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Review Article

Tight Junctions in Salivary Epithelium

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Epithelial cell tight junctions (TJs) consist of a narrow belt-like structure in the apical region of the lateral plasma membrane that circumferentially binds each cell to its neighbor. TJs are found in tissues that are involved in polarized secretions, absorption functions, and maintaining barriers between blood and interstitial fluids. The morphology, permeability, and ion selectivity of TJ vary among different types of tissues and species. TJs are very dynamic structures that assemble, grow, reorganize, and disassemble during physiological or pathological events. Several studies have indicated the active role of TJ in intestinal, renal, and airway epithelial function; however, the functional significance of TJ in salivary gland epithelium is poorly understood. Interactions between different combinations of the TJ family (each with their own unique regulatory proteins) define tissue specificity and functions during physiopathological processes; however, these interaction patterns have not been studied in salivary glands. The purpose of this review is to analyze some of the current data regarding the regulatory components of the TJ that could potentially affect cellular functions of the salivary epithelium.

1. Introduction

The intercellular junctional complex in epithelial cellular sheets consists of four major components: (1) tight junctions (TJs) [1], (2) adherens junctions [2] and desmosomes [3], (3) gap junctions [4], and (4) focal adhesions [5, 6]. Adherens junctions and desmosomes are responsible for the mechanical adhesion between adjacent cells. Of particular interest in the adherens junctions of the salivary glands are the members of cadherin family, which play a role in salivary gland development, tissue organization, and cell differentiation [7]. In early morphogenesis, E-cadherin and β-catenin are likely to participate in salivary gland remodeling [8], whereas during cytodifferentiation, they form stable cell-cell contacts and may collaborate with Rho GTPases in the establishment and maintenance of salivary cell polarity [9]. Gap junction channels, which link the cytoplasm of adjacent cells, are made up of membrane-spanning proteins, the connexins [10]. The integrity of connexins is necessary for normal glandular secretory function [11]. Previous studies have shown that connexins become uncoupled during stimulation of saliva secretion by cholinergic agonists [12]. However, the molecular mechanisms by which connexins uncouple during salivary cholinergic stimulation remain to be determined. Focal adhesion molecules interact with the extracellular matrix and play critical roles in the differentiation of many tissues [13, 14]. In salivary glands, integrins play crucial roles in embryonic and adult cell adhesion, migration, morphogenesis, growth, and differentiation [14].

TJs are essential for the tight sealing of the cellular sheets [15]. Epithelial cell TJs consist of a narrow belt-like structure in the apical region of the lateral plasma membrane that circumferentially binds each cell to its neighbor [16]. In epithelial cells, TJs are thought to be the principal structures that contribute to cell polarity by acting as an intermembrane barrier that prevents the lateral movement of membrane proteins between the apical and basolateral membranes [17, 18]. TJs also form the primary barrier against the diffusion of solutes through the paracellular cleft [19], thus maintaining selective transepithelial ion gradients. Using freeze-fracture analysis of salivary epithelium cell membranes, TJs appear as aggregates of particles that form continuous anastomosing strands [20]. The particles are composed of transmembrane proteins, embedded in plasma membranes of neighboring cells, in which extracellular domains of these TJ proteins interact to seal the intercellular junction [19]. The major TJ proteins are the transmembrane proteins claudins, occludin, and junctional adhesion molecules (JAMs) [21–23]. TJ

proteins associate with intracellular scaffold proteins, among which is the family of zonula occludins (ZOs) proteins [24]. The ZO proteins anchor TJ transmembrane proteins to the actin cytoskeleton [25].

TJs are found in tissues that are involved in polarized secretions, absorption functions, and maintaining barriers between blood and interstitial fluids [26]. The morphology, permeability, and ion selectivity of TJ vary among different types of tissues and species [27–32]. TJs are very dynamic structures that assemble, grow, reorganize, and disassemble during physiological or pathological events [33]. Several studies have indicated the active role of TJ in intestinal, renal, mammary, and airway epithelial function [27–32, 34]; however, the functional significance of TJ in salivary gland epithelium is poorly understood [35]. The purpose of this review is to analyze some of the current data regarding the TJ and its regulatory components. TJs in the salivary epithelium are necessary for salivary gland function and could potentially serve as indicators of salivary epithelial dysfunction.

2. Morphological and Functional Differences between the Salivary, Acinar, and Ductal Cells

Salivary glands consist of multiple secretory units connected to the oral cavity by a system of ducts [36]. Each secretory unit is a cluster of cells organized in secretory acini [37]. The salivary glands consist of three pairs of major salivary glands (parotid, submandibular, and sublingual), and minor salivary glands located throughout the oral cavity within the lamina propria of the oral mucosa [37]. The major salivary glands are encapsulated by a connective capsule, a feature that is absent in minor salivary glands [38]. The salivary glands are also classified according to their function in serous glands (i.e., the parotid gland) that produce almost exclusively protein [39], mucous glands that produce only a small amount of protein but a large amount of glycoprotein (e.g., the sublingual and minor salivary glands) [40], and mixed serous/mucous glands that secrete both protein and glycoprotein (i.e., the submandibular gland). In humans 90% of saliva is produced by the major salivary glands and 10% is produced by the minor salivary glands [41].

