**Abstract**

**PURPOSE:** The combination of the tight-junction transmembrane protein claudin subtypes is one of the most important determinants of variations in the tightness of individual paired tight junction strands. The barrier function of corneal epithelium is much stronger than that of conjunctival epithelium. In this study, the expression and cellular distribution of claudin species in *in vivo* human corneal and conjunctival epithelium were investigated.

**METHODS:** Reverse transcription-polymerase chain reaction was used to reveal the claudin mRNA. Immunohistochemistry was used to determine the tissue distribution of tight junction-related proteins and MUC5AC.

**RESULTS:** The transcripts for claudin-1, -2, -3, -4, -7, -9, and -14 were identified in human corneal epithelium. The transcripts for claudin-1, -2, -4, -7, -9, -10, and -14 were identified in human conjunctival epithelium. By immunohistochemistry, claudin-1, -4, and -7 were found to be localized at the membrane of human corneal and conjunctival epithelial cells. In human conjunctival epithelium, claudin-10 staining was observed at several, but not all, apical epithelial cell-to-goblet cell junctions.

**CONCLUSIONS:** Claudin-1, -4, and -7 are expressed in both corneal and conjunctival epithelia. Claudin-10 is prominent at several junctions between apical epithelial cells and goblet cells in conjunctival epithelium. Except for the claudin-10 expression in conjunctival epithelium, the claudin subtype expression of corneal and conjunctival epithelium is similar. Therefore, there must be a difference between these two epithelium types in regards to the specific ratio of claudin subtypes that are expressed or their phosphorylation status, and that the distribution of goblet cells in conjunctival epithelium also influences the difference in barrier function.

## INTRODUCTION

Corneal and conjunctival epithelium, which construct the ocular surface, form a barrier that isolates the eye from the outside environment and regulate the passive movement of fluid, electrolytes, macromolecules, and cells through the paracellular pathway. Tight junctions in the epithelium create this barrier, and together, the cornea and conjunctiva work to form this important defense for the eye. However, it is well known that the tightness of the corneal epithelium tight junction is much greater than that of conjunctival epithelium.[1,2] Corneal epithelium is constructed by epithelial cells and has 5 or 6 layers of stratification. Conjunctival epithelium is also stratified squamous epithelium, but unlike corneal epithelium, it contains goblet cells. Goblet cells are located in the apical surface of the conjunctiva and interspersed among its multiple layers of stratified epithelium.

Tight junctions are present at the apical side of epithelia and play an important role in the establishment and maintenance of the barrier function and cell polarity. The tight junction is composed of three groups of proteins; transmembrane proteins (occludin, claudin, and junctional adhesion molecules), peripheral membrane proteins (ZO-1, Z-2, ZO-3, and MUPP-1) which have PDZ domains and bind to transmembrane proteins, and cytoplasmic proteins (cingulin, 7H6 antigen, etc.) that exist around tight junctions without any direct binding.[3]

Claudin (23kDa) is comprised of a family of transmembrane proteins that form the strands of the tight junction; 24 claudins have been identified thus far. Claudins are the only junctional proteins known to have tissue specificity. Both occludin and claudins contain four transmembrane domains, with both N and C termini oriented into the cytoplasm, but these two

proteins show no sequence similarity.[4,5,6] Different mixtures of claudins and occludin create tight-junction strands that are associated laterally with strands of adjacent cells, forming paired strands that eliminate extracellular space.[7]

We previously reported the distribution of ZO-1, occludin, and claudin in *in vivo* human corneal epithelium.[8] Immunohistochemistry has shown that in human corneal epithelium, most apical cells exhibit ZO-1, occludin, and claudin-1. By the reverse transcription-polymerase chain reaction (RT-PCR) method, the transcripts for claudin-1 and several other claudin isotypes, such as claudin-2, -3, -4, -7, -9 and -14, were identified from *in vivo* human corneal epithelium.

In this report, we examine the distribution of tight-junction proteins ZO-1, occludin, and claudins from *in vivo* human conjunctival epithelium, and try to identify the difference in the tight junction property of corneal and conjunctival epithelium.

