

ANCHORING JUNCTIONS AND CYTOSKELETAL NETWORKS IN AN EPITHELIAL SHEET: MECHANICAL CHARACTERIZATION USING ELASTIC MICROFABRICATED MEMBRANES

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INTRODUCTION

Arguably, the biological basis of the mechanical properties observed in epithelial tissues resides within the complex three-dimensional arrangement of *intercellular* junctions that serve to anchor *intracellular* actin and intermediate filament networks of individual cells. Morphologically, anchoring junctions are often classified as (i) cell-cell junctions: adherens junctions and desmosomes, or (ii) cell-matrix junctions: focal adhesion complexes and hemidesmosomes. Desmosomes and hemidesmosomes are associated with intermediate filament networks, while focal adhesion complexes and adherens junctions are associated with actin networks. To date, experimental evidence is lacking that directly characterizes the mechanical function of specific anchoring junctions and organized cytoskeletal networks as they govern both tissue-level *resistance* and *response* (mechanotransduction) to mechanical deformation. As such, histology texts continue to ascribe only general mechanical functions to anchoring junctions, largely based upon rheological studies of the viscoelastic properties of experimentally isolated cytoskeletal proteins [1]. The goal of this research is to more precisely define the constitutive mechanical behavior of anchoring junctions and organized cytoskeletal networks within epithelial tissues as they relate to biological function in states of both health and disease.

MATERIALS AND METHODS

Anchoring junction formation *in vitro*

The *calcium switch* technique of human or murine keratinocyte culture [2-3] represents a well studied *in vitro* model of cell-cell and cell-matrix junction assembly during the formation of an epithelial sheet. The first step in the model consists of culturing normal keratinocytes in a minimally supplemented basal medium which contains low calcium concentrations ($[Ca^{2+}] < 0.1$ mM) to foster proliferation and expansion of basal cells. Given sufficient time, the keratinocytes will ultimately form a confluent monolayer of cells in which individual cells are anchored to the culture substrate via focal adhesion complexes, but do not express any form of cell-cell connectivity. Desmosomes, hemidesmosomes, and adherens junctions

are not present during the initial stage of culture. Next, the concentration of calcium is raised to high levels ($[Ca^{2+}] > 1.0$ mM). The calcium switch has been shown to trigger signaling cascades within keratinocytes that leads to: (i) a complete reorganization of cytoskeletal networks within individual keratinocytes; (ii) formation of adherens junctions, desmosomes and hemidesmosomes; and finally, after extended culture, (iii) stratification. Adherens junctions form within 15-30 minutes after the calcium switch, while desmosome formation requires approximately 2 hours.

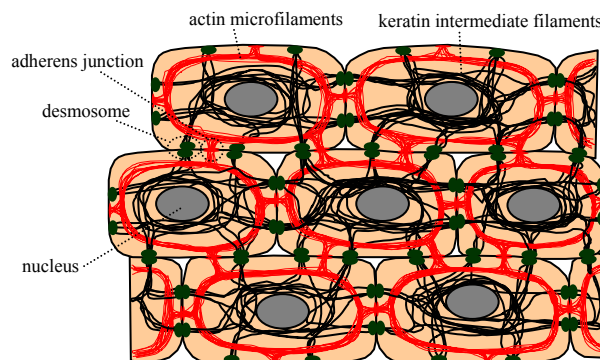


Figure 1. Keratinocyte monolayer cultured at high $[Ca^{2+}]$

Figure 1 depicts a cartoon representation of a monolayer of keratinocytes (viewed from above) cultured at high $[Ca^{2+}]$. Focal adhesion complexes and hemidesmosomes are not shown. Note how desmosomes between keratinocytes form button-like associations with keratin intermediate filaments that extend from a network that surrounds the nucleus. Adherens junctions consist of streak-like attachments of contact between short microfilament bundles that radially extend from larger peripheral bands of parallel actin bundles that form the cell cortex. The culture of normal keratinocytes *in vitro* leads to the formation of alternating desmosomes and adherens junctions during the formation of a contiguous epithelial sheet.

Experimental Apparatus

A special blister-type load-displacement cell culture apparatus has been designed and constructed for delivering and measuring dynamic differential pressures and polar deflections of a microfabricated, clamped, circular, elastic polydimethylsiloxane (PDMS) membrane on which normal human keratinocytes have been cultured into a confluent monolayer with or without specific anchoring junctions as controlled by the calcium switch. By measuring deviations in the load-deflection behavior of the composite diaphragm (PDMS membrane + keratinocyte monolayer) with respect to the behavior of the PDMS membrane measured prior to cell culture, a simple static mechanical model demonstrates that pressure-induced deflections of the composite diaphragm can be used to characterize the formation of anchoring junctions and organized cytoskeletal filament networks within the epithelial sheet.

Mechanical Model

Consider a clamped, circular, bi-layered diaphragm as shown in **Figure 2**, subjected to a static net uniform pressure, p , in which the load (acting normal to the deformed diaphragm) displaces the pole of the diaphragm a measurable distance, w_o . Assume that both the elastic membrane and the cell layer behave as isotropic, incompressible, linear-elastic materials with Young's moduli, E_m and E_c , respectively. Furthermore, assume that the membrane and the cell layer possess arbitrary uniform residual stresses, σ_m and σ_c , and that $h_m + h_c \ll a$, where h_m is the membrane thickness, h_c is the cell layer thickness, and a is the diaphragm radius.

