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Original article

Development of the evaluation system for barrier functions of engineered epithelial lumens

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ABSTRACT

We have investigated the effects of a diameter of engineered epithelial lumen on cellar architectures and a barrier function. For this investigation, we have developed a system to evaluate the barrier function of engineered epithelial lumens. To test the utility of our system, we constructed the engineered epithelial lumens by culturing Madin–Darby Canine Kidney cells (MDCK) on the gold wires with different diameters ranging from 50 μm–200 μm. Confocal laser scanning microscopy revealed that long actin stress fibers and a low focal adhesion density were observed at the gold wire diameter of 200 μm, whereas the mesh-like morphology consisted of short actin stress fibers and high focal adhesion densities were found at the gold wire diameters of 50 μm and 100 μm. The expression pattern of ZO-1 that localizes at the tight junction was independent on the gold wire diameter. The electrical impedance measurement indicates that the barrier function for the samples constructed at the gold wire diameter of 200 μm was significantly higher than those at the gold wire diameters of 50 μm and 100 μm. The difference in the barrier functions of epithelial lumens might be attributed to the changes in cellular architectures with increasing the curvature of gold wire.

Introduction

Epithelial lumens, such as blood vessel, lymphatic vessel, and kidney tubule, play the roles of transport ducts for oxygen, nutrients, cells, and waste products. An inner surface of the epithelial lumen is covered by an epithelial cell sheet. The epithelial cell sheet prevents leakage of fluid flowing in the epithelial lumens. In addition, the epithelial cell sheets are interfaces for exchanging oxygen, ions, nutrients, and waste products. The selective exchanges are regulated by the tight junction [1,2].

Introducing the epithelial lumens into engineered tissues is a prerequisite for constructing functional and healthy engineered tissues. Therefore, many methods for introducing the epithelial lumens into the engineered tissues have been developed in the tissue engineering and the regenerative medicine fields [3–6]. However, the engineered epithelial lumens without appropriate barrier functions cannot be used as transport ducts. Therefore, we must evaluate the barrier functions for the epithelial lumens introduced in the engineered tissues.

The fluid leakage can be detected by perfusing media with fluorescent dye-conjugated macromolecules, such as dextran and polystyrene latex beads, into the epithelial lumens [3,7,8]. By contrast, the barrier functions for the epithelial cell sheet have been characterized by the transepithelial resistance (TER) [9–11]. TER can be determined by analyzing the electrical impedance spectrum. However, almost systems to measure the electrical impedance spectrum have been designed for two-dimensional epithelial cell sheet. There have been only a few systems to characterize TER for epithelial lumens [11], but they were designed for epithelial lumens with large diameters and applications were also limited.

Therefore, in this study, we have developed a system for evaluating the barrier functions of the epithelial lumens. The system consists of cylindrical and central rod electrodes. The epithelial lumens can be directly constructed on the surface of the cylindrical and/or central rod-like electrodes. By selecting the diameter of the central rod-like electrode, we can investigate an effect of the diameter for the epithelial lumen on the barrier functions. Recently, it was reported that the diameter of epithelial lumens affects cellular architectures, such as an orientation of actin filaments and a focal adhesion density [12]. The diameter dependences on cellular architecture could influence the barrier functions of epithelial lumens. Therefore, we have investigated effects of diameter of

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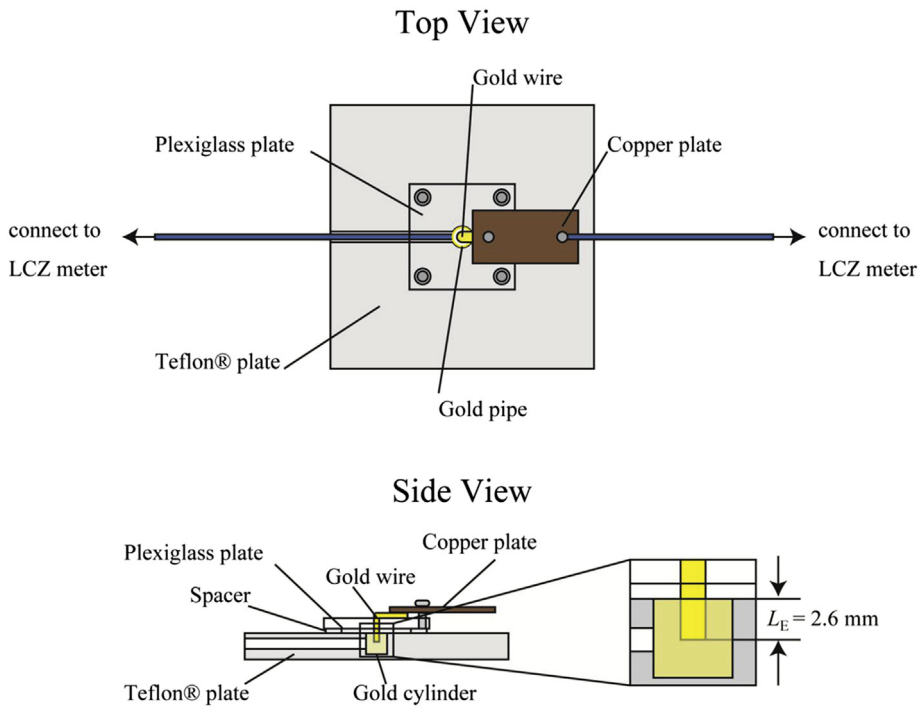

