# Role of Oral Mucosal Fluid and Electrolyte Absorption and Secretion in Dry Mouth

Guo H. ZHANG[1], Robert CASTRO[2]

*Dry mouth is induced by dehydration of the oral mucosa, resulting from an imbalance of fluid supply and clearance within the oral cavity. Saliva is the major source of oral mucosal fluid, whereas oral fluid clearance includes evaporation and swallowing. Oral mucosal fluid absorption has been suggested to play a critical role in oral fluid clearance; over-absorption of water and ions across the oral mucosa under certain conditions may be a major component for oral fluid imbalance, leading to mucosal dehydration. While numerous studies have confirmed that the oral mucosa absorbs fluid and electrolytes, the pathways and mechanisms mediating the absorption remain undefined. The transcellular pathway regulating oral mucosal epithelial absorption includes aquaporins, epithelial $Na^+$ channel and/or $Na^+/H^+$ exchanger, whereas the paracellular transport is likely to be mediated by tight junctions. The regulatory mechanisms of these pathways require further elucidation. It remains unclear whether the oral mucosa also secretes fluid and ions into the oral cavity. Although intercellular lipids secreted by epithelial cells form the major barrier to paracellular water and ion transport, the role and regulation of these lipids in oral mucosal hydration in physiological and pathological conditions need further investigation. Delineation of these mechanisms will be conducive to the development of preventive and therapeutic interventions for dry mouth.*

**Key words:** *dry mouth, mucosal fluid film, mucosal transport pathways, oral mucosal dehydration, water and ion absorption and secretion*

Dry mouth can seriously impair oral health and diminish the quality of life in patients, although its impact on overall health has long been debated. Dry mouth is not a clear term. It is often misused or incorrectly interchanged with another term, xerostomia; the latter refers to the subjective sensation of oral dryness[1,2], it can be a patient's primary complaint and is considered a symptom of dry mouth. Although the nomenclature dry mouth has been widely used to describe xerostomia and hyposalivation[3], these terms have different meanings[1]. Currently, a diagnosis of dry mouth is based primarily on the existence of hyposalivation, secondary to the hypofunction of salivary glands, determined by measuring unstimulated or stimulated salivary flow rates. An unstimulated salivary flow rate of < 0.1 ml/min is considered to be hyposalivation[4].

Xerostomia is common in the general population, especially in the elderly[5]. In a review of 24 published high quality reports from 1989 to 2009, the prevalence of xerostomia and salivary gland hypofunction in elderly individuals, taken from the general public, community dwellings, institutionalised and hospital populations, varied from 11.3% to 78.0%[6]. More recently, Benn et al[7] found that the overall prevalence of xerostomia amongst a representative sample of dentate adult community-dwelling New Zealanders (age 18 years old and over) was 13.1%. Lee et al[8] reported that the prevalence of xerostomia in Koreans undergoing physical examinations at various hospitals was 33%. In

1 National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), 6701 Democracy Boulevard, Bethesda, Maryland 20892, USA.

2 Department of Pediatrics, Division of Neonatal and Developmental Medicine, Stanford University School of Medicine, 750 Welch Road, Suite 315, MC 5713, Palo Alto, California 94304, USA.

**Corresponding author:** Dr Guo H. ZHANG, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), 6701 Democracy Boulevard, Room 1064, Bethesda, Maryland 20892, USA. Email: Zhanggu@mail.nih.gov

**Co-corresponding author:** Dr Robert CASTRO, Stanford University School of Medicine, Neonatal and Developmental Medicine, 450 E. Romie Lane, Salinas, California 93901, USA. Email: castror@stanford.edu

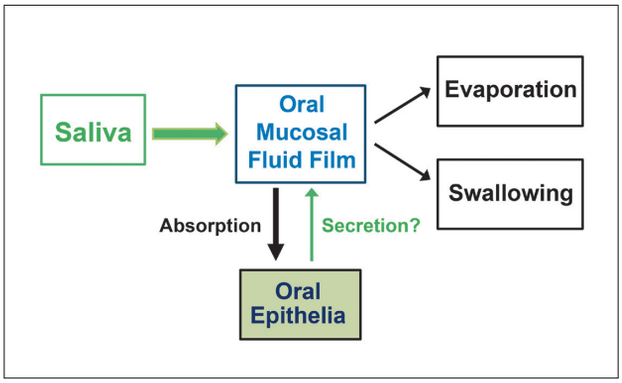


**Fig 1** The balance of oral fluid supply and clearance. The oral mucosa fluid film covers the mucosa and is in a continuous dynamic balance of fluid secretion and clearance. Saliva is the major source of the oral fluid. Fluid secretion by the mucosa may also contribute a portion of the fluid film, but further research is needed to quantify its role. Oral fluid is not only cleared by evaporation and swallowing, but mucosal absorption may constitute a major role in oral fluid clearance. Over-absorption by the mucosa may be a critical factor in the development of dry mouth.

