Advances toward Medical Nano-Imaging by High-Resolution Atomic Force Microscopy

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INTRODUCTION

The primary cause of blindness in the world is the formation of the opaque cataract in the crystalline lens of the eye. The leading factors are long-term exposure to radiation or UV light, but cataract formation can also be a consequence of certain forms of diabetes, hypertension, and of course, age. If left untreated, the disease results in progressive blindness and possibly glaucoma. Atomic force microscopy (AFM) has proven to be a valuable method for investigating structural aspects of cataract formation.

The lens of the eye is the only transparent tissue in the human body and it is avascular. The lens-specific cells are tightly packed at distances smaller than the wavelength of visible light. In addition, lens cells have degraded their organelles, such as mitochondria, and are therefore unable to carry out oxidative biochemical metabolism. Cellular nutrition and cell-cell adhesion rely on junctional microdomains in the cell membranes to connect the lens cells and their cytoplasms. These junctional microdomains in the lens cell plasma membrane contain gap junctions that ensure the transport of metabolites, ions, and water between cells, as well as thin junctions that are responsible for cell adhesion and eventually water transport. Gap junctions are formed by connexons (a complex composed of six connexin molecules),[1] whereas aquaporin-0 composes the thin junctions.[2] Mutations in both proteins result in the formation of a cataract.[3]

Since the development of atomic force microscopy (AFM),[4] dramatic improvements have been achieved in high-resolution imaging of reconstituted membranes,[5] proteins in crystalline lattices,[6] isolated native membranes,[7-9] and living prokaryotic[10] and eukaryotic cells.[11] In these studies, AFM is used as a tool that can provide structural information at sub-nanometer resolution on biological samples of interest. The use of this technique, however, remains restricted predominately to fundamental research, and concrete applications in medicine are sparse. In this application note, we demonstrate the utility of AFM in delineating the cause of cataracts. High-resolution imaging of native lens membranes and the constitutive protein components was achieved using a customized Veeco atomic force microscope.[12-14]

SAMPLE PREPARATION

Immediately after cataract surgery, the membranes were extracted from cataract debris, and pelleted by ultracentrifugation. The membrane solution was injected into a droplet of adsorption buffer (10 mM Tris-HCl pH 7.4, 150 mM KCl, 25 mM MgCl2) on top of a freshly cleaved mica sheet. After incubation, the sample was rinsed using recording buffer (10 mM Tris-HCl pH 7.4, 150 mM KCl).[13]

Imaging was performed on healthy[12] and cataract[13] lens cell membranes using a customized Veeco NanoScope™ E AFM equipped with a 130 µm J-scanner and Olympus Si3N4 (length = 100 µm; k = 0.09 N/m). The loading force was ~100 pN and the scan rate was 4–7 Hz. See references 12 and 13 for further sample preparation details.

RESULTS

AFM images of cataract membranes revealed lipid bilayer cell membranes adsorbed to the mica support. These membranes contained protein domains identified as junctional microdomains that connect adjacent lens cells. The microdomains were significantly larger in cataract membranes than those observed in membranes from healthy cells. The cataract membrane junctional microdomains were found to be composed exclusively of AQP0 transmembrane channel proteins. Image resolution was sufficient to allow identification of individual helix-connecting loops, which protrude from the membrane surface, of about four amino acids in length; and these data coincide closely with predicted models. The sub-nanometer resolution of these features was extracted from topographical images and compared to previously published data on healthy sheep lens cell membranes.[12]

A systematic structural comparison between healthy and cataract lens membranes revealed that, in healthy lens cells, AQP0 molecules are well organized in small dense patches surrounded and confined by connexons. In stark contrast, the cataract lens membranes did not contain connexons (see figure 1). As a consequence, junction arrays appeared significantly enlarged and malformed in the membranes of cataract lens cells.

It would seem that the connexons are progressively degraded during cataract development, ultimately leading to a breakdown of lens cell nutrition. From a physiological point of view, in a healthy lens cell the supramolecular assembly of AQP0 and connexons is required for cell adhesion through junction formation, as well as normal ion, metabolite, and water flow between adjacent cells through gap junctions. Moreover, the homogeneous distribution of smaller junctional microdomains allows a better connection between neighbor cells, decreasing the probability of non-adhering membrane areas. In contrast, the absence of connexons from the membranes of cataract lens cells results in a heterogeneous distribution of the adhering/non-adhering membrane areas. Finally, nutrients and ions are not delivered to cells deep inside the lens and waste products accumulate (see figure 2). These cells will become unhealthy and will not be able to maintain transparency, ultimately leading to blindness.