## MATERIALS AND METHODS

### Tissue Preparation of the Human Corneas and Conjunctivas

The experiments conducted in this study used human corneal tissue supplied from the Northwest Lion Eye Bank (Seattle, WA) or that extirpated from patients with corneal stromal opacity diseases during penetrative keratoplasty surgery, therefore the corneal epithelium was intact. Human conjunctival tissue was obtained at the time of cataract or conjunctival chalasis surgery with proper informed consent of the patients. All experiments were done immediately after obtaining the tissue. The present study had the approval of the Nantan General Hospital ethics

committee and the procedures followed the Tenets of the Declaration of Helsinki.

**Primary Antibodies**

The rabbit anti-ZO-1 polyclonal antibody, rabbit anti-claudin-1 polyclonal antibody, rabbit anti-claudin-2 polyclonal antibody, rabbit anti-claudin-7 polyclonal antibody, rabbit anti-claudin-14 polyclonal antibody, and mouse anti-claudin-15 monoclonal antibody were purchased from Zymed Laboratories (South San Francisco, CA). The goat anti-occludin polyclonal antibody, goat anti-claudin-3 polyclonal antibody, goat anti-claudin-4 polyclonal antibody, goat anti-claudin-9 polyclonal antibody, and rabbit anti-claudin-10 polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse anti-Muc5AC monoclonal antibody was purchased from Abcam (Cambridge, United Kingdom). Alexa 488-labeled goat anti-rabbit antibody, Alexa 488-labeled rabbit anti-goat antibody, and Alexa 594-labeled goat anti-mouse antibody were purchased from Invitrogen Corporation (Carlsbad, CA).

**Immunohistochemistry**

Tight-junction associated proteins were studied via indirect immunohistochemistry. For the transverse images, 7-μm cryostat sections were placed on gelatin-coated slides, air dried, and then rehydrated in phosphate buffered saline (PBS) containing 1.0 mM $MgCl^2$ and 0.1 mM $CaCl^2$ at room temperature for 15 minutes. The sections were fixed with 95% ethanol at 4°C for 30 minutes, followed by 100% acetone at room temperature for 1 minute. After several washes with PBS, sections were incubated with 1% bovine serum albumin at room temperature for 30

minutes to block nonspecific binding. Following this, the sections were incubated at 4°C for 12 hours with primary antibody, and then washed three times in PBS for 15 minutes. For negative controls, the equivalent serum was used. After staining with the primary antibodies, the sections were incubated at room temperature for 1 hour with suitable secondary antibodies and washed several times with PBS.

For the en face images, whole corneas were fixed with 95% ethanol at 4°C for 30 minutes, followed by 100% acetone at room temperature for 1 minute. After several washes with PBS, tissues were permeabilized by incubation in PBS containing 0.1%Triton X-100 for 10 minutes. Tissues were developed to the first and second antibody steps, described above. During all steps, the epithelial side was kept facing upward to avoid damage. The tissues were coverslipped using anti-fading mounting containing diamidino-2-phenylindole for nuclei staining (Vector Laboratories, Burlingame, CA). The slides were examined and we took pictures every 0.5µm slice and merged several pictures by confocal microscope (TCS SP2 AOBS; Leica Microsystems GmbH, Wetzlar, Germany).

**RNA Isolation and RT-PCR Amplification of Claudin Species**

For RT-PCR, human corneal epithelial sheets peeled from donor corneal buttons and excised conjunctiva, were directly lysed with reagent (Trizol; GIBCO BRL, Rockville, MD); total cellular RNA was isolated according to the manufacturer's instructions. RT-PCR was performed in accordance with the previous report by Yi et al.[9] In brief, complementary DNA was generated in the presence of 0.5 µg oligo (dT) from 5 mg total RNA with reverse transcriptase (SuperScript II; Life Technologies, Rockville, MD). We used PCR primers that Yi et al. designed for human

claudins-1 through -4, -7, -9, -10, -14, and -15. PCR products were examined by 2% agarose gel and ethidium bromide staining. The observed PCR products corresponded to their expected molecular weights.