2.1. Parotid Gland. The parotid gland is composed of the following: (1) serous acinar secretory end pieces [39], (2) intercalated ducts within the parotid glands, which are long and branched [36], and (3) well-developed striated ducts [36]. The secretion in the parotid gland is watery and rich in amylase, prolin-rich proteins, and peroxidase [42].

2.2. Submandibular Gland. The submandibular gland is a mixed gland composed of the following: (1) serous cells, which are attached to secretory end pieces to form a demilune, with serous acini predominating over the mucous elements [40], (2) intercalated ducts [36], and (3) long and well-defined striated ducts [36]. The secretion of the submandibular gland contains more mucus than that of the parotid gland; therefore, it is more viscous [40].

2.3. Sublingual Gland. The sublingual gland is composed of the following: (1) acinar mucus-secreting cells, some of which are capped by serous demilunes [40], (2) short to nonexistent intercalated ducts [36], and (3) striated ducts that are less developed than the submandibular and parotid glands [36]. The secretion of the sublingual gland is predominantly mucous [40].

2.4. Minor Salivary Gland. Minor salivary glands are divided into three groups as follows: (1) anterior-lingual glands that are mucous secreting glands with serous demilunes [43], (2) Von Ebner, or posterior lingual glands, which are composed of lipase-rich serous acini [44], and (3) Weber's glands, which are lingual, posterior glands that secrete mucus [45].

2.5. Saliva Secretion. The main function of the salivary gland is the production of saliva. Primary saliva secretion is elaborated by the acinar cells then it is modified as it passes through a series of progressively larger ducts [46–49]. The glandular secretion consists mostly of ions and electrolytes, as well as proteins and glycoproteins [46, 50]. Because primary acinar secretion and its modification in the ducts vary depending on the gland type, it is clear that TJ structure and function must be different between serous, mucous, and mixed acini, as well as between intercalated, striated, and excretory ducts. However, how TJ structure and function are modulated among different salivary glands during saliva secretion is little understood.

In currently accepted models of saliva secretion, the transepithelial movement of Cl^- is the primary driving force for fluid and electrolyte secretion by salivary acinar cells (Figure 1) [51]. Agonist-stimulated secretion in acinar cells is initiated by concomitant activation of Ca^{2+}-dependent apical Cl^- channels and basolateral K^+ channels [52]. The stimulated efflux of K^+ and Cl^- down their electrochemical gradients produces a transepithelial potential difference that is followed by Na^+ and water diffusion across the epithelial TJ [53] (Figure 1). The secretion from the acinar cell, in addition to fluid, contains proteins such as amylase and mucins in a solution of ions similar to the other extracellular fluids [54]. Water diffusion appears to occur by paracellular pathways and transcellular transport via water channels [51]. As the primary saliva goes through the ducts, Na^+ and Cl^- reabsorption and secretion of K^+ and HCO_3^- occur, because salivary ducts have low permeability to water, which results in a hypotonic saliva (Figure 1) [46].

TJs in salivary epithelia provide a barrier between the extracellular compartments and the lumen that is critical to normal acini functions, including the maintenance of cell polarity and normal transepithelial ion gradients [51]. A recent study indicated that apical electrolyte concentration modulates barrier function and TJ protein localization in bovine mammary epithelium [34]. Therefore, TJ protein interactions are likely to change in response to agonist-induced ion secretion in salivary glands. Proinflammatory cytokines also modulate TJ in several tissues [27, 28, 34, 56–60] including salivary epithelium [35]. Examining how TJs are modulated in response to agonists or inflammatory

