MECHANICAL CHARACTERIZATION OF MICROENGINEERED EPITHELIAL CYSTS USING ATOMIC FORCE MICROSCOPY

Yusheng SHEN†, Dongshi GUAN‡, Daniela SERIEN§, Shoji TAKEUCHI¶, Penger TONG†, Pingbo HUANG†,§ and Levent YOBAS†,¶

†Division of Biomedical Engineering, The Hong Kong University of Science & Technology (HKUST), ‡Department of Physics, HKUST, §Institute of Industrial Science (IIS), The University of Tokyo, ¶the State Key Laboratory of Molecular Neuroscience, †Department of Electronic & Computer Engineering, HKUST, Hong Kong

†Equally contributed to the work; ¶Senior authors

ABSTRACT
This paper describes a novel method for generating size controllable epithelial cysts on microplates with normal polarity and further mechanical characterization of the microengineered cysts using atomic force microscopy (AFM). By measuring the force-distance curves on MDCK-II cysts using AFM and further fitting with the Hertz’s model, we determined the apparent Young’s modulus of the cyst structure to be 1.07±0.072 kPa, considerably smaller than the reported elasticity (5-7 kPa) of individual MDCK-II cells that were probed in a cell-monolayer context. This platform may enable us to investigate the link between epithelial cyst biology and cell mechanics.

KEYWORDS: Cell mechanics, Epithelial cyst, Micropatterning, AFM

INTRODUCTION
Epithelial cysts are soccer ball-like, lumen-enclosing monolayers of polarized cells. As one of the two basic building blocks for the organogenesis of many branched epithelial organs, such lumen-enclosing structure assumes critical roles in development and normal physiology. The formation, maintenance and functionality of such structure require the coordination of mechanical forces both internally generated and externally applied. Although the characterization of the mechanical properties of epithelial cells at the single cell, multicellular and even tissue levels have begun to meet the challenge of revealing how mechanical forces are involved in complex biological processes, simple in vitro experimental platforms for characterizing cell mechanics in a more physiologically relevant context such as the cyst model, are still lacking, probably due to the fact that conventional in vitro epithelial cysts formation requires cells to be embedded in a three dimensional (3D) gel which renders the cysts inaccessible to most probes. Here, we demonstrate the generation of size controllable and probe accessible epithelial cysts on microplates and further mechanical characterization of the micro-tissue using AFM (Figures 1 and 2).

EXPERIMENTAL
The process flow of producing parylene-based microplates and procedure of cell culture were shown in Figure 2. Briefly, following the coating of fibronectin on the microplates, MDCK-II cells were seeded at a density of approximately $1 \times 10^4$ cm$^{-2}$ on each sample and cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12, Invitrogen) supplemented with 10% fetal bovine serum (FBS). After incubating for 12 hours, non-adhered cells were washed out and the culture medium was further supplemented with 2% Matrigel (Corning). MDCK-II cysts with lumens will normally form after 7 days’ cell culture and were subsequently used in the AFM experiment.

In the AFM experiment, a colloidal probe cantilever was used for the cysts mechanical characterization (Figure 1). The AFM measurements were carried out in contact mode with a maximum indentation force about 200 nN. The measured Force–distance curves were recorded and fitted with the Hertz model to calculate the modulus.
RESULTS AND DISCUSSION

First, we generated MDCK-II cyst structures with mature lumen within 7 days by geometrically confining cells on parylene-based microplates in an “in-3D” culture condition (Figure 2). This method avoided the involvement of the 3D gel and the massive assembly of basement membrane around the cyst, therefore, allowing direct mechanical measurement of the microengineered tissue by micropipette and AFM.

The morphology and polarity of the cyst are identical to those formed in 3D gel, with a F-actin belt present at the apical side of the cyst facing the lumen and a very thin layer of laminin wrapping the whole cyst via the connection by β1 integrin (Figures 3 and 4). This resemblance makes the microengineered cyst a suitable model to explore the role mechanical forces may play during normal physiological process such as mechanical control of lumen expansion.

Figure 3: Confocal images of a typical MDCK-II cyst formed on the microplate. Left, top and side views; right, top view. Scale bar, 20 µm.

Figure 4: Confocal images of MDCK-II cysts formed on the microplates and stained with different domain specific markers. Scale bar, 20 µm.

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As a platform demonstration of the model, we measured the force-distance curves on MDCK-II cysts using AFM with a colloidal probe (Figure 1) and further fitted to the Hertz’s model to obtain the apparent Young’s modulus of the cyst structure (Figure 5). We determined the apparent Young’s modulus of the cyst structure to be 1.07±0.072 kPa, dependent on the indentation velocity, considerably smaller than the reported elasticity (5-7 kPa) of individual MDCK-II cells that were probed in a cell-monolayer context. This technique enables us to monitor cyst mechanics during biological processes occurred within the cyst such as cyst softening during a hypotonic challenge (Figure 6). Such mechanical response and regulation have largely remained unknown and the knowledge may help our understanding of the pathology of some diseases like autosomal-dominant polycystic kidney disease (ADPKD).

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REFERENCES


CONTACT

Correspondence and requests for materials should be addressed to Pingbo HUANG (email: bo-huangp@ust.hk) and Levent YOBAS (email: eelyobas@ust.hk).