## RESULTS

### Distribution of ZO-1 and Occludin in Human Conjunctival Epithelium

In the transverse sections, occludin and ZO-1 were localized at the apical superficial epithelial cell tight junctions, as well as the epithelial-cell to goblet-cell tight junctions (Fig. 1A, C). In the en face sections, occludin and ZO-1 antibodies showed as bands that corresponded to the junctional complex (Fig. 1B, D). This occludin staining was different from that of the cornea, as it is not continuous and is presented in a dot-like pattern along the cell junctions.[8]

### Claudin Subtype Expression in Human Conjunctival Epithelial Cells Detected by RT-PCR

We previously reported that the transcripts for claudin-1 and several other claudin isotypes, such as claudin-2, -3, -4, -7, -9, and -14, were identified from human corneal epithelium.[8] In this study, we determined the presence of transcripts of claudin subtypes in human conjunctival epithelial cells by the same RT-PCR method. The transcripts for claudin-1, -2, -4, -7, -9, -10, and -14 were identified from conjunctival epithelium (Fig. 2). Claudin-3 was not identified in human conjunctival epithelium which existed in human corneal epithelium. On the other hand, claudin-10 was identified in conjunctival epithelium but not in corneal epithelium. No amplified claudin

mRNA was observed without reverse transcriptase (RT) (data not shown).

**Distribution of Claudins in Human Conjunctival and Corneal Epithelium**

Not all claudin subtypes of which transcripts were identified were expressed in conjunctival and corneal epithelium. The corneal epithelial cells through all cell layers were stained by claudin-1, -4, and -7. No staining was observed by claudin-2, -3, -9, -10, -14, and -15 (Fig. 3). In the en face images, claudin-1, -4, and -7 antibodies showed as bands that corresponded to the junctional complex (Fig. 4). In the conjunctival epithelium, claudin-1 and -4 staining was observed in all cell layers. Claudin-7 staining was observed in superficial cells (Fig. 5). In the en face images, those three claudin subtype antibodies showed as bands that corresponded to the junctional complex (Fig. 6). In addition, it was worth mentioning that some openings of goblet cells which were identified by positive reactivity with anti-MUC5 antibody showed claudin-10 staining (Fig. 5 and 6). No staining was observed by claudin-2, -3, -9, -14, and -15 (Fig. 6).

**DISCUSSION**

The ocular surface consists of corneal and conjunctival epithelia, both of which are stratified non-keratinized epithelium. The corneal epithelium is a transparent and flat stratified squamous epithelium devoid of goblet cells with a cuboid basal layer lying on the avascular corneal stroma by the Bowman's layer. The conjunctival epithelium is populated by goblet cells. Sequencing of mucin genes has led to the identification of two categories of mucins: secreted, and membrane associated. The conjunctival goblet cells express one of the secreted gel-forming mucins,

MUC5AC.[10] Like epidermis and other surface-lining mucosa, corneal and conjunctival epithelia serve as barriers of the ocular surface. This barrier is crucial for maintaining the homeostasis of fluid and solutes between the intraocular milieu and precorneal tear film. Although both corneal and conjunctival epithelia provide barrier functions at the ocular surface together, the barrier function of corneal epithelium is much stronger than that of conjunctival epithelium.[1,2] There are many differences between corneal and conjunctival proteome. For example, it is widely known that differentiated human corneal epithelial cells express cytokeratin 3 and cytokeratin 12. In addition, other cytokeratins, including cytokeratin 14 and cytokeratin 19, are expressed as minor components of the cytoskeleton in basal and/or suprabasal human corneal epithelial cells. On the other hand, conjunctival epithelium uniformly expresses cytokeratin 19, but not cytokeratin 12.[11] The distribution of alpha sub-chains of type IV collagen in the basement membrane is also different between corneal and conjunctival epithelium. The conjunctival basement membrane contains collagen alpha2(IV), but not collagen alpha5(IV). By contrast, in the corneal basement membrane, there is the collagen alpha5(IV), but not the collagen alpha2(IV). [12]