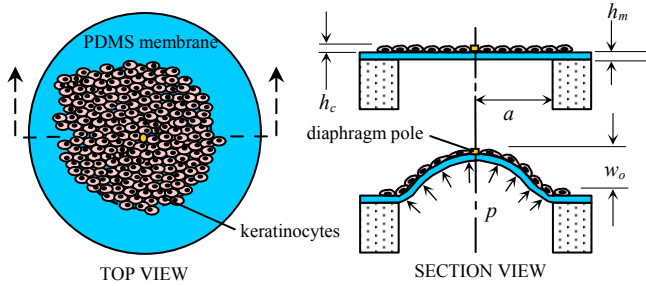


Figure 2. Microfabricated composite diaphragm

Both layers of the diaphragm are assumed to undergo identical displacements (and strains), bending stresses are ignored and only in-plane stresses are considered, provided they are uniform across each layer of the composite diaphragm. If the circumferential strain is assumed to be negligible, one can proceed to derive a non-dimensional equation governing the load-deflection behavior of the composite diaphragm for small polar displacements:

$$\tilde{p} = \frac{32}{9} (1 + \gamma \phi) \tilde{w}_o^3 + 4 \sigma \mu^2 (1 + \eta \phi) \tilde{w}_o, \quad (1)$$

where

$$\tilde{p} = \frac{p a^4}{E_m h_m^4}, \quad \tilde{w}_o = \frac{w_o}{h_m}, \quad \sigma = \frac{\sigma_m}{E_m}, \quad (2)$$

$$\gamma = \frac{E_c}{E_m}, \quad \phi = \frac{h_c}{h_m}, \quad \eta = \frac{\sigma_c}{\sigma_m}, \quad \text{and} \quad \mu = \frac{a}{h_m}. \quad (3)$$

RESULTS AND DISCUSSION

In **Figure 3**, (a) and (b) depict two possible mechanisms by which biological activity within the keratinocyte monolayer can alter the mechanical load-deflection behavior of the composite diaphragm.

Select values for the non-dimensional parameters required in equations (1)-(3) were chosen to reflect the experimental setup. The mechanical model is approximately valid for $\tilde{w}_o/\mu \leq 0.13$. (a) During the formation of the initial focal adhesion complexes at low $[\text{Ca}^{2+}]$, contractile bundles of actin within the keratinocytes can locally compress the membrane, resulting in apparent changes in σ_m and σ , whereas $\eta = \gamma \approx 0$. The PDMS membrane must have a finite tensile residual stress in excess of the compressive attachment forces of the cell layer to preclude any buckling deformations. (b) The reorganization of actin and intermediate filaments and subsequent formation of adherens junctions and desmosomes within the keratinocyte layer can be attributed to finite values of E_c and σ_c , hence $\gamma, \eta \neq 0$. Because of the known temporal sequence of anchoring junction formation, values for E_c can be defined for a monolayer with adherens junctions alone or for a composite E_c resulting from the presence of both adherens junctions and desmosomes. Finally, as depicted in (c), the necessity of experimentally matching length scales across the diaphragm thickness can be established. For values of $\phi \ll 1$, the mechanical behavior of the membrane will dominate the load-deflection behavior of the composite diaphragm, and changes in γ are unlikely to be detected. For $\phi \approx 1$, changes in γ result in load-deflection deviations that are finite and measurable. Thus, the use of a microfabricated, low modulus membrane (5-10 μm thick) is clearly justified. Mechanical load-deflection experiments and immunofluorescence studies of the coordinated movements of actin microfilaments, keratin intermediate filaments, and the formation of anchoring junctions are currently ongoing.

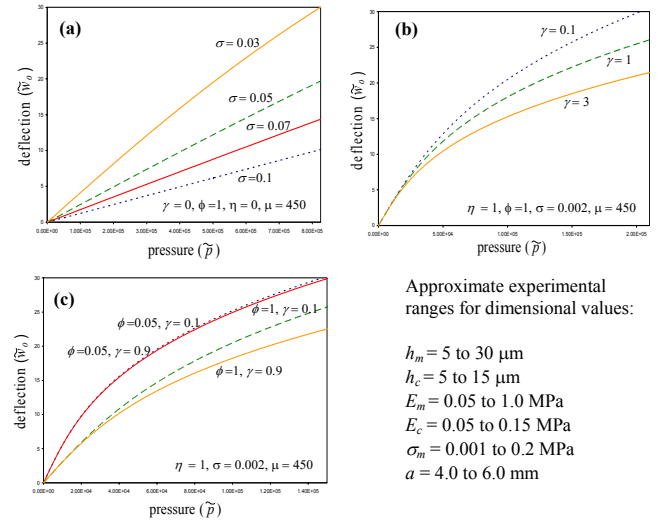


Figure 3. Load-deflection curves of a composite diaphragm

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