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Expression pattern of adhesion molecules in junctional epithelium differs from that in other gingival epithelia

S. Hatakeyama[1], T. Yaegashi[2], Y. Oikawa[1], H. Fujiwara[2], T. Mikami[1], Y. Takeda[1], M. Satoh[1]
Departments of [1]Oral Pathology and [2]Periodontology, School of Dentistry, Iwate Medical University, Morioka, Japan

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Background and Objective: The gingival epithelium is the physiologically important interface between the bacterially colonized gingival sulcus and periodontal soft and mineralized connective tissues, requiring protection from exposure to bacteria and their products. However, of the three epithelia comprising the gingival epithelium, the junctional epithelium has much wider intercellular spaces than the sulcular epithelium and oral gingival epithelium. Hence, the aim of the present study was to characterize the cell adhesion structure in the junctional epithelium compared with the other two epithelia.

Material and Methods: Gingival epithelia excised at therapeutic flap surgery from patients with periodontitis were examined for expression of adhesion molecules by immunofluorescence.

Results: In the oral gingival epithelium and sulcular epithelium, but not in the junctional epithelium, desmoglein 1 and 2 in cell–cell contact sites were more abundant in the upper than the suprabasal layers. E-cadherin, the main transmembranous molecule of adherens junctions, was present in spinous layers of the oral gingival epithelium and sulcular epithelium, but was scarce in the junctional epithelium. In contrast, desmoglein 3 and P-cadherin were present in all layers of the junctional epithelium as well as the oral gingival epithelium and sulcular epithelium. Connexin 43 was clearly localized to spinous layers of the oral gingival epithelium, sulcular epithelium and parts of the junctional epithelium. Claudin-1 and occludin were expressed in the cell membranes of a few superficial layers of the oral gingival epithelium.

Conclusion: These findings indicated that the junctional epithelium contains only a few desmosomes, composed of only desmoglein 3; adherens junctions are probably absent because of defective E-cadherin. Thus, the anchoring junctions connecting junctional epithelium cells are lax, causing widened intercellular spaces. In contrast, the oral gingival epithelium, which has a few tight junctions, functions as a barrier.

Setsuko Hatakeyama, PhD, Department of Oral Pathology, School of Dentistry, Iwate Medical University, 19-1 Uchimaru, Morioka 020-8505, Japan
Tel: +81 19 651 5111
Fax: +81 19 621 3321
e-mail: hsetsuko@iwate-med.ac.jp

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The epithelium adjacent to a tooth can be classified into three anatomical types: the oral gingival epithelium; the sulcular epithelium; and the junctional epithelium (1). The oral gingival epithelium is composed of a keratinizing stratified epithelium and covers the external surface of the gingiva, while the sulcular epithelium is a

nonkeratinizing epithelium that lines the inner aspect of the gingival sulcus. In contrast, the junctional epithelium is structurally and functionally unique. Namely, the junctional epithelium is located at a strategically important interface between the gingival sulcus and the underlying soft and mineralized connective tissues of the periodontium (2), contains a nonkeratinizing epithelial layer at the free surface, and has wider intercellular spaces. These structures may thereby provide a pathway for fluid and transmigrating leukocytes from the gingival connective tissue to the gingival sulcus (3,4), and even for micro-organisms moving in the opposite direction (5,6). However, recent research suggests that the junctional epithelium is a critical structure contributing to periodontal host defenses against infection (7). Thus, characterization of the adhesion structures that support the multilayered architecture of the junctional epithelium, and that create wide intercellular spaces, is an important step in understanding the normal functioning of the junctional epithelium.

In general, cell–cell junctions are classified into three functional groups: occluding junctions (tight junctions); communicating junctions (gap junctions); and anchoring junctions at cell–cell contact sites (8–10). Desmosomes and adherens junctions act as anchoring adhesion structures in various tissues, including the stratified epithelium. Thus, in the present study, immunohistochemical localization of the cell adhesion molecules constituting desmosomes and adherens junctions within the oral gingival epithelium, sulcular epithelium, and junctional epithelium was examined. Connexin 43 [the main gap junction-associated protein (11)] and claudin-1 and occludin [major tight-junction-associated proteins (12)] were also studied.