Junctional microdomain in healthy lens membranes

Junctional microdomain in cataract lens membranes

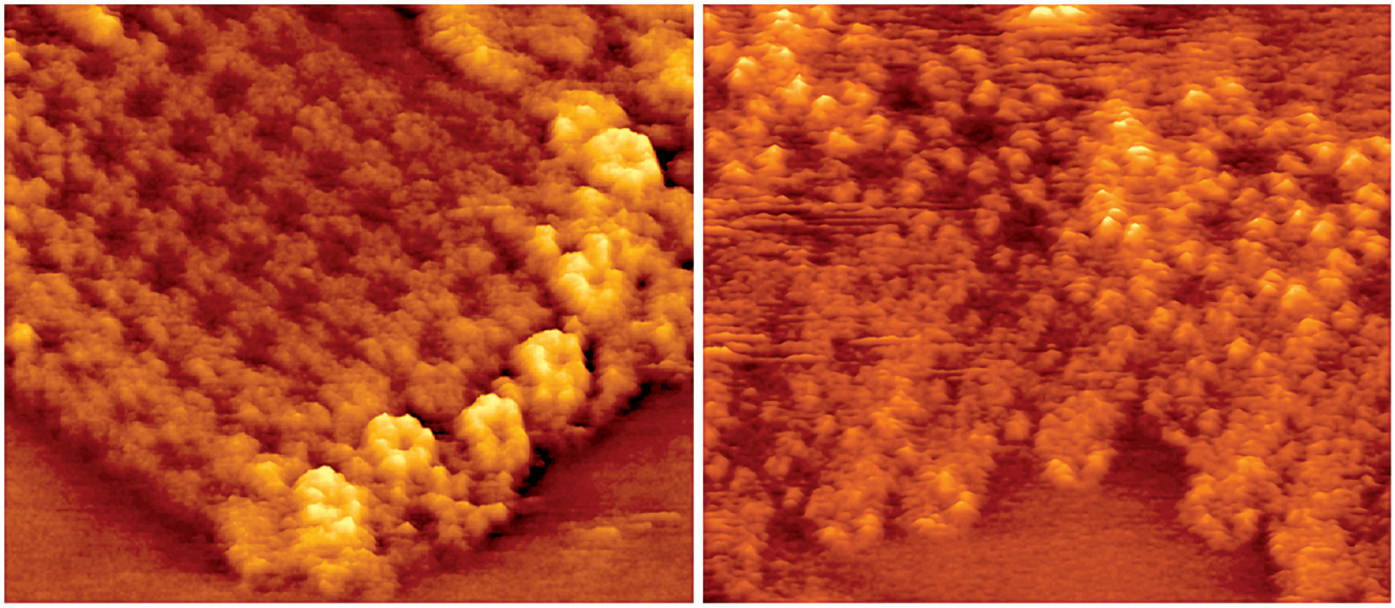

Figure 1: Contact mode high-resolution AFM topography images showing substructure on individual transmembrane channels in healthy sheep (left) and human cataract (right) lens cell membranes. In the healthy case, AQP0 molecules (cross-shaped tetrameric proteins with a diameter of 6nm) form small and regular patches edged by connexons (flower-shaped hexameric proteins with a diameter of 8nm) that delimit the AQP0 microdomains. In the pathological case, connexons are lacking.

