Barrier Function in the Ocular Surface: From Conventional Paradigms to New Opportunities

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ABSTRACT By providing a physical and immunological barrier, the ocular surface serves the important function of protecting the vision apparatus. The barrier function keeps the cornea relatively dehydrated, preserving transparency for transmission of light. In many instances, dysfunction of this barrier leads to clinical diseases, such as dry eye, infectious keratitis, allergic keratoconjunctivitis, chemical injury, and persistent epithelial defects. Herein, we review the components of the epithelial barrier in the ocular surface, i.e., the transcellular and paracellular pathways, and describe the methodologies for measuring barrier function in vitro, in animals, and in clinical studies. The usefulness and limitations of these techniques are discussed. Recent studies in the regulation of individual tight junction proteins, the occludins, the zonula occludens, and the claudins, are also reviewed. Several potential interventional strategies based on the knowledge gained from these studies are noted.

KEY WORDS barrier function, cornea, dry eye, epithelium, inflammation, signaling, tight junctions

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I. INTRODUCTION

The ocular surface provides a barrier to noxious stimuli, thus protecting the vision apparatus.[1] In addition, the barrier function keeps the cornea relatively dehydrated and hence preserves transparency for transmission of light.[2] Dysfunction of this barrier leads to disease. Clinical diseases that involve barrier impairment include dry eye, infectious keratitis, allergic keratoconjunctivitis, chemical injury, and persistent epithelial defects. These collectively contribute to a very significant portion of ocular morbidity and cornea-related blindness.

II. DEFINITION OF BARRIER FUNCTION AND COMPONENTS OF BARRIER

We define barrier function as *the permeability of the cornea and conjunctival epithelium to water and major solutes including ions*. Loss of barrier may also allow passage of larger entities, such as pathogens. This permeability is determined by the epithelial and subepithelial components. In the corneal epithelium, there are two major pathways that may potentially provide passage of water and solutes. First, epithelial components, such as mucin layer and glycocalyx,[3] would potentially be a barrier to transcellular transport. Second, there is the paracellular pathway, which is composed of tight junctional complexes consisting of predominantly the proteins occludins, zonula occludens, and claudins.[4] Additionally, a relatively acellular epithelial basement membrane serves as an important barrier in the cornea.[5]

The measurement of barrier function has important applications in medical practice and research. It plays a role in toxicity and environmental studies.[6,7] It is a major functional outcome in ocular surface inflammation and wound healing research, for example, in dry eye-related studies.[1,8-10] The epithelial barrier has to be overcome for efficient drug delivery to deeper structures of the eye.[11,12] Finally, the development of junctional complexes is an important parameter for the differentiation and function of artificial corneal constructs in tissue engineering.[13-15]

III. TRANSCELLULAR PERMEABILITY

On the apical surfaces of epithelial cells, cell surface glycans bind carbohydrate-binding proteins to promote

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formation of highly dynamic complexes that receive exogenous signals. Examples of such protective molecules are heavily O-glycosylated transmembrane mucins MUC1, MUC4, and MUC16. These important components of cellular membrane are increasingly recognized as a shield that not only modifies the response of epithelial cells to pathological processes, but also limits the penetration of drugs.

In stratified human corneal epithelial cell cultures, it has been shown that targeting MUC16 using siRNA increased adherence of *Staphylococcus aureus* bacteria, suggesting that mucins have the physiological role of preventing pathogen adhesion. Indeed, some bacteria can secrete proteases that induce MUC16 shedding, reducing the barrier for infection.[1] Nevertheless, the literature on transcellular transport in the ocular surface is very limited apart from those studies dealing with pathogens and externally applied molecules, such as drugs.

IV. FUNCTION OF TIGHT JUNCTION PROTEINS

Tight junctions are one of the four major types of cellular connections, the others being desmosomes, gap junctions, and adherens junctions. They may encircle epithelial cells like a circumferential belt and thereby attach epithelial cells to other neighboring cells. Transport of solute molecules in the paracellular pathway may be multi-stepped and even involve specific regulated changes to the tight junction proteins and cytoskeletal reorganization, with consequent widening of the intercellular space.

Inflammatory signaling has been found to be responsible for alterations of the corneal epithelial barrier.[16] The tight junction complex is a highly dynamic structure. In the ocular surface, disturbances have been reported in the transcript and protein levels of the individual tight junction proteins, as well as their subcellular localization. The spatial expression of the tight junction proteins differ in different parts of the stratified corneal epithelium. For instance, occludin, zonula occludens-1 (**ZO-1**) and zonula occludens-2 (**ZO-2**) were found to be at the cell borders of the superficial layer, whereas claudin-1 was localized mainly in the basal and wing cell layers of rat corneal epithelium.[17] The signaling pathways that lead to changes in specific tight junction proteins are described below (Figure 1).

A. Occludin

Occludin is a 65-kDa tetraspan integral membrane protein that contributes to the stability of the tight junction and maintains optimal barrier function. Tissue level of occludin can be modified at both post-transcriptional and post-translational levels. Notably, these events have affected tight junction dynamics and epithelial homeostasis. Under the influence of oxidative processes, occludin can self-oligomerize or combine with other molecules to form tight junction strands.

Occludin can be regulated by phosphorylation at multiple sites. On phosphorylation of occludin within a highly conserved motif (**Y**ETD**Y**TT) containing tyrosines, there may be disruption of binding to ZO-1 and ZO-2/3, leading to separation of occludin from the membrane tight junctions. These processes cause barrier dysfunction, oxidative stress, and damage in human colorectal adenocarcinoma cells or in lung epithelial cells exposed to cigarette smoke.[18,19]

The role of matrix metalloproteinases (**MMP**s) in the regulation of occludins in the ocular surface has been well documented. The processes may be dependent on galectin[20] or regulated by the extracellular MMP-inducer EMMPRIN.[21] It has been reported that under low-humidity conditions, the c-jun N-terminal kinase 2 (**JNK2**) pathway can trigger occludin dysfunction through MMPs. In this situation, proinflammatory cytokines such as interleukin (**IL**)-1α and IL-1β, as well as the cornified envelope proteins involucrin and the small proline-rich protein-2a, may also be involved.[22] A study by Pelegrino et al suggests that occludin may have a bearing on dry eye disease in humans.[22] Consistent with this study, Setala et al found that in experimentally induced dry eye in mice, occludin expression was decreased and epithelial barrier was impaired.[23] In such a model, the phospholipid transfer protein may be protective for the barrier, as knocking out this molecule worsens the loss of barrier after dry eye induction.[23]

Through affecting the expression of occludin, vitamin D is critically involved in the remodeling of the cornea after injury. Yin et al found an increase in epithelial barrier and occludin expression when serum-starved corneal epithelial cells were stimulated with vitamin D metabolites 25(OH)D_3 and its active metabolite 1,25$(OH)_2D_3$.[24] The authors found that vitamin D receptors and the 1α-hydroxylase enzyme were present in corneal epithelial cells and may be important for barrier function.[24] Previously, Suzuki et al had reported that administration of topical 1,25$(OH)_2D_3$ suppressed inflammation by inhibiting Langerhan cell migration into diseased corneas.[25] It is not certain if the vitamin D effects act via differentiation pathways; nevertheless, enhancing barrier function by vitamin D supplementation is a potentially beneficial therapeutic strategy in ocular surface disease.