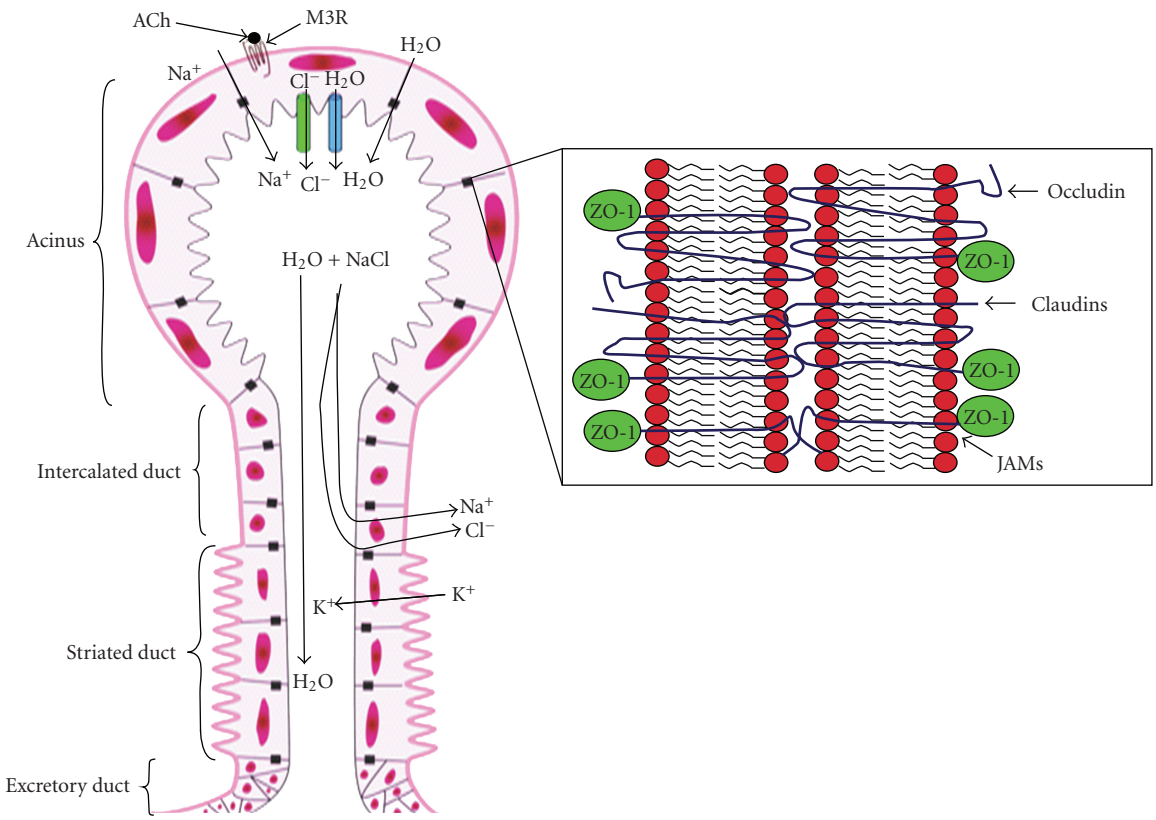

Figure 1: *Diagram representing acinar salivary secretion and TJ proteins.* Activation of basolateral M3 muscarinic receptors by neurotransmitters (e.g., acetylcholine) initiates signaling cascades that stimulate apical Ca^{2+}-dependent Cl^- channels. The stimulated efflux of Cl^- produces a transepithelial potential difference that drives Na^+ and H_2O transport across the TJ. Alternatively, H_2O can reach the lumen by water channels. These events create a plasma-like primary secretion in the lumen. As the primary saliva passes through the ducts, Na^+ and Cl^- are reabsorbed and K^+ is secreted into the lumen. Inset (adapted from [55]) indicates the TJ proteins occludin, claudin, and JAM linked to the cytoskeleton via cytoplasmic ZO proteins. Clearly TJ structure varies depending on the cell function; the question is how combinations of TJ proteins define function in acinar and ductal cells.

mediators is a significant step in defining the signaling pathways that regulate TJ integrity in salivary glands.

3. Transmembrane TJ Proteins

3.1. Claudins. Claudins are members of a multigene family, with < 24 members in humans/rodents [61], presenting a unique tissue expression pattern [62]. Claudins are integral transmembrane proteins that range from 22 kDa to 27 kDa [63]. Claudins span the cellular membrane 4 times, with both N-terminal and C-terminal ends located in the cytoplasm [21, 63, 64]. The C-terminal end diverges among different claudin subtypes, has potential phosphorylation sites, and has a putative PDZ binding domain of ZO proteins [64–66]. Claudins have two extracellular loops, which show a high degree of conservation [67]. The first loop is larger than the second, and it is involved in homophilic and heterophilic interactions [63, 64].

When claudin-1 or -2 is expressed in L-fibroblasts, which lack the endogenous claudins, they are able to reconstitute a well-developed network of strands (similar to TJ strand networks) [68, 69]. Conversely, occludin by itself cannot develop TJ strands [69], indicating that claudins are the backbone of TJ. Alterations of claudin expression strongly affect epithelial paracellular permeability (for a review see [67]). Analysis by freeze-fracture electron microscopy revealed an increase in number, depth, and complexity of TJ fibrils when claudins were overexpressed in epithelial cells [70, 71]. Claudin-null L-cells, transfected to express different claudins, have been used to demonstrate that claudin-1 heterotypically binds to claudin-3, but not to claudin-2 or claudin-5 [72]. Conversely, claudin-2 and claudin-5 heterotypically bind to claudin-3, but not to claudin-1 [72]. Thus, the compatibility of claudins for head-to-head binding is not easily predicted. The claudin-null HeLa cells stably expressing single or multiple claudins have been used to examine the ability of claudin-1, claudin-3, claudin-4, and claudin-5 to interact with each other [73]. Although the extracellular loop domains of claudin-3 and claudin-4 are highly conserved, claudins that interact with claudin-3 do not heterotypically bind to claudin-4 [73]. However, claudin-3 and claudin-4 do form heteromeric complexes [73]. To date, all of the known heterotypic claudin-claudin interactions appear to involve claudin-3 [72,

73]. However, since a limited subset of the claudins has been examined, future studies are necessary to determine other potential heterotypic claudin-claudin interactions. In contrast, homotypic claudin-claudin interactions appear to be universal [72]. Claudin-claudin interactions in salivary glands have not yet been defined; however, different claudin combinations may give functional specificity in acinar and ductal cells (and may help to better understand salivary gland functions). Kinetic analysis of GFP-claudin-1-containing strands in renal epithelial cells indicated that, although claudins are not mobile within paired strands, claudin-1-containing strands are dynamic: strands occasionally break and anneal, dynamically associating with each other in both an end-to-side and side-to-side manner [74]. Analysis by freeze-fracture electron microscopy revealed an increase in number, depth, and complexity of TJ fibrils when claudins were overexpressed in Madin-Darby canine kidney (MDCK) cells [70, 71]. In salivary glands, claudins are likely to regulate salivary gland functions by allowing cell polarity and by maintaining the transepithelial gradient necessary to establish unidirectional secretion [35]. To date, from the 24 known claudins, only claudins-1, -8, -10, -11, and -16 have been detected in salivary glands. These claudins (i.e., those that express in salivary glands) will be discussed in this section and are summarized in Table 1.