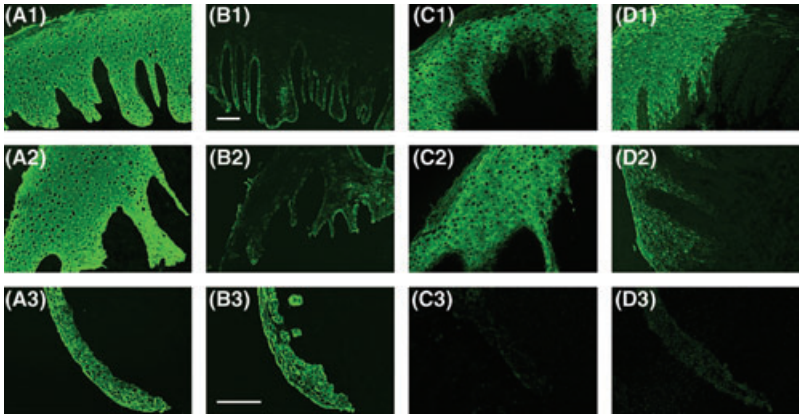

Fig. 1. Expression patterns of keratin 14 (A1, A2, A3; green), keratin 19 (B1, B2, B3; green), keratin 13 (C1, C2, C3; green) and keratin 4 (D1, D2, D3; green) in the gingival epithelium. Oral gingival epithelium, A1, B1, C1, D1; sulcular epithelium, A2, B2, C2, D2; junctional epithelium, A3, B3, C3, D3. Keratin 14 is expressed in all cells of the oral gingival epithelium (A1), sulcular epithelium (A2) and junctional epithelium (A3). Keratin 19 is present in the basal cells of the oral gingival epithelium (B1) and sulcular epithelium (B2) and in all cells of the junctional epithelium (B3). Keratin 13 is present to a greater degree in the upper layers than in the suprabasal cells in the oral gingival epithelium (C1) and sulcular epithelium (C2), but is not present in the junctional epithelium (C3). Keratin 4 is present in the marginal portion of the oral gingival epithelium (D1) and sulcular epithelium (D2), but not in the junctional epithelium (D3). Bar, 100 μm. Panels A1, A2, B1, B2, C1, C2, D1 and D2 are at the same magnification and A3, B3, C3 and D3 are at the same magnification.

Materials and methods

Tissue samples

Samples of gingival tissues were obtained from adult patients with periodontitis (six men and eight women,

Table 1. Immunofluorescence of cell-adhesion molecules in human gingival epithelia

	Molecules	Junctional epithelium	Sulcular epithelium	Oral gingival epithelium
Occluding junctions				
Tight junction	claudin-1	(−)	(−)	(+) uppermost layers
	occludin	(−)	(−)	(+) uppermost layers
Anchoring junctions				
Adherens junction	actin	(±)	(+)	(+)
	E-cadherin	(−)	(+)	(+)
	P-cadherin	(+) all layers	(+) only basal layer	(+) only basal layer
	α-catenin	(+)	(+)	(+)
Desmosome	keratin 19	(+) all layers	(+) only basal layer	(+) only basal layer
	keratin 14	(+) all layers	(+) all layers	(+) all layers
	keratin 13	(−)	(+) prickle cells	(+) prickle cells
	kerain 4	(−)	(+)	(−)
	desmoglein 1 & 2	(−)	(+) prickle cells	(+) prickle cells
	desmoglein 3	(+) all layers	(+)	(+)
	γ-catenin	(+)	(+)	(+)
Communicating junctions				
Gap junction	Connexin 43	(±)	(+) prickle cells	(+) prickle cells

(+), positive; (±), weakly positive; (–), negative.

mean age 56.2 ± 6.1 years, range: 48–66 years) undergoing a flap operation at the Dental Hospital of Iwate Medical University. Written informed consent was obtained from all patients prior to the operation, and all protocols were approved by the Ethical Committee of Iwate Medical University. All patients had a detailed clinical record and radiographs, and tissue samples were obtained from various sites. Excised samples were immediately cut along the vertical axis of the tooth and embedded in OCT Compound (Tissue-Tek, Sakura Finetechnical Co. Ltd, Tokyo, Japan) before being snap-frozen in acetone solution cooled with dry ice, and 5-μm sections were cut.