Tight junctions are present at the apical side of epithelia and play an important role in the establishment and maintenance of barrier function and cell polarity. The barrier characteristics of tight junctions vary considerably among different types of epithelium and endothelium depending on physiological requirements.[13]

Occludin (60kDa) was the first transmembrane protein identified at tight junctions,[14] but its precise cellular functions remain unclear. Occludin-deficient mice are viable; the tight junction ultrastructure appears unaltered, and isolated intestinal tissues demonstrate normal trans epithelial resistance (TER) and permeability to mannitol.[15, 16] However, blocking the

extracellular loops[17] and reducing the protein content of occludin[18] alter paracellular permeability in a number of cell systems. On the other hand, the function of occludin in regulating epithelial cell division has been suggested by the ability of exogenous occludin expression to revert the phenotype of raf-transformed rat salivary gland epithelial cells.[19]

Claudin (23 KDa) is comprised of a family of transmembrane proteins that form the strands of the tight junction.[4] Both occludin and claudins contain four transmembrane domains, with both N and C termini oriented into the cytoplasm, but these two proteins show no sequence similarity. Twenty-four claudins have been identified thus far. Sequence analysis of claudins has led to differentiation into two groups, designated as classic claudins (claudins 1-10, 14, 15, 17, 19) and non-classic claudins (claudins 11-13, 16, 18, 20-24), according to their degree of sequence similarity.[20]

Claudins are the only junctional proteins known to have tissue specificity. Different mixtures of claudins create tight junction strands that are associated laterally with strands of adjacent cells, thus forming paired strands that eliminate extracellular space. However, it has been postulated that ion-selective pores occur within paired tight junction strands.[5, 7, 21] All claudins have two extracellular loops. The first extracellular loop consists of ~ 50 amino acids with two conserved cysteines and the distribution of the charged amino acid residues in the first extracellular loop of claudins is crucial for determining the charge selectivity of the aqueous pores of tight junction strands.[22] The second extracellular loop usually has ~ 25 amino acids and may associate with itself and possess a holding function, narrowing the paracellular cleft.[23]

Claudin-13 has no human expressed sequence tags (ESTs), and most murine ESTs for claudin-13 are from embryonic DNA libraries, thus suggesting that these genes may not be expressed in adult tissues. Claudin-6 is developmentally restricted and not expressed in adult tissues.[24]

Claudin-11 has been found only in oligodendrocytes and Sertoli cells in the testis.[25] Morita et al. reported that claudin-5/TMVCF is only expressed in the endothelial cells of blood vessels.[26] Claudin-16/parecellin-1 is exclusively expressed in the thick ascending limb of Henle and might form aqueous pores that function as $Mg^{++}$ paracellular channels.[27] We eliminated those subtypes from our experiment.

The human corneal epithelial cells through all cell layers were stained by claudin-1, -4, and -7. No staining was observed by claudin-2, -3, -9, -10, -14, and -15. In the en face images, claudin-1, -4, and -7 antibodies showed as bands that corresponded to the junctional complex. In the human conjunctival epithelium, claudin-1 and -4 staining were observed in all cell layers. Claudin-7 staining was observed in superficial cells. In the en face images, those three claudin subtype antibodies showed as bands that corresponded to the junctional complex. In addition, it was worth mentioning that some openings of goblet cells showed claudin-10 staining. In the present study, we investigated the mRNA expression of claudins by RT-PCR and localization by immunofluorescence microscopy. There were discrepancies between mRNA and the protein expressions of claudins. The transcripts for claudin-1, -2, -3, -4, -7, -9, and -14 were identified from human corneal epithelium. The transcripts for claudin-1, -2, -4, -7, -9, -10, and -14 were identified from human conjunctival epithelium. There are several possibilities for these discrepancies including low levels of the translation of claudin mRNAs into proteins, rapid protein turnover, or low amounts of claudin proteins in tissues.