Table 1: *Localization of TJ proteins in salivary glands*. This table summarizes TJ detected to date in acinar and ductal cells. * indicates that this protein is present only in serous acini. $^{\triangle}$ denotes an unusual basolateral or cytoplasmic localization where TJs do not exist.

Species/Cell Type	Acinar	Ductal	References
Human Major Salivary Glands	Claudin-1* Claudin-2 Claudin-3 Claudin-4 Claudin-16$^{\triangle}$ Occludin JAM-A ZO-1	Claudin-1 Claudin-2 Claudin-3 Claudin-4 Claudin-16 Occludin JAM-A ZO-1	[79, 109]
Human Minor Salivary Glands	Claudin-3 Claudin-4	Claudin-1 Claudin-3 Claudin-4 Claudin-7 Claudin-11$^{\triangle}$	[77]
Rat Major Salivary Glands	Claudin-3 Claudin-10$^{\triangle}$	Claudin-1 Claudin-3 Claudin-4	[78]
Mouse Major Salivary Glands	Claudin-4 Claudin-3	Claudin-6 Claudin-8 Claudin-10	[89]

3.1.1. Claudin-1. Although the precise physiological role of claudin-1 is unclear, newborn claudin-1 deficient mice develop severe dehydration and die within 1 day of birth [75], indicating that claudin-1 plays a fundamental role within TJ. Additionally, overexpression of claudin-1 increased transepithelial electrical resistance (TER) and decreased paracellular permeability to 4–40 kDa FITC dextran in MDCK cells [76], further indicating the important role of claudin-1 in TJ formation.

Claudin-1 seems to be present only in ducts from human minor salivary gland [78] and rats (see Table 1) [79]. However, in human major salivary glands, claudin-1 was also found in serous acini (see Table 1) [80]. These studies indicate that claudin-1 expression varies among species and cell type. In polarized rat parotid gland Par-C10 cell monolayers [81] that endogenously express claudin-1 [35] treatment with proinflammatory cytokines TNFα and/or IFNγ caused a reduction of claudin-1 expression. The observed claudin-1 downregulation was associated with disruption of TJ structure and function [35]. These findings indicate that claudin-1 may contribute to TJ integrity in salivary epithelium, a condition that is necessary for cell polarity and unidirectional ion secretion.

3.1.2. Claudin-2. The commonly used experimental MDCK strains (i.e., types I and II) differ in TER when they form monolayers [68]. MDCK I cell monolayers do not express claudin-2 and have a very high TER [68, 82]. However, MDCK II cell monolayers have a 10- to 100-fold lower TER than MDCK I cell monolayers do, and they express claudin-2 in the intercellular space [68, 82]. Both cell strains express the TJ proteins claudin-1, -3, and -4, as well as ZO-1 and occludin [68]. These studies revealed that incorporation of claudin-2 converts the tight TJ strands to leaky strands in MDCK I cell monolayers. Other studies indicate that exogenous claudin-2 expression in MDCK-C7 cells (a twin to MDCK strain I cells) induces cation-selective channels in the TJ [77, 83]. Additionally, in the kidney, claudin-2 expression is restricted to leaky epithelium in the proximal tubule and thin descending limb of Henle [84]. Furthermore, claudin-2 is absent in the remaining distal nephron, which is considered to be a tight epithelium [84]. These studies indicate that claudin-2 causes leakiness within the TJ.

Claudin-2 has been detected in both acinar and ductal cells from human major salivary glands (see Table 1) [80]. However, claudin-2 was not detected in human minor salivary glands and rodents (see Table 1) [78, 79]. As for claudin-1, discrepancies in claudin-2 detection may exist due to the cell type and species. Although the role of claudin-2 in salivary gland is not known, high levels of claudin-2 in adult salivary acinar cells could contribute to the typical leakiness of salivary acinar cells (e.g., high permeability to water and Na^+).

3.1.3. Claudin-3. Claudin-3 is found in equal amounts in two strains of MDCK cell monolayers (MDCK-C7 and MDCK-C11); however, these strains show different levels of transepithelial resistance [83]. In addition, transfection experiments showed no relationship between electrical transepithelial resistance and claudin-3 expression in MDCK I cell monolayers [68]. In contrast, claudin-3 knockdown by siRNA in the gastric polarized epithelial cell line (MKN28) caused a significant decrease in TER and increased dextran permeability [85]. The studies indicate that claudin-3 function varies depending on the cell type.

Claudin-3 has been detected in both acinar and ductal cells from human major and minor salivary glands and in the rat parotid gland (see Table 1) [78–80] as well as in Par-C10 cell monolayers [35]. The specific function of claudin-3 in epithelial cells of the acini and ducts in the salivary glands has not been elucidated. However, a recent study showed that claudin-3 expression decreased in the parotid glands of female mice lacking Aquaporin 5 ($AQP5^{-/-}$), the major transcellular water transporter in salivary acinar cells [86]. This study suggests a possible link between claudin-3 and paracellular water secretion in salivary glands.

