CELL-MATRIX ADHESIONS AS SENSORS OF THREE-DIMENSIONAL EXTRACELLULAR MATRIX MICROSTRUCTURE AND COMPOSITION

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INTRODUCTION

The communication between cells and their three-dimensional (3D) extracellular matrix (ECM) is crucial in the determination of tissue form and function. One aspect of cell-ECM communication involves the conversion of micro-mechanical information provided by the ECM into biochemical responses experienced by the cell. Ultimately, the transduction of information from a mechanical to a biochemical format serves as a regulator of fundamental cellular behavior including proliferation, differentiation, and migration. A critical aspect of the transmission of mechanical forces from the ECM to the cell cytoskeleton and ultimately to the cell nucleus is the formation of cell-matrix adhesions [1].

Previous studies have focused on the visualization of cell-matrix adhesions in a two-dimensional (2D) format consisting of cells grown on 2D substrates [2]. To date, studies are lacking regarding the dependence of cell-matrix adhesion characteristics on specific structural-mechanical properties of a cell's ECM microenvironment, especially in a 3D, physiologically relevant context. This work determines and compares cell-matrix adhesion composition and distribution within cells grown on ECM substrates in 2D and 3D formats. In addition, the dependence of cell-matrix adhesion characteristics on ECM microstructure and macromolecular composition is determined. Cell-matrix adhesion characteristics investigated include localization of specific integrins subunits, cytoplasmic proteins, and actin cytoskeleton organization.

METHODS

Growth of Cells on 2D and Within 3D ECMs

Swiss mouse 3T3 fibroblasts from the American Type Culture Collection (Manassas, VA) were maintained and propagated in Dulbecco's modified Eagle's medium supplemented with 1.5 g/L $NaHCO_3$, 10% calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 m/M L-glutamine. Low-passage human dermal fibroblasts were obtained from Clonetics Corporation (San Diego, CA) and propagated in fibroblast basal medium supplemented with 1 µg/ml human recombinant fibroblast growth factor, 5 mg/ml insulin, 50 mg/ml gentamicin, 50 mg/ml amphotericin-B, and 2% fetal bovine serum.

Two-D ECMs were air-dried coatings of either fibronectin, fibrillar collagen type I or fibrillar collagen type I in combination with specific ECM macro-molecular components (e.g., hyaluronic acid). 2D fibronectin ECMs were prepared by coating poly-L-lysine (Sigma Chemical Co., St. Louis, MO) treated slides with bovine plasma fibronectin (Sigma Chemical Co., St. Louis, MO) [3]. Fibroblasts were then seeded on the 2D fibronectin substrates at densities between 10^4 and 10^5 cells/ml. To produce 2D fibrillar ECM substrates (coatings) with different microstructures, native type I collagen was neutralized under different conditions and in the presence of specific ECM components as previously described [4,5]. Neutralized collagen-based solutions were pipetted onto coverslips and air-dried. Fibroblasts were seeded on the 2D fibrillar ECM substrates at densities between 10^4 and 10^5 cells/ml.

To form 3D tissue constructs consisting of cells seeded within a 3D ECM, collagen was polymerized under different conditions and in the presence of specific ECM components as previously described [4,5]. Fibroblasts at a density of 10^5 cells/ml were added as the last component to the neutralized solutions and the suspension was polymerized in chambered coverslips in a humidified environment at 37°C. Immediately after polymerization, the 3D ECMs were incubated in complete medium at 37°C in a humidified environment of 5% CO_2 in air.

A tissue-derived biomaterial representing the submucosal layers of the porcine small intestine (known as intestinal submucosa) was obtained from Cook Biotech Inc. (West Lafayette, IN). Intestinal submucosa represents a 3D extracellular micro-environment with all the structural and compositional complexity found naturally *in vivo*. Fibroblasts were seeded on intestinal submucosa at a density of 4×10^4

cells / 0.785 cm^2 and grown for 18 hours in complete medium at 37°C in a humidified environment of 5% CO_2 in air.