Fig. 1. Schematic illustration of the electrode chamber to measure the electrical impedance of epithelial lumen.

engineered epithelial lumens on the cellular architectures and the electrical impedance by using our system.

Materials and methods

Madin–Darby Canine Kidney (MDCK) cells were provided from RIKEN CELL BANK. DMEM (Nacalai tesque) supplemented with 10% fetal bovine replacement (Equitech-Bio) and 1% penicillin–streptomycin was used for culturing MDCK cells. Dulbecco's phosphate buffered saline (PBS(-)) was used for washing samples. Sodium chloride was dissolved with MilliQ water at various concentrations ranging from 2 mM to 1000 mM. Gold wires with the different diameters ranging from 50 μm–200 μm were purchased from Nilaco Co. Ltd., and they were used as a central

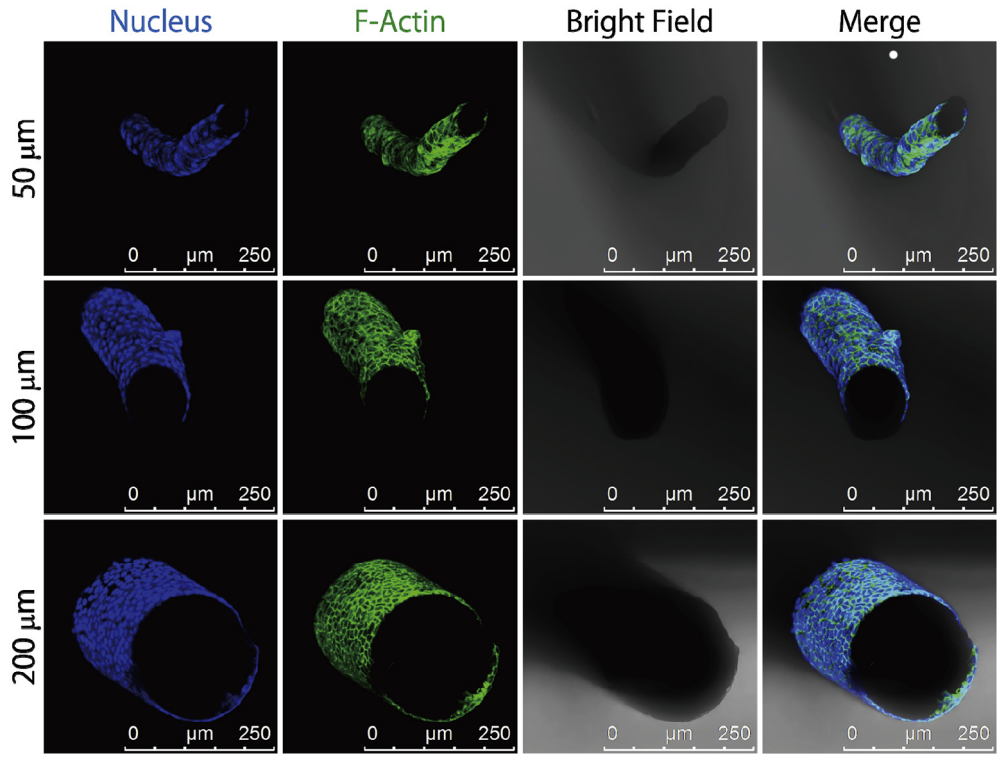

Fig. 2. Confocal laser scanning microscope images for the epithelial lumens constructed on the gold wires with various diameters.