3.1.4. Claudin-4. The overexpression of claudin-4 in MDCK cells decreases transepithelial conductance by decreasing paracellular Na^+ permeability, without affecting permeability to Cl^- or flux for a noncharged solute [71]. In cultured pig kidney epithelial cells (LLC-PK1), knockdown of claudin-4 expression decreased Cl^- permeability and caused the TJ to lose the anion selectivity [87]. When claudin-4 in MDCK I cells is removed, there is a decrease in the number of TJ strands and a reduction of TER [88]. These studies suggest that claudin-4 may be responsible for both conductance and ionic selectivity.

Claudin-4 has been detected in both acinar and ductal cells from human major and minor salivary glands [78, 80] (see Table 1). Claudin-4 has also been detected by Western blot analysis in Par-C10 polarized cell monolayers [35]. However, claudin-4 was detected only in ductal cells from rat parotid glands and mouse submandibular glands when using immunostaining in frozen sections (see Table 1) [79, 89]. Overexpression of claudin-4 increased TER and decreased epithelial permeability (to 70-kDa dextran, as compared to untransfected controls) in SMIE cell monolayers [90]. These results indicate that claudin-4 may function as a regulator of TJ barrier function in rat submandibular glands.

3.1.5. Claudin-5. Claudin-5 has been reported to be primarily present in TJ of endothelia, suggesting a role in the control of the endothelial barrier [91]. Indeed, mice deficient in claudin-5 show barrier failure, which is size selective and limited to the endothelium of the blood-brain barrier [92]. Claudin-5 stable transfection to human epithelial colorectal adenocarcinoma cells (Caco-2) increased TER and decreased paracellular permeability to mannitol (as compared to untransfected controls that lack claudin-5 and normally display low TER) [93]. However, when claudin-5 null cells displaying high TER (MDCK-C7) were transfected with claudin-5, no changes of barrier function were detected [93]. These findings suggest that claudin-5 contributes to the TJ sealing.

In human and rats major salivary glands, the expression of claudin-5 seems to be restricted to endothelial cells that surround acinar and ductal cells [79, 80]. Since previous studies showed that claudin-5 controls paracellular solute and water movement across endothelial monolayers [91], and because of its location in endothelial cell surrounding salivary epithelium [79, 80], it is tempting to speculate that claudin-5 could be involved in controlling nutrients supply from blood to salivary glands. However, studies are needed to elucidate the functions of claudin-5 in salivary glands.

3.1.6. Claudin-6. Claudin-6 was first identified through searching expressed sequence tag (EST) databases from embryos [61]. However, claudin-6 expression has not been detected in adult tissues [61, 94], indicating that claudin-6 may be regulated developmentally. Consistent with these studies, claudin-6 is expressed and concentrated at TJ only in the ducts at E16 (in mice submandibular glands) [89]. Conversely, it is almost completely absent after birth [89] (see Table 1), suggesting that claudin-6 is developmentally regulated in salivary glands.

3.1.7. Claudin-7. Claudin-7 is localized in the distal and collecting tubules, as well as in the thick ascending limb of Henle in porcine and rat kidneys [95]. Overexpression of claudin-7 in LLC-PK1 cells resulted in increased TER and a dramatic reduction in dilution potentials [95] due to a concurrent decrease in the paracellular conductance to Cl^- and an increase in the paracellular conductance to Na^+ [95]. These results indicate that claudin-7 may form a paracellular barrier to Cl^- while acting as a paracellular channel to Na^+.

Claudin-7 is expressed in ductal cells from early developmental stages through adulthood in human minor salivary glands (see Table 1) [78]. Similar to claudin-3, claudin-7 protein expression also decreased in parotid glands from female $AQP5^{-/-}$ mice, further indicating a relationship between claudins and water transport during saliva secretion [86].

3.1.8. Claudin-8. Claudin-8 is expressed along the aldosterone-sensitive distal nephron, including the entire collecting duct [59]. Induction of claudin-8 expression in MDCK II cells reduced permeability, not only to protons, but also to ammonium and bicarbonate [96, 97]. These studies suggest that claudin-8 probably limits the passive leak of these three ions via paracellular routes, thereby playing a permissive role in urinary net acid excretion.

Claudin-8 has been detected in the ducts of mouse submandibular glands, during both the pre- and post-natal stages (see Table 1) [89]. However, further research is needed to determine the role of claudin-8 in salivary glands.

3.1.9. Claudin-10. Although the exact function of claudin-10 is unknown, its expression has been associated with hepatocellular carcinoma recurrence [98] and papillary thyroid carcinoma [99], suggesting that claudin-10 contributes to cancer progression. Claudin-10 is expressed in the terminal tubules developing mouse submandibular glands, where this claudin-10 is colocalized with ZO-1 [89]. However, studies in rat major salivary glands indicated that claudin-10 is also present at the basolateral region of acinar cells, showing an ectopic subcellular localization where TJ strands do not exist (see Table 1) [76]. The role of claudin-10 in salivary glands remains to be determined.

