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Research Paper

Mechanical load and mechanical integrity of lung cells – Experimental mechanostimulation of epithelial cell- and fibroblast-monolayers

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ABSTRACT

Experimental mechanostimulation of soft biologic tissue is widely used to investigate cellular responses to mechanical stress or strain. Reactions on mechanostimulation are investigated in terms of morphological changes, inflammatory responses and apoptosis/necrosis induction on a cellular level. In this context, the analysis of the mechanical characteristics of cell-layers might allow to indicate patho-physiological changes in the cell–cell contacts.

Recently, we described a device for experimental mechanostimulation that allows simultaneous measurement of the mechanical characteristics of cell-monolayers. Here, we investigated how cultivated lung epithelial cell- and fibroblast-monolayers behave mechanically under different amplitudes of biaxial distension. The cell monolayers were sinusoidally deflected to 5%, 10% or 20% surface gain and their mechanical properties during mechanostimulation were analyzed. With increasing stimulation amplitudes more pronounced reductions of cell junctions were observed. These findings were accompanied by a substantial loss of monolayer rigidity. Pulmonary fibroblast monolayers were initially stiffer but were stronger effected by the mechanostimulation compared to epithelial cell-monolayers.

We conclude that, according to their biomechanical function within the pulmonary tissue, epithelial cells and fibroblasts differ with respect to their mechanical characteristics and tolerance of mechanical load.

1. Introduction

In the living organism almost all cell types are exposed to mechanical stimulation in form of shear stress or distension. Therefore, various in vitro studies focused on the biological behavior of cell-layers after mechanical stimulation (Tschumperlin et al., 2000; Hsu et al., 2010), analyzing inflammatory responses (Wirtz and Dobbs, 1990), cytoskeleton

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reorganization (Lee et al., 2010), and cell death (Hammerschmidt et al. 2007). Other studies focused on the mechanical properties of cells (Fletcher and Mullins, 2010; Harris et al., 2012). However, studies that combine both the biological and the mechanical response on mechanical stress are rare (Chaudhuri et al., 2009; Dassow et al., 2010).

In most organ systems mechanical stimulation plays an essential role for the physiological function. Particularly in the lung, mechanical stimulation within a physiological range provides the basis for maturation, tissue regeneration and functionality (Schittny et al., 1998; Gomes et al., 2001) whereas mechanical stimulation above the physiological range is responsible for the development of lung injury (Pugin, 2003). Thereby the mechanisms underlying these processes are yet not fully understood. Recently our group could demonstrate that during experimentally induced lung injury the increase in global lung stiffness is associated with a locally reduced stiffness of the parenchyma (Schwenninger et al., 2012).

We hypothesized that the reduced stiffness of the lung parenchyma is associated with a structural loss of mechanical integrity on the cellular level. Therefore, we investigated the instant cellular responses on experimental mechanostimulation in monolayers of human lung epithelial carcinoma cells (A549), rat lung epithelial cells (RLE-6TN), normal human bronchial epithelial cells (NHBE), and primary human lung fibroblasts (IMR-90) and furthermore analyzed the monolayers' mechanical characteristics at increasing stimulation amplitudes.

2. Materials and methods

2.1. Measurement of mechanical distensibility

For mechanostimulation and synchronous measurement of the mechanical distensibility, cells were grown on highly flexible silicone membranes and deflected sinusoidally using an earlier described mechanostimulator (Schumann et al., 2008; Gamerdinger et al., 2012). In brief, the mechanostimulator consists of a pressure chamber that is air-tightly sealed on top by the cell-layer-carrying membrane to which the investigated sample is attached by means of protein binding via RGD-labeling of the membrane. If a certain amount of fluid volume is displaced into the pressure chamber the membrane is deflected into the shape of a spherical cap (bubble inflation technique) (Fig. 1). Thereby the pressure inside the pressure chamber increases depending on the mechanical properties of the cell-monolayer/membrane construct.

