

Development of a device to stretch tissue-like materials and to measure their mechanical properties by scanning probe microscopy

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Abstract

We have developed a new stretch device to investigate the biomechanical responses to an external loading force on a tissue-like material consisting of cells and a collagen gel. Collagen gel, a typical matrix found abundantly in the connective tissue, was attached to an elastic chamber that was precoated with a thin layer of collagen. Madin–Darby canine kidney cells that were cultured on the collagen gel were stretched in a uniaxial direction via deformation of the elastic chamber. Changes in the morphology and stiffness of the tissue-like structure were measured before and after the stretch using wide-range scanning probe microscopy (WR-SPM). The change in cellular morphology was heterogeneous, and there was a twofold increase in the intercellular junction due to the stretch. In addition to the WR-SPM measurements, this device enables observation of the spatial distribution of cytoskeletal proteins such as vimentin and α -catenin using immunofluorescent microscopy. We concluded that the stretch device we have reported in this paper is useful for measuring the mechanical response of a tissue-like material over a range of cell sizes when exposed to an external loading force.

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1. Introduction

An external loading force has crucial effects on the functions and structure of tissues. For example, bones require an external loading force to maintain their mechanical resistance; additionally, muscles and tendons need an external loading force to conserve their mechanical strength [1]. The external loading force is transmitted into the tissues in a heterogeneous manner based on the structure and mechanical properties of the tissue; therefore, the response of each region in the tissue to the external loading force is spatially different. The heterogeneity in the transmission of the external loading force into the tissue is essential for the maintenance of the tissue structure [2,3]. However, it is difficult to examine the biomechanical effects of the external loading force on the tissues

because there is no apparatus that can measure the local surface strain and local stiffness simultaneously.

The cellular contractile force also plays an important role in maintaining the tissue structure and functions by providing them with mechanical support. For example, this force is involved in physiological processes such as cellular migration [4], proliferation [5] and differentiation [6]. The activities of these physiological processes are strongly influenced by external loading forces [7–9]. However, the relationship between the external loading force and cellular mechanics remains unclear. Previously, we measured the changes in cellular stiffness in stretched or uniaxially compressed fibroblasts cultured on an elastic substrate using mechanical scanning probe microscopy (M-SPM) [10]; these changes corresponded with the contractile force of the stress fibers but not with the tension on the membrane. Thus, we concluded that the contractile force of a single fibroblast is maintained constant against an external loading force. The next issue that will be addressed by

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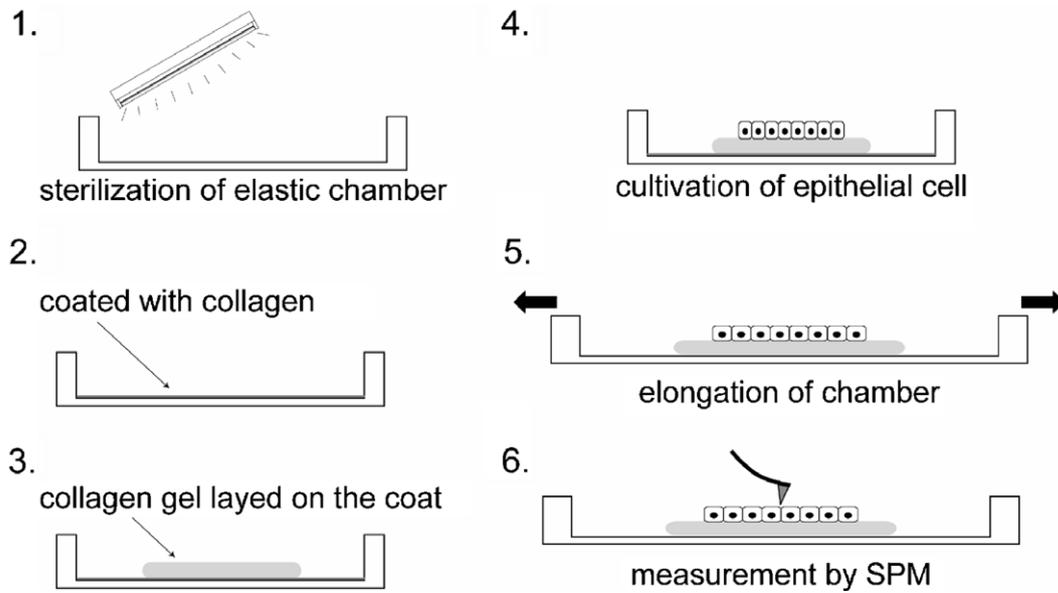


Fig. 1. Procedure of sample preparation and SPM measurements. A sterilized elastic chamber was coated with a collagen solution diluted with HCl prior to overlaying a collagen gel. Epithelial cells were cultured on the collagen gel. The cells were stretched uniaxially during the SPM measurements.