Claudin-1 is ubiquitous and common. In mammalian skin, continuous tight junctions circumscribing the keratinocytes of the granular cell layer were reproducibly identified and claudin-1 and -4 were concentrated in these tight junctions. Claudin-1-deficient mice were born alive, but died within one day of birth accompanied by excessive water loss from the skin.[28]

*Clostridium perfringens* enterotoxin (CPE) is a single polypeptide and can cause food poisoning in humans. Katahira et al. identified the receptor on the cell membrane for CPE (CPE-R).[29] Because of the significant sequence similarity for claudin-1 and -2, Morita et al. found that CPE-R was identical to claudin-4. [30] The treatment of the cells with C-CPE reduces the TER. [31] Claudin-4 has a tightening potential. Claudin-4 increased TER ~300% when expressed in low-resistance Madin-Darby canine kidney (MDCK) II cells and decreased the paracellular permeability for $Na^+$ more than $Cl^-$.[32] On the other hand, paracellular cation pores are formed by claudin-7 for $Na^+$. The mouth of the channel, which is constituted by extracellular domains of claudin-7 from opposing cells, is negatively charged and hinders $Cl^-$ entry while allowing $Na^+$ to go through.[33]

Claudin-10 expression in the inner ear, mouse prostate, most segments of nephron, endothelial cells of restricted blood vessels, colon epithelium, and exocrine glands has been reported.[34-37] In exocrine glands including the submandibular, sublingual, parotid, and lacrimal glands, claudin-10 was expressed along lateral membranes in addition to apical tight junction strands.[37]

As mentioned above, claudins are tight junction forming transmembrane proteins. Electron-microscopic freeze-fracture observation and horseradish peroxidase permeability study revealed that the tight junction exists only between superficial epithelial cells in corneal epithelial cells.[38,39] However, our study demonstrated the existence of claudins at all cell layers. There are several reports describing claudin proteins as being expressed not only at tight junctions, but also along the lateral membrane. For example, Claudin-1,-4, and -7 were localized along the lateral membrane in the airway epithelium.[40] Although the biological significance of the localization of claudin proteins in the lateral membrane is unknown, we speculate that since the surface of corneal and conjunctival epithelium is always exfoliating and since the turnover of the

epithelium is 7 to 10 days, claudin proteins might exist at the membrane that allows rapid formation of tight junction strands.

In conclusion, the results of our study showed that claudin-1, -4, and -7 were expressed in both corneal and conjunctival epithelia. We also found that claudin-10 was prominent at several junctions between apical epithelial cells and goblet cells in conjunctival epithelium. Since variations in the tightness of individual paired tight junction strands are determined by the combination of claudin species, and since the barrier function of corneal epithelium is much stronger than that of conjunctival epithelium, we speculated at the beginning of this experiment that the subtype expression in these two types of epithelium might be different. However, and except for the claudin-10 expression in conjunctival epithelium, the claudin subtype expression of corneal and conjunctival epithelium is similar. Therefore, we posit that there must be a difference between these two types of epithelium in regards to the specific ratio of claudin subtypes that are expressed or their phosphorylation status, and that the distribution of goblet cells in conjunctival epithelium and claudin-10 expression between epithelial cells and goblet cells also influence the difference in barrier function that exists between these two types of epithelium. The elucidation of claudin subtype expression in specific tissue is an important first step for developing a strategy for regulating drug absorption or for preventing some diseases. In this report, we demonstrated the claudin subtype expression in corneal and conjunctival epithelium. Further investigations into the regulation of the pores which are made by those claudins will be required.

**Acknowledgments**

The authors thank Chikako Mochida for technical assistance and John Bush for editing the manuscript.