For the measurement of the mechanical distensibility, cell-monolayers of A549, RLE-6TN, NHBE and IMR-90 cells were deflected sinusoidally for 10 min at a rate of 15 min^{-1} to a relative surface gain of 5%, 10% or 20%. The distensibility of the cell-monolayer plus underlying membrane (D_{comb}) was calculated as the ratio of the displaced volume (ΔV) and the resulting pressure increase (Δp) inside the pressure chamber.

$$D_{comb} = \frac{\Delta V}{\Delta p} \quad (1)$$

D_{comb} is a combination of the membrane's distensibility (D_{memb}) and of the cell-monolayer's distensibility (D_{cells}). For our approach, the properties of every membrane (D_{memb}) were measured separately (Dassow et al., 2013). Consequently we were able to calculate D_{cells} from the two known distensibilities D_{comb} and D_{memb}:

$$D_{cells} = \frac{1}{(1/D_{comb}) - (1/D_{memb})} \quad (2)$$

As for these measurements a certain deflection is required, measurements with 5% surface gain served as baseline for comparison of mechanical characteristics.

2.2. Membranes

Silicone membranes were produced using a spin coating process (Armbruster et al., 2009). To allow cell adherence, membrane hydrophobicity was reduced by coating with 0.5 mg/ml Sulfo-SANPAH and treatment with RGD-peptide (Li et al., 2006). The average D_{memb} was 265 ± 27 µl/mbar.

2.3. Cell cultivation

A549 cells (ATCC, CCL-185) were cultured in Dulbecco's modified Eagle's medium (DMEM, high Glucose with L-Glutamine, Life Technologies GmbH, Darmstadt, Germany) containing 10% fetal calf serum (FCS, PAA Laboratories GmbH, Coelbe, Germany) at 37 °C in a humidified 5% CO_2 atmosphere.

RLE-6TN cells (ATCC, CRL-2300) were cultured in HAM-s F12 (Lonza Sales Ltd., Verviers, Belgium) containing 10% FCS, 0.01 mg/ml bovine pituitary extract (BPE), 0.005 mg/ml Insulin, 2.5 ng/ml Insulin growth factor (IGF), 1.25 µg/ml Transferrin, 2.5 ng/ml Epithelial growth factor (EGF, all supplements from Life Technologies, Darmstadt, Germany) at 37 °C in a humidified 5% CO_2 atmosphere.

NHBE cells (Lonza Sales Ltd., Verviers, Belgium) were cultured in bronchial epithelial cell growth medium (BEGM, media and all supplements from Lonza Sales Ltd., Verviers, Belgium) at 37 °C in a humidified 5% CO_2 atmosphere.

IMR-90 fibroblasts (ATCC, CCL-186) were cultured in α-MEM (Life Technologies, Darmstadt, Germany) containing 10% FCS and 1% Pen/Strep solution at 37 °C in a humidified 5% CO_2 atmosphere. A549, RLE-6TN and IMR-90 cells were grown up to 90% confluence in 75 cm^2 tissue culture flasks and in 175 cm^2 tissue culture flasks respectively (BD Biosciences, Heidelberg, Germany). NHBE cells were grown up to 70% confluence in 175 cm^2 tissue culture flasks. After the culturing period, the cell lines were removed from the flasks by treatment with 3 ml Trypsin–EDTA (PAA Laboratories GmbH, Coelbe, Germany) solution for 5 min in a humidified incubator at 37 °C and 5% CO_2, washed in PBS (Lonza, Sales Ltd., Verviers, Belgium) with 5% FCS, and resuspended in the corresponding culture media. The primary cells were trypsinized with Trypsin EDTA (Life Technologies, Darmstadt, Germany (NHBE), Sigma-Aldirch, Munich, Germany (IMR-90), respectively) for 7 min. Subsequently, cells were washed in PBS containing 5% FCS and resuspended in the corresponding culture media.

For cell seeding the membranes were placed in six well plates. In case of A549 or RLE-6TN 8×10^4 cells ($=2 \times 10^4$ cells/cm^2) and in case of NHBE or IMR-90 5×10^4 cells ($=1.3 \times 10^4$ cells/cm^2) were spread on the surface of the membrane, each in 1.3 ml corresponding culture medium. The membranes were then carefully transferred to the incubator to allow cell