Effect of substrate mechanical properties on alveolar epithelial cells

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***Abstract*— A549 epithelial adenocarcinoma cells are usually chosen as a model of type II pulmonary epithelial cells for in vitro toxicological evaluations of air born microparticles. These cells are normally cultured in conventional two-dimensional (2D) systems on a rigid support with the consequent lost of typical alveolar cell features. The aim of this work was to study the effect of substrate mechanical properties on A549 cells morphology and function. 2D static culture of A549 cells were seeded and cultured on rigid conventional polyester Thermanox™ coverslips and on elastic PDMS membrane. A549 cells grown for 72 hours on the rigid support did not show the typical alveolar cell features with rounded shape and microvilli, that were present in cells cultured on the elastic PDMS membrane. In addition, cells cultured on Thermanox™ coverslips did not show production of surfactant, while an aboundant surfactanct present was observed in cells cultured on the elastic substrate. These findings give evidence that the elastic properties of the substrate importantly affect cell morphology and functions of these epithelial cells. The use of an artificial support with adequate mechanical properties it is important for the use of in vitro tests during cell toxicity evaluations.**

***Keywords* — Cell mechanics, Mechanobiology, Substrate stiffness, Elastic module.**

I. Introduction

THE alveolar epithelium is predominantly composed of type I and type II epithelial cells [1]. Type II pulmonary cells are usually chosen for toxicity studies at alveolar level in determining the toxic effects of air born microparticles (PM). In particular, the A549 epithelial adenocarcinoma cell line is a common option to reproduce in vitro the respiratory membrane in contact with air [2]. These cells are usually cultured in conventional two-dimensional (2D) systems based on adherent cell monolayers in a static dish culture.

It is well known that the function of cells is dependent on factors including chemical, physical, and mechanical stimuli [3]. Particularly, the rigidity ("stiffness") of the extracellular matrix (ECM) defined by its elastic modulus (E), has a significant effect on epithelial cell growth, proliferation and differentiation [4, 5]. Mechanical inputs, due to the elasticity of the extracellular matrix, regulate also the transduction of important transcriptional regulators [6, 7]. As a consequence, the mechanical properties of cell substrate can importantly affect cell function, protein expression and cell structure [8].

The aim of our work was to investigate whether the culture of A549 cells on substrate with different elastic properties affects cell morphology and function.

II. Methods

A549 cells (ATCC® CCL-185™) were cultured in FK12 medium (ATCC, Middlesex, UK) supplemented with 10% inactivated fetal bovine serum (Thermo Fisher Scientific, Waltham, USA). Cells were grown in T75 flasks at 37°C with 5% CO_2 and trypsinized for passage three times a week. Two substrate surfaces were used for A549 cell culture in standard conditions, the rigid surface of Thermanox™ Coverslips (Thermo Fisher Scentific, Waltham, Massachusetts, USA) and a polydimethylsiloxane (PDMS) artificial elastic membrane (Elastosil® Film, Silex Ltd, Hampshire, UK). We determined the elastic module of Elastosil® Film by a tension test. A rectangular tensile specimen was gripped between the clamps of a tensile-testing machine and subjected to a controlled tension until failure. The elongation of the gauge section and the applied force were used to calculate the average elastic modulus of E = 0.53 MPa. Differently, the elastic modulus of polyester Thermanox™ Coverslips is reported to be approximately of 1,000 MPa.

Cells were seeded at concentration of $1*10^4$ cells/cm^2 on Thermanox™ Coverslips and Elastosil® Film and maintained in culture for 72h. Every 24 hours images were digitized using a phase-contrast microscope to monitor cell adherence and growth. At the end of the observation period, samples were fixed in 0.5 glutaraldehyde or 2% paraformadehyde (Società Italiana Chimici, Milano, Italy) in 4% sucrose, for scanning electron microscopy (SEM) and immunofluorescence analysis, respectively. For SEM observation cells were postfixed with osmium tetroxide, dehydrated through a series of passages in increasing ethanol baths and dried in pure hexamethyldisilazane (HMDS, Fluka Chemie AG, Buchs, Switzerland). At the end, samples were mounted on stubs, coated with gold in a sputter coater (Agar Scientific, Stansted, UK) and then examined on a Cross-Beam 1540EsB electron microscope (Carl Zeiss GmbH, Oberkochen, Germany).

To perform immunohistochemical, analysis samples fixed in paraformadehyde, permeabilized using triton X-100 and incubated in 3% bovine serum albumin. A549 cells were then treated with rhodamine-labeled phalloidin for 45 minutes at room temperature and counterstained with DAPI (1 mg/ml) for 20 minutes at room temperature. Samples were finally examined by laser confocal microscopy (LSM 510 Meta, Carl Zaiss, Germany).