References

1. Huang AJ, Tseng SC, Kenyon KR. Paracellular permeability of corneal and conjunctival epithelia. *Invest Ophthalmol Vis Sci* 1989;30(4):684-689.
2. Prausnitz MR, Noonan JS. Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. *J Pharm Sci* 1998;87(12):1479-1488.
3. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol* 2001;2(4):285-293.
4. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol* 1998;141(7):1539-1550.
5. Morita K, Furuse M, Fujimoto K, Tsukita S. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc Natl Acad Sci U S A* 1999;96(2):511-516.
6. Tsukita S, Furuse M. Occludin and claudins in tight-junction strands: leading or supporting players? *Trends Cell Biol* 1999;9(7):268-273.
7. Furuse M, Furuse K, Sasaki H, Tsukita S. Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. *J Cell Biol* 2001;153(2):263-272.
8. Ban Y, Dota A, Cooper LJ, et al. Tight junction-related protein expression and distribution in human corneal epithelium. *Exp Eye Res* 2003;76(6):663-669.
9. Yi X, Wang Y, Yu FS. Corneal epithelial tight junctions and their response to lipopolysaccharide challenge. *Invest Ophthalmol Vis Sci* 2000;41(13):4093-4100.
10. Gipson IK. Distribution of mucins at the ocular surface. *Exp Eye Res* 2004;78(3):379-388.
11. Kasper M, Moll R, Stosiek P, Karsten U. Patterns of cytokeratin and vimentin expression in the human eye. *Histochemistry* 1988;89(4):369-377.
12. Fukuda K, Chikama T, Nakamura M, Nishida T. Differential distribution of subchains of the basement membrane components type IV collagen and laminin among the amniotic membrane, cornea, and conjunctiva. *Cornea* 1999;18(1):73-79.
13. Powell DW. Barrier function of epithelia. *Am J Physiol* 1981;241(4):G275-288.
14. Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S. Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol* 1993;123(6 Pt 2):1777-1788.
15. Saitou M, Furuse M, Sasaki H, et al. Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol Biol Cell* 2000;11(12):4131-4142.
16. Schulzke JD, Gitter AH, Mankertz J, et al. Epithelial transport and barrier function in occludin-deficient mice. *Biochim Biophys Acta* 2005;1669(1):34-42.
17. Nusrat A, Brown GT, Tom J, et al. Multiple protein interactions involving proposed extracellular loop domains of the tight junction protein occludin. *Mol Biol Cell* 2005;16(4):1725-1734.
18. Yu AS, McCarthy KM, Francis SA, et al. Knockdown of occludin expression leads to diverse phenotypic alterations in epithelial cells. *Am J Physiol Cell Physiol* 2005;288(6):C1231-1241.

19. Wang Z, Mandell KJ, Parkos CA, Mrsny RJ, Nusrat A. The second loop of occludin is required for suppression of Raf1-induced tumor growth. *Oncogene* 2005;24(27):4412-4420.
20. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE. Structure and function of claudins. *Biochim Biophys Acta* 2008;1778(3):631-645.
21. Goodenough DA. Plugging the leaks. *Proc Natl Acad Sci U S A* 1999;96(2):319-321.
22. Colegio OR, Van Itallie C, Rahner C, Anderson JM. Claudin extracellular domains determine paracellular charge selectivity and resistance but not tight junction fibril architecture. *Am J Physiol Cell Physiol* 2003;284(6):C1346-1354.
23. Piontek J, Winkler L, Wolburg H, et al. Formation of tight junction: determinants of homophilic interaction between classic claudins. *Faseb J* 2008;22(1):146-158.
24. Turksen K, Troy TC. Barriers built on claudins. *J Cell Sci* 2004;117(Pt 12):2435-2447.
25. Morita K, Sasaki H, Fujimoto K, Furuse M, Tsukita S. Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. *J Cell Biol* 1999;145(3):579-588.
26. Morita K, Sasaki H, Furuse M, Tsukita S. Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells. *J Cell Biol* 1999;147(1):185-194.
27. Simon DB, Lu Y, Choate KA, et al. Paracellin-1, a renal tight junction protein required for paracellular Mg2+ resorption. *Science* 1999;285(5424):103-106.
28. Furuse M, Hata M, Furuse K, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* 2002;156(6):1099-1111.
29. Katahira J, Inoue N, Horiguchi Y, Matsuda M, Sugimoto N. Molecular cloning and functional characterization of the receptor for Clostridium perfringens enterotoxin. *J Cell Biol* 1997;136(6):1239-1247.
30. Sonoda N, Furuse M, Sasaki H, et al. Clostridium perfringens enterotoxin fragment removes specific claudins from tight junction strands: Evidence for direct involvement of claudins in tight junction barrier. *J Cell Biol* 1999;147(1):195-204.
31. Kondoh M, Masuyama A, Takahashi A, et al. A novel strategy for the enhancement of drug absorption using a claudin modulator. *Mol Pharmacol* 2005;67(3):749-756.
32. Van Itallie C, Rahner C, Anderson JM. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. *J Clin Invest* 2001;107(10):1319-1327.
33. Alexandre MD, Lu Q, Chen YH. Overexpression of claudin-7 decreases the paracellular Cl- conductance and increases the paracellular Na+ conductance in LLC-PK1 cells. *J Cell Sci* 2005;118(Pt 12):2683-2693.
34. Sakai N, Chiba H, Fujita H, et al. Expression patterns of claudin family of tight-junction proteins in the mouse prostate. *Histochem Cell Biol* 2007;127(4):457-462.
35. Kitajiri SI, Furuse M, Morita K, et al. Expression patterns of claudins, tight junction adhesion molecules, in the inner ear. *Hear Res* 2004;187(1-2):25-34.
36. Kiuchi-Saishin Y, Gotoh S, Furuse M, Takasuga A, Tano Y, Tsukita S. Differential expression patterns of claudins, tight junction membrane proteins, in mouse nephron segments. *J Am Soc Nephrol* 2002;13(4):875-886.
37. Inai T, Sengoku A, Guan X, Hirose E, Iida H, Shibata Y. Heterogeneity in expression and subcellular localization of tight junction proteins, claudin-10 and -15,