Immunofluorescence

We examined the following molecules: (i) the desmosome-associated proteins (desmoglein 1, desmoglein 3 and γ-catenin) and the intracellular cytoskeletal keratins; and (ii) the adherens junction-associated proteins (E-cadherin, P-cadherin and α-catenin) and the intracellular cytoskeletal actin. Single and double immunoreactions were carried out using monoclonal mouse antibodies and polyclonal rabbit or goat antibodies. Sections were fixed in ethanol before immunoreaction with the primary antibody against keratin 4 (6B10; Progen, Heidelberg, Germany; diluted 1 : 50), keratin 13 (MIC7; Cappel, Aurora, OH, USA; diluted 1 : 50), keratin 14 (IL002; Yelm, Rome, Italy; diluted 1 : 50), keratin 19 (RCK108; Dako, Tokyo, Japan; diluted 1 : 50), desmoglein 1 (DG3.10; Progen; diluted 1 : 50), desmoglein 3 (Santa Cruz Inc., Santa Cruz, CA, USA; diluted 1 : 50), or γ-catenin (Santa Cruz; diluted 1 : 50) for 10 min at 4°C. Other sections were fixed in 4% paraformaldehyde for immunoreaction with primary antibodies against E-cadherin (G-10; Santa Cruz; diluted 1 : 50), P-cadherin (Santa Cruz; diluted 1 : 50), α-catenin (Santa Cruz; diluted 1 : 100), claudin-1 (Santa Cruz; diluted 1 : 50), occludin (Santa Cruz; diluted 1 : 50) and connexin 43 (D-17; Santa Cruz), for 15 min at 4°C. Incubation with primary antibodies was performed for 2 h at room temperature. Then, after washing (three times for 10 min each) in phosphate-buffered saline (PBS) containing Tween 20 (PBST), sections were incubated with a 1 : 100 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse anti-body (Kirrkegaard & Petty Laboratories Inc., Gaithersburg, MD, USA), Alexa Fluor 488®-conjugated goat anti-rabbit immunoglobulin G (IgG), or Alexa Fluor 594®-labeled donkey anti-goat IgG (Molecular Probes Inc., Eugene, OR, USA) for 1 h at room temperature. Sections were