3.1.10. Claudin-11. Previous studies determined that claudin-11 is present in the central nervous system and is involved in nerve conduction [100]. Claudin-11 is also present in Sertoli cells and apparently is involved in spermatogenesis [101]. Claudin-11 typically forms "pure" TJs, in the sense that other claudins are not present in these junctions [100]. In claudin-11 null mice, compartmentalization (established by claudin-11-based TJ in *stria vascularis)* is required for hearing through generation of endocochlear potential [102]. Overexpression of claudin-11 induces proliferation and enhances migration in an oligodendrocyte cell line [103].

In human minor salivary glands, claudin-11 has been detected in the cytoplasm of ductal cells unlike other claudins [78]. Claudin-11 is not expressed in acinar cells (see Table 1) [78]. The reason why claudin is expressed in a place where TJs do not exist (e.g., membrane-cytoplasmic) is unknown. The function of claudin-11 and its expression pattern in human major salivary glands remain to be determined. Claudin-11 is expressed in the terminal tubules and ducts of the mouse developing submandibular gland where it is colocalized with ZO-1 [89].

3.1.11. Claudin-16. Claudin-16 is expressed in the kidney of several mammalian species (e.g., in rodents, cattle, and humans) [104–107] and in mammary glands from mice [108]. When claudin-16 is missing, magnesium does not return from the renal tubule to the blood. Consequently, it is lost in the urine, which leads to hypomagnesemia [104]. These studies indicate that claudin-16 (*a.k.a.,* paracellin-1) provides a cation-selective channel in the renal tubule [104].

Claudin-16 has been detected in the ducts of major human salivary glands (where claudin-16 colocalizes with the scaffold protein ZO-1 or with occludin) [109]. However, in acinar cells, claudin-16 was detected at the basolateral side of the cells (between cells of the same acinus and/or between cells of neighboring acini) (see Table 1) [109]. Consequently, the significance of claudin-16 expression pattern (in acinar cells) has yet to be determined.

3.2. Occludin. Occludin is a transmembrane protein that forms part of the TJ [22] which contributes to TJ barrier function and to formation of aqueous pores within TJ strands [15]. Occludin has a molecular mass of 60–65 kDa, two extracellular loops [110], and four transmembrane domains. Both the N- and C-terminal ends of occludin are located in the cytoplasm [110]. The N-terminal region is involved in sealing and barrier properties [111]. The C-terminal domain is rich in charged amino acids and binds specifically to a complex of ZO-1 and ZO-2 [110]. The extracellular loops have a high content of tyrosine and glycine residues that are thought to be involved in the regulation of paracellular permeability and cell adhesion [110].

Occludin-deficient embryonic stem cells are able to differentiate into polarized epithelial cells with functional TJ [112]. L-fibroblasts exhibited no cell-cell adhesion as a result of induced occludin expression [69], suggesting that occludin is not necessary for TJ formation. Expression of occludin in MDCK II cells increased TER and paracellular flux of a small molecular dextran tracer [113], suggesting a role for occludin in the formation of selective pores (despite high electrical resistance).

Four differentially spliced occludin-specific mRNA transcripts have been identified, which are the result of post-transcriptional events [114]. Expression of the translated proteins altered subcellular distribution of occludin and loss of colocalization with ZO-1 for two of the four splice variants [114]. Two splice variants (i.e., occludin types II and III) lack the fourth transmembrane domain [114]. It was observed that occludin types II and III did not co-localize with ZO-1, which highlights the significance of the fourth transmembrane domain in directing occludin to the TJ [114]. An occludin isoform lacking the fourth transmembrane domain (close to the C-terminal domain) was discovered in human embryo tissues [115]. Occludin 1B has been identified as a transcript encoding a longer form of occludin with a unique N-terminal sequence of 56 amino acids [116]. Immunostaining for occludin 1B shows that its distribution pattern in MDCKs is identical to that of occludin. The detection of occludin 1B in a range of epithelial tissues (and the preservation across species) implies that occludin 1B may be a significant player in the modulation of TJ barrier properties [116]. These findings also suggest that occludin and its isoforms may be a multigene family.

In human major salivary glands, occludin has been detected in ductal and acinar cells (see Table 1) and in endothelial cells surrounding the salivary epithelium [80]. Occludin has also been detected in cell lines of salivary gland origin such as the polarized Par-C10 and SMIE cell monolayers [35, 90]. Mice lacking occludin showed loss of cytoplasmic granules in striated ducts from salivary glands [117]; however, the significance of this observation remains to be determined.

The functional role of occludin in salivary gland TJ has been demonstrated through studies involving polarized cell lines. In a rat parotid gland epithelial cell line (Pa-4, similar to Par-C10 but different clone), transfection of an oncogenic Raf-1 resulted in a complete loss of TJ function (and the acquisition of a stratified phenotype that lacked cell-cell contact growth control) [118]. The expression of occludin and claudin-1 was downregulated, and the distribution patterns of ZO-1 and E-cadherin were altered. Introduction of the human occludin gene into Raf-1-activated Pa-4 cells resulted in reacquisition of a monolayer phenotype and the formation of functionally intact TJ [118]. These studies indicate that not only occludin is a critical component of functional TJ in salivary epithelium, but that it also controls the phenotypic changes associated with epithelium oncogenesis.