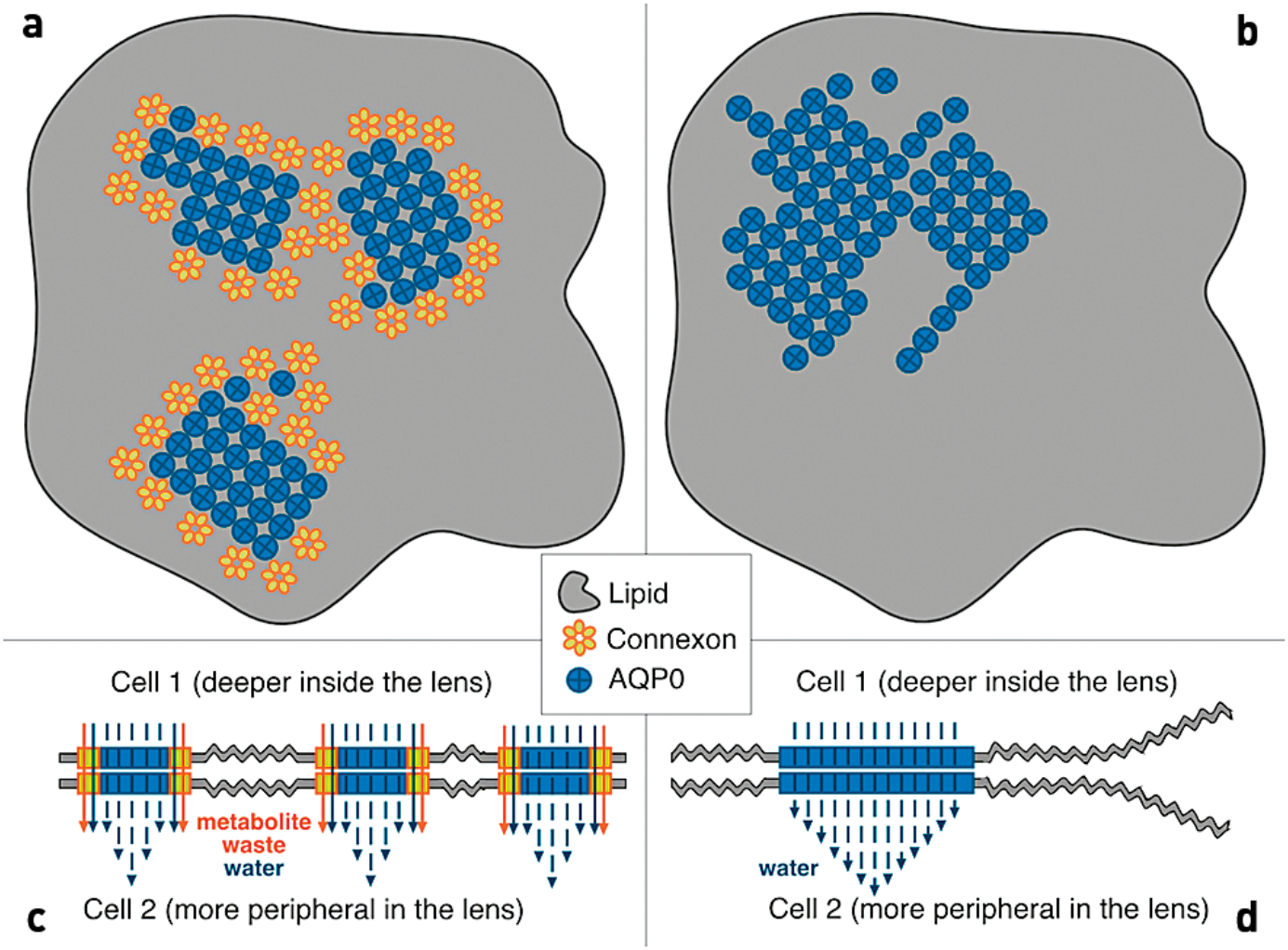

Figure 2: Representation of the structural differences between healthy (a) and cataract (b) membranes. In healthy membranes, themomogeneous distribution of contact areas ensures normal communication between neighboring cells (c) whereas in cataract membranes, the lack of connexons results in abnormal cell-to-cell adhesion (d). Furthermore, the absence of connexons in the unhealthy tissue results in cellular starvation and waste accumulation.

CONCLUSION

High-resolution AFM imaging provides an ideal means to investigate the structural differences between healthy and cataract lens cell membranes. This is a very promising result in the ongoing push to utilize SPM technology in the investigation of disease causes at the molecular level. AFM has an established capability to analyze individual molecules. Since it is now well accepted that many pathologies originate from molecular disorders, it can be expected that the AFM technique will become increasingly important in medical imaging in the near future.

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