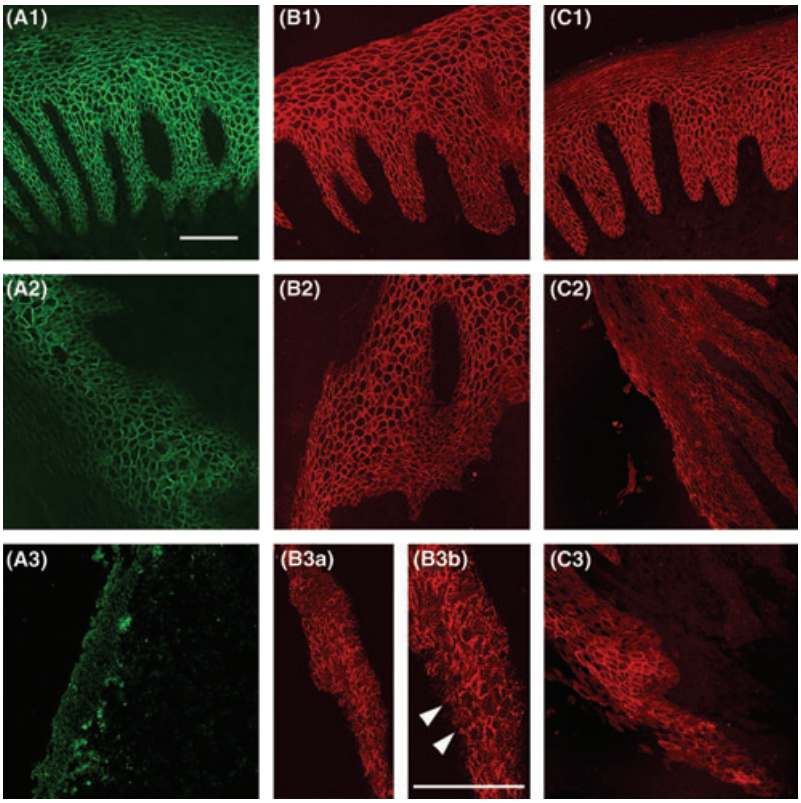

Fig. 2. Expression patterns of desmoglein 1 and 2 (A1, A2, A3; green), desmoglein 3 (B1, B2, B3; red) and γ-catenin (C1, C2, C3; red) in the gingival epithelium. Oral gingival epithelium, A1, B1, C1; sulcular epithelium, A2, B2, C2; junctional epithelium, A3, B3, C3. Desmoglein 1 and 2 are more abundant in areas of cell–cell contact in spinous layers of the oral gingival epithelium (A1) and sulcular epithelium (A2) but is absent in the junctional epithelium (A3). Desmoglein 3 is expressed in cell–cell contact sites in almost all epithelial cells (B1, B2, B3), although its expression was discontinuous in the junctional epithelium (B3b arrowheads). The cell membrane facing the extracellular matrix was negative for desmogleins. Localization of γ-catenin corresponds to that of desmoglein 3. Bars, 100 μm. All figures, except the panel of B3b, are at the same magnification.

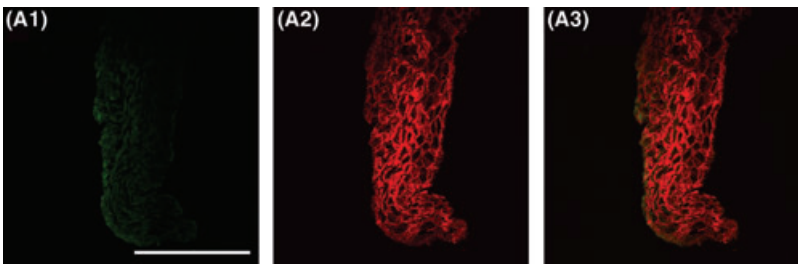

Fig. 3. Double labelings of both desmoglein 1 and desmoglein 3 in junctional epithelium. Desmoglein 1 and 2 (A1; green) are not present, but desmoglein 3 (A2, red; A3, merge) is present in the junctional epithelium. Bar, 100 μm.

then washed again with PBS(T). Filamentous actin (F-actin) was visualized by binding with Alexa Fluor 594®-labeled phalloidin (Molecular Probes Inc.) after treatment with 0.1% Triton-X-100 in PBST. Finally, specimens were mounted in a mounting medium (Prolong® gold antifade reagent; Molecular Probes) and then inspected with a laser-scanning confocal microscope (LSM510; Zeiss, Göttingen, Germany). Negative controls consisted of incubation with nonimmune serum, without primary antibody, prior to the addition of the appropriate secondary antibodies.

Results

Inspection of areas of healthy gingival epithelia revealed intact oral gingival epithelia, sulcular epithelia and junctional epithelia, and a small number of chronic inflammatory cells in the subepithelial connective tissue. The portions of healthy gingival epithelia in excised tissues were used in the present study and all applicable tissues showed similar results. In every case, positive immunofluorescence was not observed in negative control stains. The expression of adhesion molecules in healthy gingival epithelium is summarized in Table 1.

Keratin 4, 13, 14 and 19

Different patterns of keratin expression were present in the three regions of the gingival epithelium. In each specimen, the cytoplasm of all layers of oral gingival epithelium, sulcular epithelium and junctional epithelium cells exhibited strong staining with anti-keratin 14 immunoglobulin (Fig. 1: A1, A2, A3). In contrast, only basal cells of the oral gingival epithelium and sulcular epithelium, and almost all cells in the junctional epithelium, were clearly positive for keratin 19 (Fig. 1: B1, B2, B3). Immunostaining for keratin 13 was found in prickle cells in the oral gingival epithelium and sulcular epithelium (Fig. 1: C1, C2), but not in the junctional epithelium (Fig. 1: C3). Finally, immunostaining for keratin 4 was present in the marginal portion of the oral gingival epithelium (Fig. 1: D1) the sulcular epithelium (Fig. 1: D2), but not in the junctional epithelium (Fig. 1: D3).