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III. RESULTS

Phase-contrast images of A549 cells cultured on Thermanox™ Coverslips or Elastosil® Film showed a uniform cell adherence and growth on both substrates. On Thermanox™ Coverslips cells reached confluence after 72 hours while and formed a uniform monolayer. On Elastosil® Film A549 cells remained distributed in clusters, they did not spread over the membrane surface and remained attached in the same position, despite their growth. SEM analysis highlighted important differences in morphology and function of A549 cells depending on whether the cells were cultured on rigid or on elastic support (as shown in Fig. 1 and 2). Through SEM analysis, it was observed that A549 cells grown on Thermanox™ Coverslips remained flat, expressing a low number of microvilli. A549 cells cultured on Elastosil® Film maintained the typical alveolar cell morphology, with rounded shape and large number of microvilli on cell surface. In addition these cells produced an important amount of surfactant (see Fig. 2), an important lipoprotein complex for the prevention of lung collapse in vivo.

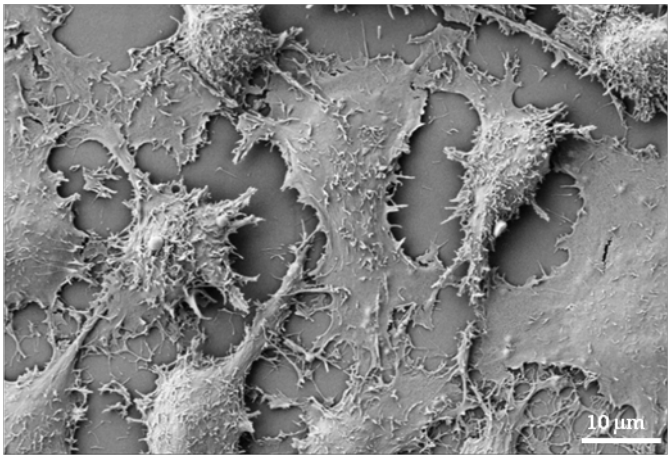

Figure 1. SEM images of A549 cells cultured on Thermanox coverslips after 72h of culture.

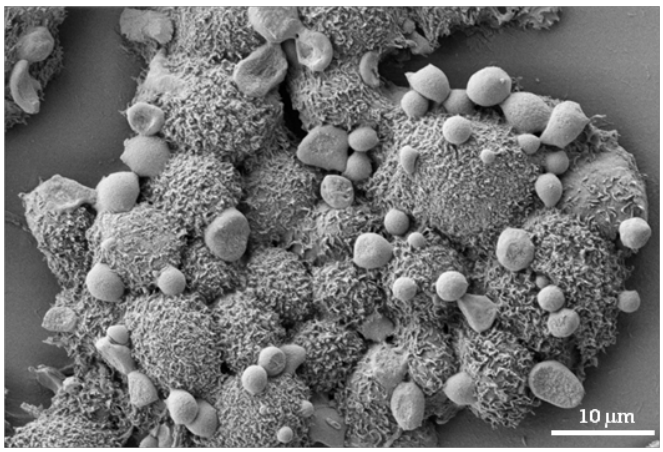

Figure 2. SEM images of A549 cells cultured on Elastosil film after 72h of culture.

F-actin staining revealed that the elastic properties of the substrate importantly affect cells cytoscheleton. As shown in Fig. 3, A549 cells seeded on Thermanox™ Coverslips have a random distribution of F-actin fibers, with a few elongated fibers crossing the entire cellular bodies. In contrast, cells grown up on Elastosil® Film displaied a randomly oriented cobblestone shape with F-Actin fibers mostly organized in dense peripheral actin bands.

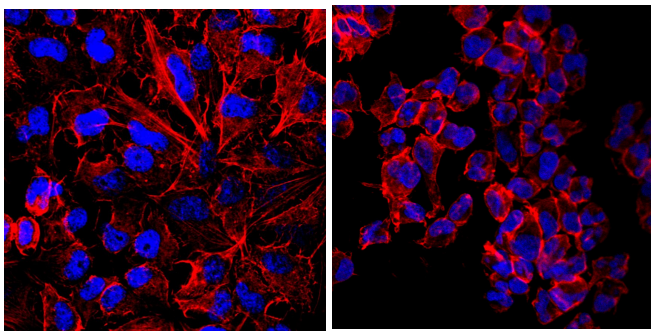

Figure 3. Immunofluorescence images of A549 cells stained for F-Actin (red) cultured on Thermanox coverslips (left) and on Elastosil film (right) after 72h of culture. Magnification 40X.

IV. CONCLUSION

Our findings indicate that A549 cells cultivated on substrates with different elastic properties develop important differences in cell morphology and functionality. Cells in contact with a soft elastic support showed acquired phenotype similar to the physiological one of alveolar type II cells. Thus, to assess the toxicity of substances that can arrive in contact with alveolar membrane in the lungs, in vitro test should be based on an elastic culture supports instead of the traditional rigid culture dishes.

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