Visualization of Cell-Matrix Adhesions

All specimens were fixed briefly then immuno-labeled for specific adhesion molecules using standard procedures. Three-D images of cellular morphology and ECM microstructure were obtained simultaneously using confocal microscopy in a reflection mode [5]. Localization of cytoplasmic proteins and integrin receptors was conducted using fluorescence labeling techniques in conjunction with confocal microscopy.

RESULTS AND DISCUSSION

As a first approach to determine the effect of ECM microstructure and composition on cell-matrix adhesion formation, β1 integrin was localized in Swiss mouse 3T3 fibroblasts grown on 2D fibrillar collagen substrates, within 3D collagen type I ECMs, and within the tissue-derived ECM, intestinal submucosa. β1 integrin is known to be involved in the adhesion of fibroblasts to type I collagen. Immuno-fluorescence results showed that β1 integrins within fibroblasts grown on tissue culture plastic coated with fibrillar collagen demonstrated a high density, punctate staining pattern and were polarized to the surface of the cell that was in intimate contact with the 2D substrate (Figure 1, panels A and B). In contrast, fibroblasts grown within a 3D type I collagen ECM (Figure 1, panels C and D) and a 3D tissue-derived ECM, intestinal submucosa (Figure 1, panels E and F), demonstrated β1 integrin pattern that appeared decreased in number, increased in length, and distributed over the entire surface area of the cell. Similar findings were also reported by Cukierman et al. [2] who determined and compared cell-matrix adhesion properties of fibroblasts grown on cell-derived 3D ECMs with those observed on traditional 2D fibronectin substrates

CONCLUSIONS

In summary, these results demonstrate that cell-matrix adhesion formation is dependent upon ECM composition and microstructure. Continued investigations of cell-ECM interactions within a 3D context are necessary to advance our knowledge of the physiologic relevance of adhesion formation as well as its involvement in the process of mechanotransduction. It is plausible that cell-matrix adhesions of distinct composition and morphology provide a cell with the ability to differentially sense and respond to biophysical cues (e.g., microstructure and mechanical loads) provided by the ECM. Therefore, the integration of mechanical and biochemical measurements allow for the identification of these mechanisms involved in signaling between the cells and its ECM. The significance of this research is to lay the foundation for directed repair of damaged tissues by identifying specific structural and mechanical properties of the ECM micro-environment that influence fundamental cell behavior.

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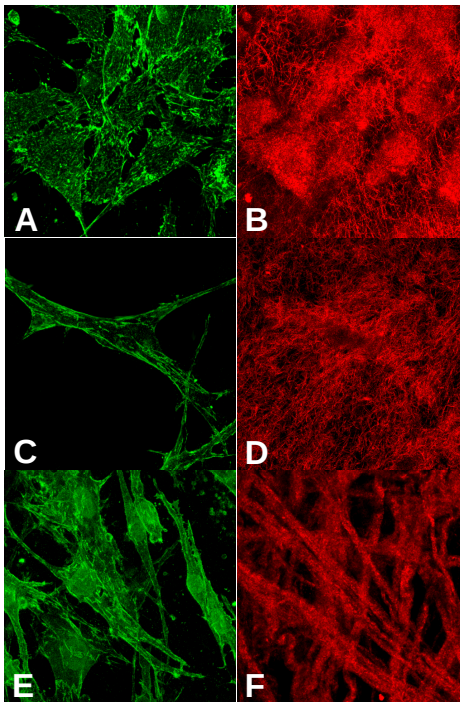

Figure 1. Confocal images showing differential β1 integrin distribution within fibroblasts grown on 2D fibrillar collagen (A and B), within a type I collagen ECM (2 mg/ml) (C and D), and within a 3D tissue-derived ECM, intestinal submucosa (E and F). Panels A, C, and E represent β1 integrin-specific fluorescence and panels B, D, and F represent ECM microstructure.

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