The desmosomal proteins: desmoglein 1 and 2, desmoglein 3 and γ-catenin

As the antibody against desmoglein 1 used in this study also had cross-reactivity for desmoglein 2, the positive immunostaining for this antibody indicated the presence of desmoglein 1 and 2. Namely, desmoglein 1 and 2 were abundant in the cell–cell contact sites of the spinous and suprabasal layers of the oral gingival epithelium and sulcular epithelium (Fig. 2: A1, A2), but were not present in the basal layer, or in the junctional epithelium (Fig. 2: A3). In contrast, desmoglein 3 was present in the cell–cell contact sites in all layers except the uppermost layers of the oral gingival epithelium and sulcular epithelium (Fig. 2: B1, B2), and also

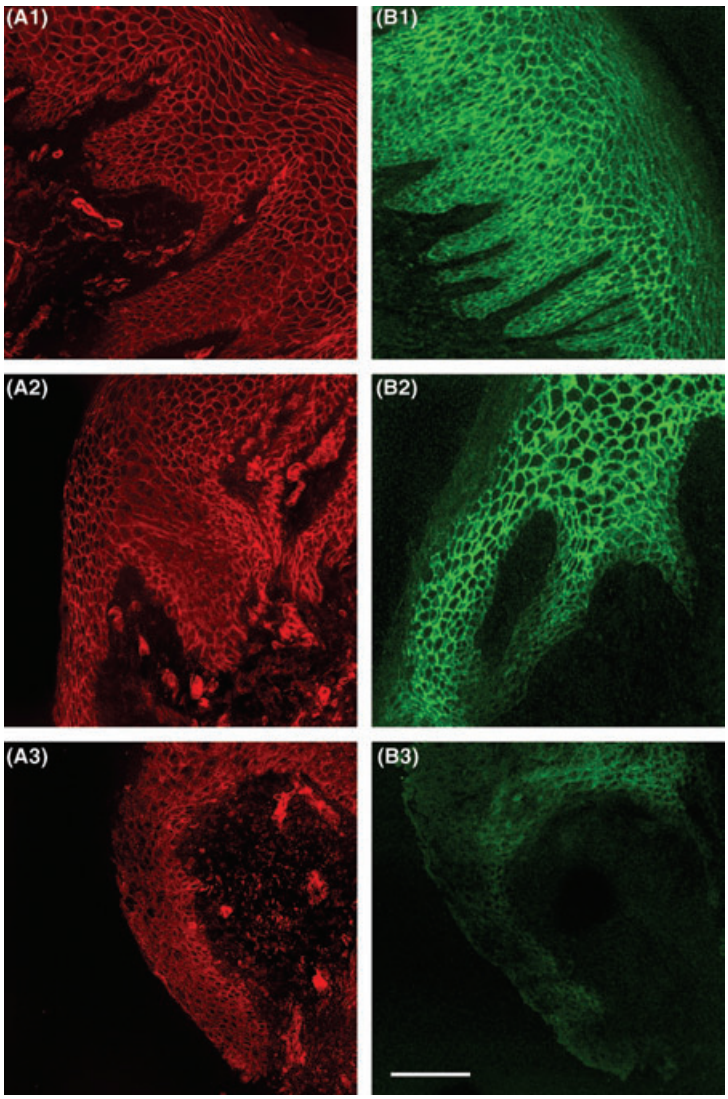

Fig. 4. Expression of F-actin (A1, A2, A3; red) and E-cadherin (B1, B2, B3; green) in the gingival epithelium. Oral gingival epithelium, A1, B1; sulcular epithelium, A2, B2; junctional epithelium, A3, B3. F-actin is present at cell–cell contact sites, but not at cell membranes facing the connective tissue in the oral gingival epithelium (A1), sulcular epithelium (A2) and junctional epithelium (A3). E-cadherin is expressed at cell–cell contact sites in the spinous layers and is obscure in the basal layers of the oral gingival epithelium (B1) and sulcular epithelium (B2), whereas it is scarce in the junctional epithelium (B3). Bar, 100 μm. All figures are at the same magnification.

all layers in the junctional epithelium with its discontinuous expression pattern (Fig. 2: B3a, B3b arrowheads). The uppermost layers of the sulcular epithelium and oral gingival epithelium were negative for desmoglein 1, 2, and 3 (Fig. 2: A1, A2, B1, B2). Finally, γ-catenin was present in cell–cell contact sites in all layers of the oral gingival epithelium, sulcular epithelium and junctional epithelium (Fig. 2: C1, C2, C3). Furthermore, desmoglein 3 and γ-catenin were not present in the cell membrane facing the basement membrane of the basal cells. Double staining using two antibodies against desmogleins confirmed the absence of desmoglein 1 and 2, but the presence of desmoglein 3 in the junctional epithelium (Fig. 3: A1, A2, A3).