In murine submandibular gland carcinoma cells (CSG), the expression of an N-terminally truncated occludin construct decreased TER and paracellular permeability to 4–42 kDa tracers [111]. These studies suggest that occludin may be a regulator of the paracellular pathway, rather than a structural or functional component of the TJ, given that TJs form and appear functionally normal in the absence of occludin [69, 112].

3.3. Junctional Adhesion Molecules (JAMs). JAMs are members of the immunoglobulin superfamily of proteins and are expressed in epithelial cells [119]. JAMs are subdivided into a group consisting of JAM proteins (JAM-A, JAM-B, JAM-C) and another group consisting of Coxsackievirus-adenovirus receptor (CAR), Coxsackie- and adenovirus receptor-Like Membrane Protein (CLMP), Endothelial cell adhesion molecule (ESAM), and JAM-4 [69, 112, 120, 121]. In epithelia, JAM-A and JAM-C localize to the TJ, whereas JAM-B exists along the lateral membrane [122]. Unlike occludin and claudins, JAM protein family members have a single transmembrane domain, an extracellular domain containing two Ig-like motifs, and a cytoplasmic tail [123]. The extracellular domains of JAM-A, JAM-B, and JAM-C contain dimerization motifs that play a role in their interactions [123, 124]. The C-terminal end has a putative PDZ binding domain, which interacts with the PDZ domains of accessory proteins (e.g., ZO-1) [125]. These studies indicate that JAM-A might be involved in the propagation of signal cascades, resulting from homophilic and heterophilic TJ protein interactions. JAM-A apparently plays a role in the adhesion and transmigration of monocytes through endothelial cells [23]. JAM-A functional significance in epithelial cells is less clear; however, inhibition of JAM-A with a monoclonal antibody caused a decrease in TER, and defects in TJ assembly, in intestinal epithelial T84 cells [126]. These studies indicate that JAM-A is important for TJ integrity in these cells.

Among the JAM protein family, JAM-A is the only member that has been detected in acinar and ductal cells from human major salivary glands (see Table 1) [80]. Further studies are needed, both to characterize its overall function and to determine the process by which it is regulated in salivary epithelium.

4. Multiprotein Complexes at the TJ

Three major protein complexes involve one or more scaffolding proteins: (1) the ZO protein complex [127], (2) the protein associated with Lin Seven (Pals1)-ALS1-associated TJ protein (PATJ) complex [128], and (3) the partitioning defective-3 (PAR)-3-atypical protein kinase C (aPKC)-PAR-6 complex [129]. To date, only the ZO protein complex has been described in mammalian salivary gland epithelium; the details of which will be described below.

ZO-1 is a classical scaffolding protein of the membrane-associated guanylate kinases (MAGUKs) family. It has three PDZ domains, one SH3 domain, and one guanylate kinase (GuK) domain [127]. Unlike other TJ proteins, ZO-1 is not a transmembrane protein; rather, it is a large cytosolic phosphoprotein [130]. This role is critically important for interaction with integral membrane proteins at TJ [131]. ZO-1 also interacts with other cytoplasmic proteins (e.g., ZO-2 and ZO-3 homologs of ZO-1) and with the actin cytoskeleton [120]. ZO-1 forms complexes with ZO-2 and ZO-3 [132]. Moreover, both ZO-2 and ZO-3 interact with F-actin (and also with occludin and claudins) [65, 132, 133]. Collectively, the ZO complex is the major link to the actin cytoskeleton at the TJ. The absence of ZO-1 results in a slight delay in TJ formation but does not impair the formation of functional TJ in MDCK cells [134] or in the mouse mammary epithelial cell line Eph4 [135]. This indicates redundancy in the roles of ZO family members, as each can accomplish the role of other members. However, if all ZO family members are lost in mammary epithelial cells, TJ formation is blocked (i.e., TJ strands are lost and barrier function is disrupted) [131]. Together, these findings indicate a critical role for the ZO protein family in the development of TJ strands, probably by forming the physical scaffold for the strand-forming proteins (e.g., claudins and occludin).

In human major salivary glands, ZO-1 is present in acini, ducts, and interglandular endothelial cells [80, 109]. Additionally, ZO-1 is colocalized with claudin-16 at the excretory duct of human major salivary glands [109]. ZO-1 has been widely used as a marker to achieve salivary gland differentiation [136]; however, studies are needed to determine the molecular mechanisms by which ZO-1 modulates TJ in salivary glands.

5. Functional Approaches Available to Study TJ in Salivary Glands

Epithelial cell lines from rat salivary glands (e.g., Par-C10, SMIE, and CSG), exhibiting a high degree of differentiation when plated on permeable supports or on Matrigel, are currently available. The rat parotid Par-C10 cells are able to form polarized monolayers when cultured on permeable supports (i.e., in a two-dimensional culture) [35]. Par-C10 cells also form acinar spheres when single cells are grown on Matrigel (i.e., in a three-dimensional culture) [137] (Figure 2). These models have proven useful to study TJ morphology by freeze-fracture analysis [35], TJ organization through confocal microscopy (Figure 2), and TJ protein expression through Western blot analysis [35]. Par-C10 polarized monolayers allow for the study of TJ function by TER and agonist-induced short circuit current measurements [35]. Par-C10 acinar spheres make possible the study of TJ integrity (by measuring intrasphere changes in potential difference in response to relevant secretory agonists) [137].