examined by RT-PCR and immunofluorescence microscopy. *Arch Histol Cytol* 2005;68(5):349-360.

38. McLaughlin BJ, Caldwell RB, Sasaki Y, Wood TO. Freeze-fracture quantitative comparison of rabbit corneal epithelial and endothelial membranes. *Curr Eye Res* 1985;4(9):951-961.

39. Tanaka M, Ohnishi Y, Kuwabara T. Membrane structure of corneal epithelium: freeze-fracture observation. *Jpn J Ophthalmol* 1983;27(3):434-443.

40. Coyne CB, Gambling TM, Boucher RC, Carson JL, Johnson LG. Role of claudin interactions in airway tight junctional permeability. *Am J Physiol Lung Cell Mol Physiol* 2003;285(5):L1166-1178.

**Figure legends**

**Figure 1. Distribution of ZO-1 and occludin in human conjunctival epithelium by immunofluorescence staining.** In the transverse sections, ZO-1 (A) and occludin (C) were localized at the apical superficial epithelial-cell tight junctions, as well as the epithelial-cell to goblet-cell tight junctions. In the en face images, ZO-1 (B) and occludin (D) antibodies showed as bands that corresponded to the junctional complex. Red: goblet cells labeled by mucin staining with anti-MUC5AC antibody. Blue: nuclear counterstaining. Scale bar: 50μm.

**Figure 2. Claudins expressed in human corneal and conjunctival epithelial cells detected by RT-PCR.** The transcripts for claudin-1, -2, -3, -4, -7, -9 and -14 were identified from human corneal epithelium. The transcripts for claudin-1, -2, -4, -7, -9, -10, and -14 were identified from conjunctival epithelium. M lane: molecular weight marker, Bar: 500 bp.

**Figure 3. Claudin subtype expression in human corneal epithelium in the transverse sections by immunofluorescence staining.** The corneal epithelial cells through all cell layers were stained by claudin-1, -4, and -7. No staining was observed by claudin-2, -3, -9, -10, -14, and -15. Blue: nuclear counterstaining. Scale bar: 50μm.

**Figure 4. Distribution of Claudin-1, -4, and -7 in human corneal epithelium in the en face images by immunofluorescence staining.** Claudin-1, -4, and -7 antibodies showed as bands that corresponded to the junctional complex. Blue: nuclear counterstaining. Scale bar: 50μm.