Actin filaments and adherens junction-associated proteins: F-actin, E-cadherin, P-cadherin and α-catenin

Phalloidin experiments demonstrated that F-actin was present in cell–cell contact sites in all layers of the oral gingival epithelium and sulcular epithelium, and weakly in the junctional epithelium (Fig. 4: A1, A2, A3). Immunostaining experiments demonstrated that E-cadherin was more abundant in the upper layers than in the suprabasal layers of the oral gingival epithelium and sulcular epithelium (Fig. 4: B1, B2). In contrast, E-cadherin was absent in the junctional epithelium (Fig. 4: B3). P-cadherin was mainly present in the cell–cell contact sites in the basal layer of the oral gingival epithelium and sulcular epithelium (Fig. 5: A1, A2). In the junctional epithelium, the expression of P-cadherin was strong in the basal layers and weak in the other layers (Fig. 5: A3). Moreover, α-catenin was present in all layers of the oral gingival epithelium, sulcular epithelium and junctional epithelium, with the exception of the uppermost layers of the oral gingival epithelium and sulcular epithelium (Fig. 5: B1, B2, B3). P-cadherin and α-catenin were not present in the cell membrane facing the basement membrane.

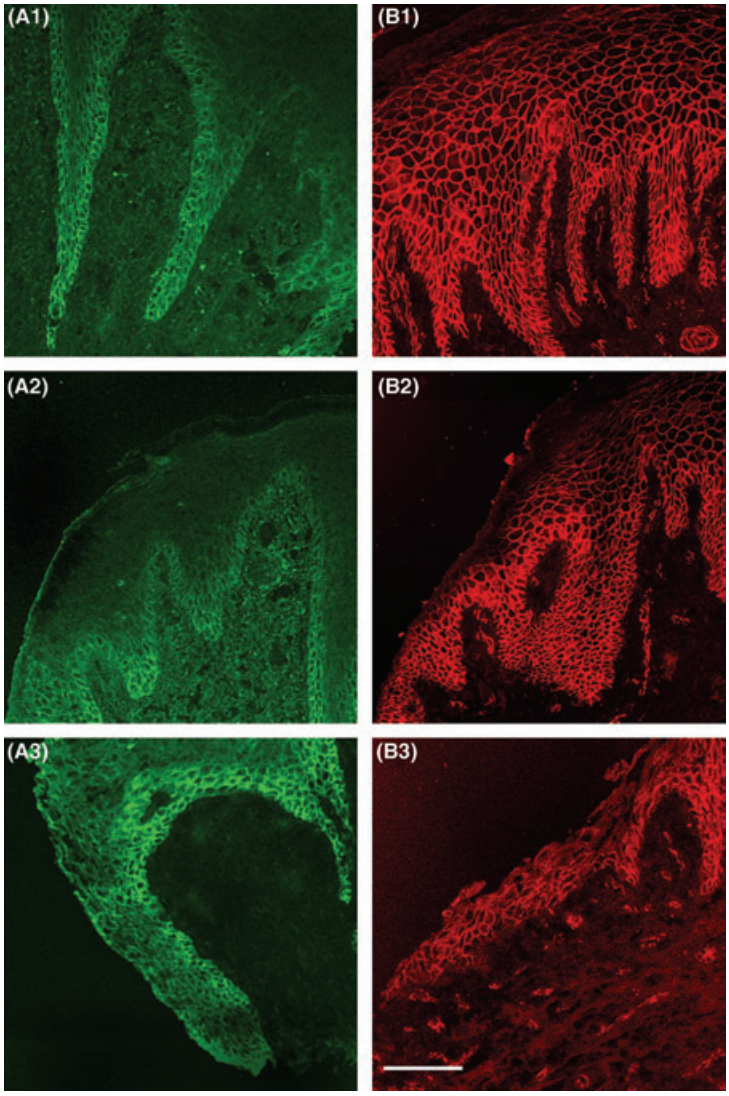

Fig. 5. Expression of P-cadherin (A1, A2, A3; green) and α-catenin (B1, B2, B3; red) in the gingival epithelium. Oral gingival epithelium, A1, B1; sulcular epithelium, A2, B2; junctional epithelium, A3, B3. P-cadherin is localized at cell–cell contact sites mainly in basal layers in the oral gingival epithelium (A1), sulcular epithelium (A2) and junctional epithelium (A3), and weakly positive in other layers of the junctional epithelium. α-catenin (B1–3) is present at cell–cell contact sites, but not at cell membranes facing the connective tissue in the oral gingival epithelium (B1) sulcular epithelium (B2) and junctional epithelium (B3). Bar, 100 μm. All figures are at the same magnification.