another study not included in the review by Liu et al[6], 34% of subjects from 18 to 92 years of age complained of xerostomia[9]. Overall, it has been well-established that aetiological factors for xerostomia include old age, being female, certain autoimmune disorders, specifically Sjögren's syndrome[10], medications reducing saliva secretion[11-15], and radiation exposure to the head and neck areas[1,16-20]. Additional factors also include smoking[21] and wearing dentures[22].

Since the lack of sufficient saliva production is the primary mechanism, current research has focused on the various aetiological factors resulting in hyposalivation. Xerostomia is associated with reduced rates of saliva secretion[10,23-27]. However, not all studies confirm that all subjects who complain of xerostomia exhibit hyposalivation[5,9,15,28-30]. For example, Ohara et al[28] found that in 34.8% of the 65- to 84-year old subjects complaining of xerostomia, only 11.5% had hyposalivation. Similar results were reported in separate studies[9,30]. Additional factors, other than hyposalivation, must play a substantial role in the pathogenesis of dry mouth.

The oral mucosa is a complex surface lining of the oral cavity, consisting of various structures within different regions of the mouth. Based on its function, the oral mucosa is divided into three subtypes: the lining (60%), masticatory (25%), and specialised (15%) mucosa[31]. The major function of the oral mucosa includes protection of deeper tissues, the sensation of temperature, the maintenance of pH and hydration. The oral mucosa also secretes required substances that are not clearly defined. Specialised sensory and neuromuscular properties of the oral mucosa cause the unique sensation found within the intraoral environment, such as temperature, taste, and touch, as well as to provide information regarding the size, texture, moisture, and the movement of foods in the mouth. The oral mucosa is continuously exposed to a variety of physical, mechanical, chemical, and biological challenges, which includes compression, stretching, shearing, abrasion, temperature variation, pH changes, toxic substances and microbial invasions. Therefore, it is extremely important to maintain a well moisturised, buffered, and protected oral cavity surface and the significance of maintaining a healthy oral mucosa cannot be overemphasised.

## Oral mucosal hydration: Balance between fluid secretion and clearance

Amongst a number of elements that influence oral mucosal health, the key component is maintaining appropriate mucosal hydration. The healthy oral mucosa is in a continuous dynamic balance of fluid secretion and clearance (Fig 1)[32,33]. Dawes[34] first proposed that if the sum of fluid loss, including from absorption, evaporation, and swallowing, is greater than the salivary flow rate, affected individuals will experience the sensation of dry mouth. To sustain a fully moist surface of the oral cavity, the oral mucosa requires specific and distinct regulatory mechanisms supplying a fluid comprising of the appropriate water content and concentrations of electrolytes, lipids and proteins. Three major pairs of salivary glands, which include the parotid, submandibular, and sublingual glands, provide the largest component of fluid from distinct anatomical positions during eating and mastication. There are approximately 800 to 1,000 minor salivary glands located within the submucosa of different oral locations, including the buccal, labial, lingual, soft and hard palatal sites, the floor of the mouth, and the tongue, which continuously supply appropriate moisture, and inorganic and organic substances. In addition, sebaceous glands in the oral mucosa may also play a role in secreting lipids and other materials.

### *Residual saliva and oral mucosal fluid film*

It is widely acknowledged that an unstimulated salivary flow rate of < 0.1 ml/min is consistent with hyposalivation[35,36]. However, it has also been shown that not all patients with this reduced unstimulated salivary flow