The polarized rat submandibular SMIE cells are useful to study TJ structure and function [90] and allow the monitoring of transepithelial fluid movement in vitro [138]. The murine submandibular gland carcinoma cell line CSG is another polarized cell line that has been utilized to determine a role for occludin on paracellular permeability and the cytoplasmic plaque of TJ associated proteins in salivary epithelium [111]. To our knowledge, SMIE or CSG cells have not been yet grown on Matrigel; however, further study is warranted to determine whether they are able to form acinar spheres.

Previous reports described methods for culturing human primary submandibular cells that formed functional TJs [139, 140]. These in vitro cell systems could be used to study TJ function and to understand TJ formation during

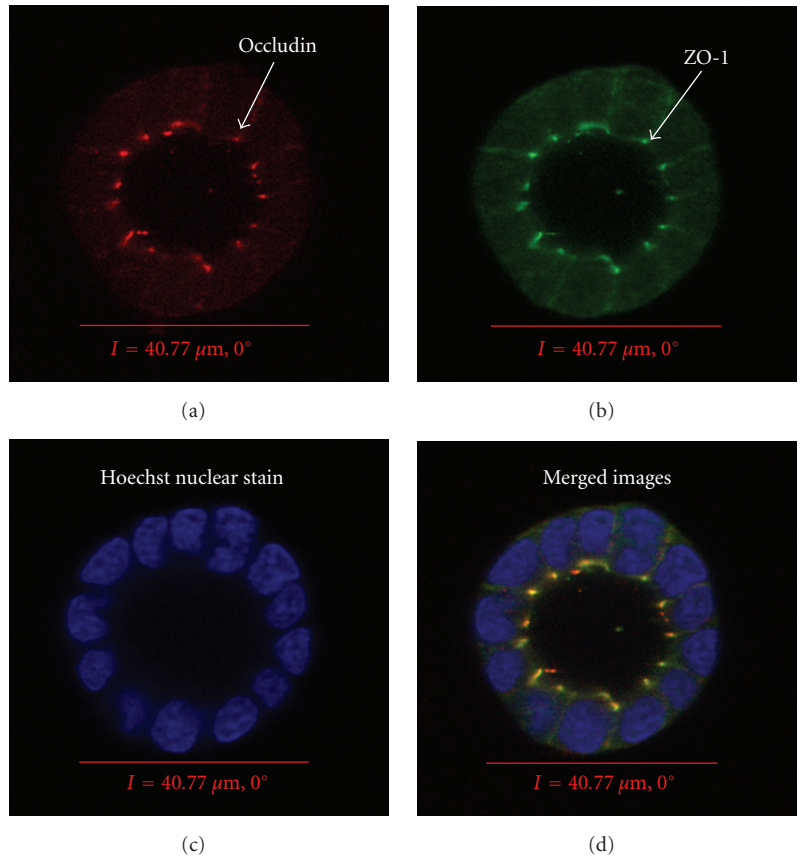

Figure 2: *Three-dimensional Par-C10 acinar-like spheres express TJ as markers of acinar differentiation.* Protein expression was detected using immunofluorescence microscopy with goat antimouse occludin (a and d: red), rabbit anti-ZO-1 (b and d: green), followed by Hoechst nuclear stain (c and d: blue). Images were obtained and analyzed using a Carl Zeiss 510 confocal microscope. Sphere diameter taken at the widest point of the xy plane (I) is shown in μm. This model has been published [137].

salivary gland regeneration. Other studies have been able to reconstitute three-dimensional human salivary gland tissues that exhibit TJ and secrete α-amylase [136]; however, due to the difficulty of obtaining human tissue samples, these approaches are limited.

The identification of molecular components of TJ has enabled researchers to analyze TJ functions by generating knockout mice of the several TJ proteins, a review of which can be found in reference number [141] of the current review. In addition, positional cloning has identified mutations in the genes of several TJ components in hereditary human and cattle diseases, further demonstrating critical roles for TJ in various organs [141]. TJ proteins in salivary glands (e.g., for claudin-1, -5, -16, occluding, and ZO-1) [141] may be studied in knockout mouse models. However, because salivary gland structure and function have not been yet studied in mice models, future studies involving in vivo models are warranted.

6. Concluding Remarks

Interactions between different combinations of the TJ family (each with their own unique regulatory proteins) define tissue specificity and functions during physiopathological processes; however, these interaction patterns have not been studied in salivary glands. Therefore, further research should determine how external signals modulate TJ structure and function in salivary glands.

At sites of epithelial cell damage, loss of TJ integrity represents a signal for cells to begin a repair program involving proliferation and migration activities at the wound edge, ending with the reassembly of TJ to reform an intact epithelial layer [142]. In Sjögren's syndrome (an autoimmune secretory disorder), lymphocytic infiltrates cause damage in ductal and acinar epithelial cells, which leads to salivary gland dysfunction [143]. Furthermore, Sjögren's syndrome-related proinflammatory cytokines compromised TJ integrity and resulted in salivary epithelial dysfunction [35]. Therefore, dysregulation cell-cell contacts by TJ alteration must occur in Sjögren's syndrome.

Additional studies are required to understand TJ regulation, both in healthy and diseased salivary glands. Such research could result in improved detection (i.e., early markers for salivary epithelial dysfunction) and better treatments (e.g., modulation of TJ to improve secretion).

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