The gap junction protein, connexin 43, and the occluding junction proteins, claudin-1 and occludin

Connexin 43 was present in the cell–cell contact sites in the spinous layers of the oral gingival epithelium and sulcular epithelium, and in parts of the junctional epithelium (Fig. 6: A1, A2, A3). Claudin-1 was present only in the uppermost intermediate layers of the oral gingival epithelium (Fig. 6: B1 arrowheads) and occludin was also present in the cell membrane of surface layers of the oral gingival epithelium and in the cytoplasm of some of these cells (Fig. 6: B2, arrows).

Discussion

Stratified squamous epithelium is characterized by the presence of abundant desmosomes. Specific desmoglein molecules are expressed at different stages of keratinocyte differentiation (13,14). Desmoglein 1, which is a target protein in the autoimmune disease, pemphigus

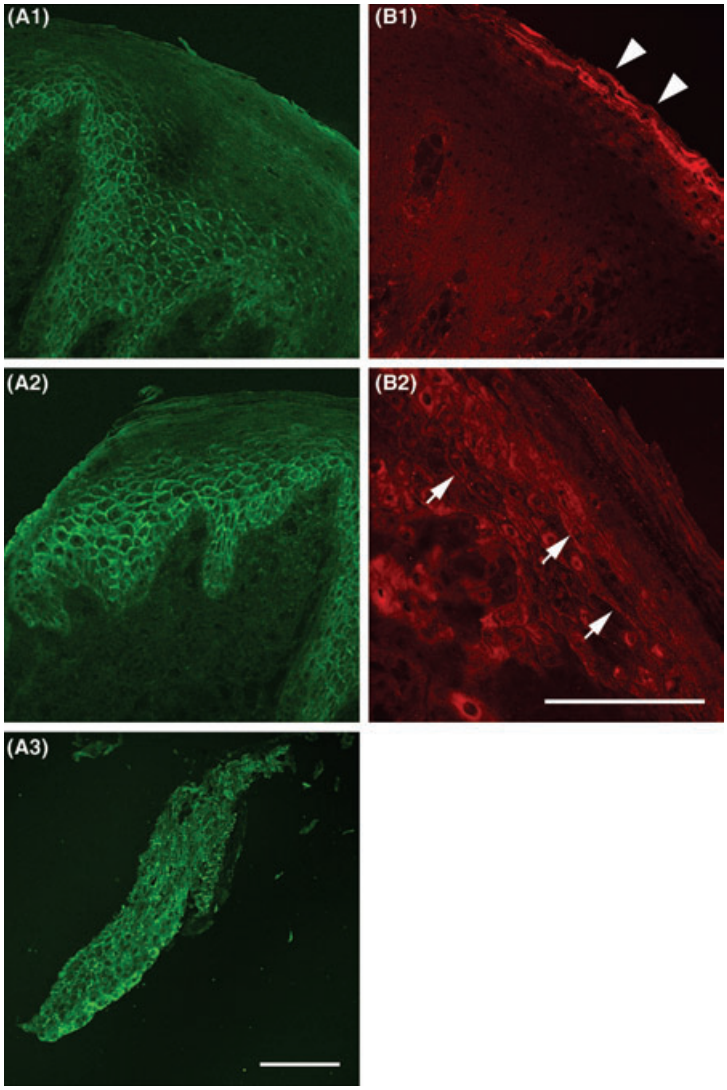

Fig. 6. Expression of connexin 43 (A1, A2, A3; green), claudin-1 (B1; red), and occludin (B2; red) in the gingival epithelium. Oral gingival epithelium, A1, B1, B2; sulcular epithelium, A2; junctional epithelium, A3. Connexin 43 is present in cell–cell contact sites in the spinous layer in the oral gingival epithelium (A1), sulcular epithelium (A2) and in part of the junctional epithelium (A3). Claudin-1 (B1; arrowheads) is present in the intermediate spaces of the uppermost layers, and occludin (B2; arrows) is present at the cell membrane in a few surface layers. Bar, 100 μm. Panels A1, A2, and A3 are at the same magnification and panels B1 and B2 are at the same magnification.