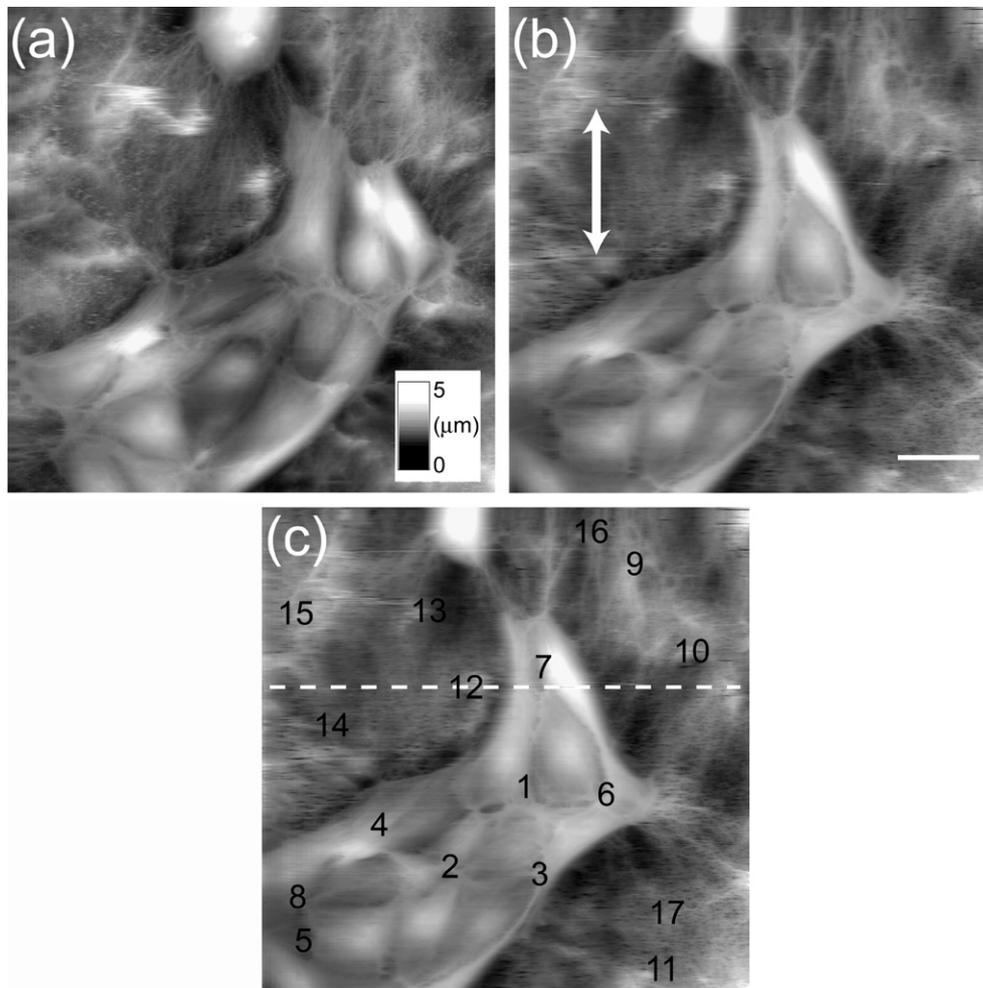


Fig. 2. Change in the topographic images of epithelial cells cultured on a collagen gel exposed to uniaxial stretch. The cells on the surface of the collagen gel were measured by SPM before (a) and after (b) the stretch. The direction of the uniaxial stretch was represented by a set of arrows. The length of the bar represents 50 μm . Some points on the image were numbered to analyze strain fields (c). The dashed line was defined as origin of the stretch.

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