**Figure 5. Claudin subtype expression in human conjunctival epithelium in the transverse sections by immunofluorescence staining.** Claudin-1 and -4 staining in all cell layers of human conjunctival epithelium were observed. Claudin-7 staining was observed in superficial cells. Some openings of goblet cells showed claudin-10 staining (arrow). Red: goblet cells labeled by mucin staining with anti-MUC5AC antibody. Blue: nuclear counterstaining. Scale bar: 50μm.

**Figure 6. Claudin subtype expression in human conjunctival epithelium in the en face images by immunofluorescnece staining.** Claudin-1, -4, and -7 antibodies showed as bands that corresponded to the junctional complex. In addition, some openings of goblet cells showed claudin-10 staining (arrow). No staining was observed by claudin-2, -3, -9, -14, and -15. Red: goblet cells labeled by mucin staining with anti-MUC5AC antibody. Blue: nuclear counterstaining. Scale bar: 50μm.

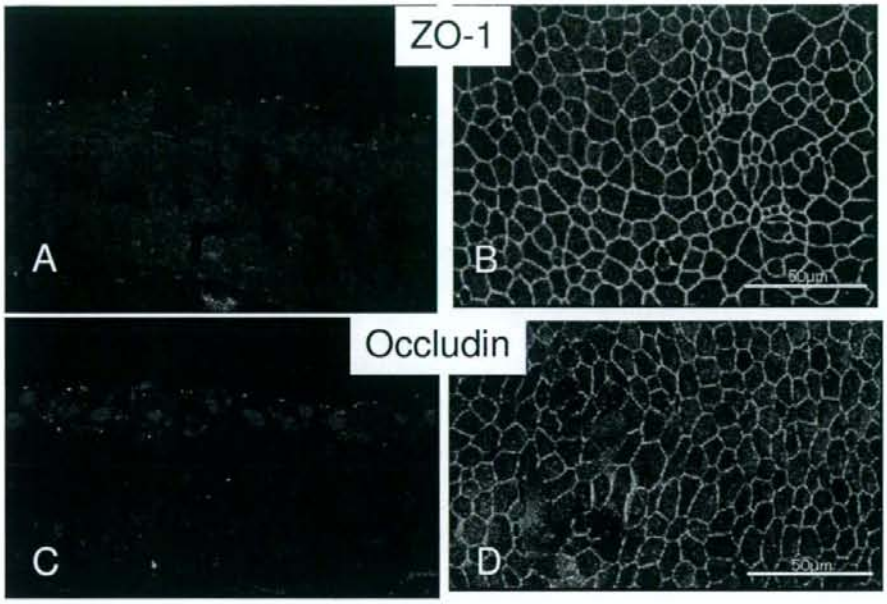


Figure 1

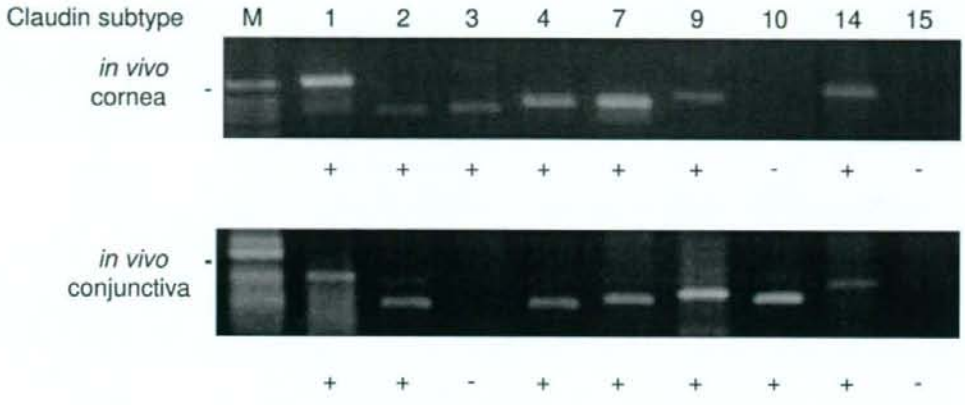
Claudin subtype
M
1
2
3
4
7
9
10
14
15
in vivo
cornea
+ + + + + + - + -
in vivo
conjunctiva
+ + - + + + + + -

**Figure 2**