foliaceus (15), is restricted to the upper spinous layers of the epidermis. In contrast, desmoglein 3, which is involved in the pathogenesis of pemphigus vulgaris (16), is mainly localized in the basal and spinous layers of the epidermis. The present study is the first to demonstrate that desmoglein 3, but not desmoglein 1 and 2, was localized to the junctional epithelium. This indicates that desmosomes in the junctional epithelium are comprised of desmoglein 3, but not desmoglein 1 or 2, and seems consistent with the small size and number of desmosomes present in the junctional epithelium when compared with the oral gingival epithelium (4,17). The keratin expression of stratified epithelia varies according to the stage of cellular differentiation (18). In the gingival epithelium, keratin 19 was localized to the basal cells of the oral gingival epithelium and sulcular epithelium and was present in all cells of the junctional epithelium, while keratin 4 and keratin 13, known as mucosal-type keratins were not expressed in the junctional epithelium. These data indicate that the junctional epithelium cells were not differentiated to prickle cells and maintained the nature of basal keratinocytes. This conclusion is consistent with the histological feature of the junctional epithelium that the tooth enamel end and the connective tissue end of this epithelium are lined by the basal lamina.

Cadherins are transmembranous proteins that connect one cell to another through calcium ion-dependent homophilic binding, and are subdivided into several subtypes (19). E-cadherin is expressed on the cell surfaces at all epidermal layers, while P-cadherin is expressed on the surface of basal cells (19). E-cadherin serves as an important component of adherens junctions (20–22), where it is intracellularly linked to actin via α-, β- and γ-catenin (23). Furthermore, the adherens junction is a critical structure for stratification of squamous epithelia (24). Colocalization of actin and E-cadherin in the oral gingival epithelium and sulcular epithelium in the present study indicated the presence of adherens junctions in these epithelia, where they allow development of a stratified squamous epithelial layer. In contrast, in almost all cases, E-cadherin was scarcely found in the junctional epithelium, although in previous studies, there has been a discrepancy concerning the presence of E-cadherin in the junctional epithelium. For example, E-cadherin was weakly expressed in human junctional epithelium of clinically healthy gingiva (24), while it was absent in the junctional epithelium of either rats or mice (25). The absence of E-cadherin indicates that integrated adherens junctions are not generally present in the junctional epithelium, despite the presence of α-catenin, γ-catenin and P-cadherin. The distribution of desmogleins and keratins and the absence of E-cadherin confirms that the junctional epithelium is indeed functionally a simple epithelium, notwithstanding the formation of multilayers, as stated previously, according to the distribution of membrane surface carbohydrates and other types of keratins (1).

Defects in the adherens junction can result in widened spaces between epithelial cells in the junctional epithelium

and subsequent transmigration of fluid and leukocytes (7), which may prevent the entry of bacteria into the connective tissue from the gingival sulcus, and consequent bacterial challenge. Recent studies have demonstrated that junctional epithelium cells may play a much more active role in innate defense mechanisms by synthesizing various antimicrobial molecules, such as α-defensins and members of the cathelicidin family (7,17). These data imply that the wide intercellular space may actually serve as a reservoir of antibacterial factors.

Gap junction-mediated intercellular communication allows cellular homeostasis and control of growth and differentiation via the exchange of ions and small molecules. In human skin, connexin 43 is abundant in the spinous and granular layer, but relatively sparse or absent in the basal epidermal layer (11). In the present study of the human gingival epithelium, connexin 43 was present in the oral gingival epithelium and sulcular epithelium. Although the relationship between gap junctions and E-cadherin function has been characterized (24), the present study supported the presence of gap junction structures formed with connexin 43 in the gingival epithelium.

Tight junctions function to seal cells together, to maintain polarity of cells (10) and to separate the luminal space from the mesenchymal space. Constitutive tight junction proteins and tight junction-related structures have also been identified in squamous stratified epithelia, including the epidermis (12,26), where they are predominantly present in the stratum granulosum. In this study, claudin-1 and occludin were present in the intermediate portion of a few of the uppermost epidermal layers of oral gingival epithelium. These data suggest that the tight junction is probably a typical structure in the uppermost layer of oral gingival epithelium, is equivalent to the stratum granulosum in stratified epithelium, and functions as a barrier (27).

Acknowledgements

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