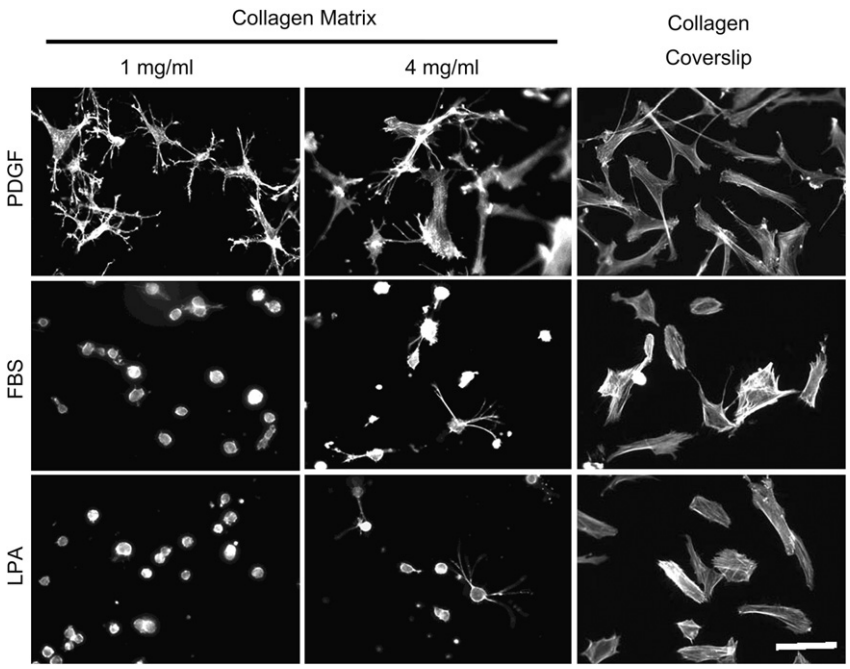

Fig. 1. Fibroblast spreading on collagen matrices and collagen-coated coverslips. BR5 fibroblasts were cultured 4 h in PDGF, FBS and LPA-containing medium on 1 mg/ml and 4 mg/ml collagen matrices and collagen-coated coverslips as indicated. At the end of incubations, samples were fixed and stained for actin. Scale bar, 100 μm.

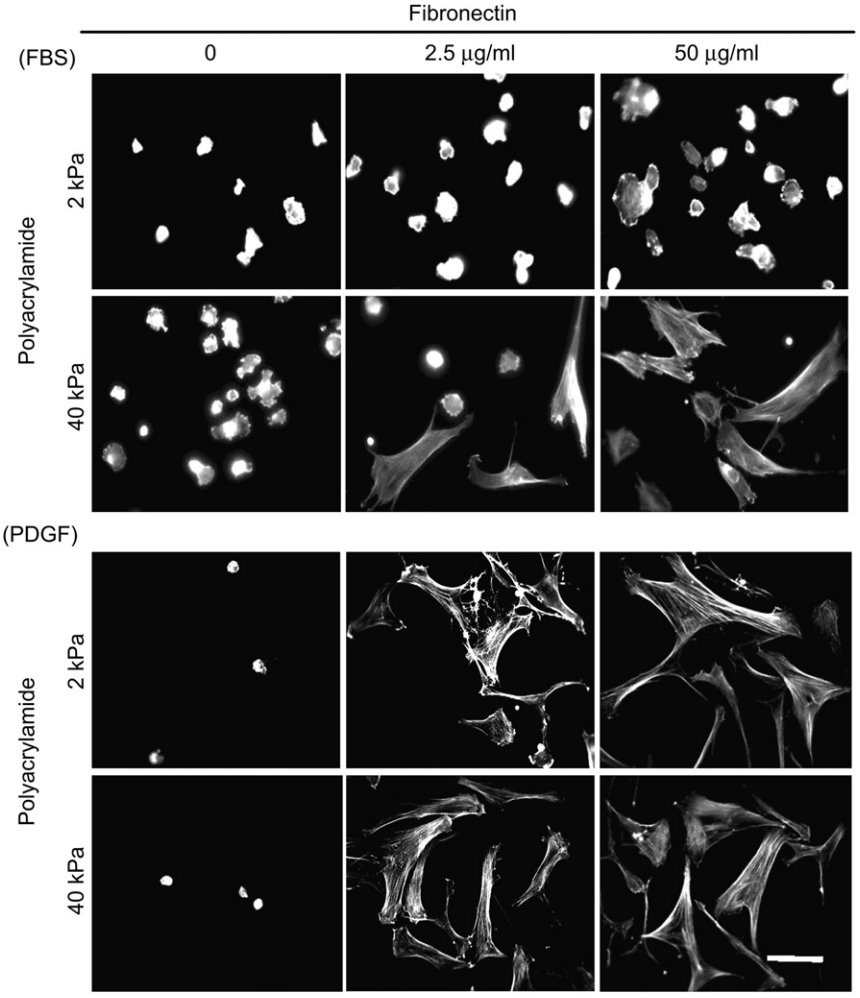

Fig. 2. Fibroblast spreading on fibronectin cross-linked PA gels. BR5 fibroblasts were cultured 4 h in FBS and PDGF-containing medium on 2 and 40 kPa polyacrylamide gels with fibronectin cross-linked at the concentrations indicated. At the end of incubations, samples were fixed and stained for actin. Scale bar, 100 μm.