rate experience symptoms of xerostomia[37]. Similarly, as previously described, not all individuals with xerostomia have hyposalivation[9,28,30]. Furthermore, it has been reported that the severity of dry mouth symptoms often does not correlate directly with the reduction in salivary flow rates[29,38,39]. It is widely accepted that dry mouth occurs when the fluid influx, primarily from salivary gland secretion, is exceeded by the fluid loss from the mouth[32,33]. In other words, the sensation of a dry mouth in those individuals is due to insufficient mucosal hydration[40-44]. On the oral tissue surfaces, there is a thin layer of remaining fluid following saliva swallowing, termed residual saliva. It is probably more accurate to call it 'residual fluid', since a major component may not be actual saliva. The mean saliva volume in the mouth, as measured by Lagerlöf and Dawes[45], was approximately 1.07 ml and 0.77 ml prior to and after swallowing, respectively, with large ranges for individual variations. The residual fluid forms a thin film covering the oral surface tissues, termed either oral mucosal fluid film, oral mucosal film, oral salivary film or simply saliva film. One of the major functions of the film is to maintain hydration or wetness of the oral tissue surfaces. Appropriate hydration status provided by this film protects the oral mucosa from feeling dry.

The relationship between the oral mucosal fluid film and the oral mucosal pellicle remains undefined. The latter is a protein film that is bound tightly to the mucosal cells. It is widely known that the acquired pellicle found on the teeth forms a protective and lubricating film[46]. In contrast, the mucosal pellicle on the epithelial cells of the oral mucosa forms a physical barrier, prevents pathogen adhesion and lubricates oral soft tissues[47]. Although the acquired pellicle on the teeth has been extensively studied, the formation, components and function of the mucosal pellicle require further characterisation. Salivary mucins, specifically membrane-bound mucin (MUC1)[48] and gel-forming mucins (MUC5B[49] and MUC7[50]), are major components of the mucosal pellicle, which strongly adhere to the oral epithelial cell surface. Other bound proteins, including cystatin S, carbonic anhydrase VI, and IgA, do not strongly adhere to the surface and can easily be washed off[50]. Mucins appear to provide lubrication, preventing abrasion damage to the oral mucosa, whereas IgA may play a role in the so-called immune reservoir in the fight against invasion of pathogens[50]. When the oral mucosal fluid film is inadequate and there are symptoms of dry mouth, the protein concentrations remaining on the oral mucosa surface is significantly increased[43,44]. Although these proteins may not play a functional role within the mucosal pellicle, the significance or consequence of this derangement in the protein concentrations needs further investigation. Currently, it is not clear whether the oral mucosal pellicle has any influence on oral mucosal fluid absorption and whether the pellicle acts as a barrier to fluid transport via the mucosal cells.

An index often utilised to assess the hydration or wetness status is the oral mucosal film thickness, which is considered a better criterion for dry mouth evaluation[51]. This film thickness is calculated by measuring residual fluid. Collins and Dawes[52] determined that the total surface area of the oral cavity in adults was approximately 215 $cm^2$ and the area of the oral mucosa was estimated to be 178 $cm^2$, without significant differences between males and females. The wetness of the oral mucosa or the thickness of oral mucosal film depends mainly on unstimulated whole salivary flow rates, and to a lesser extent, the salivary secretion rates from minor salivary glands[44]. Increasing evidence indicates that a negative correlation exists between the thickness of oral mucosal film and the severity of dry mouth[41,44,53].

Oral mucosal film has several characteristics. The film thickness varies with intraoral locations (Table 1). In general, the mucosa at the hard palate and the upper lip has the thinnest film, in contrast to the thicker surface films of the tongue and the floor of the mouth (Table 1). This suggests that the perception of mucosal dryness is most likely generated on the hard palate and the upper lip, although the process and mechanisms of oral dryness sensation are considerably complex and remain poorly understood. Children have a similar thickness of oral mucosal film in comparison to adults. For example, the average film thickness in 5-year-old children is not significantly different from measured adult values, despite lower salivary flow rates in children[54]. It has been shown that the moisture content in epidermal stratum corneum decreases with age[55]. Similarly, there is a negative correlation in the lower labial mucosa between age, mucosal moisture and the labial mucosa function indices[56].

In contrast to salivary flow rates, the thickness of the oral mucosal film does not appear to correlate with circadian rhythms. The thickness measured in 20 individuals did not significantly vary between the middle morning and afternoon hours[40]. While further investigations on potential differences between daytime and night-time are lacking, this observation indicates that oral mucosal film or thickness is constant during daytime hours.

The most important clinical significance of the mucosal fluid film is its close association with oral dryness. In three groups of subjects (25 individuals each) with unstimulated mean salivary flow rates of 0.04,

**Table 1** The thickness of oral mucosal film in human subjects with normal salivary flow rates.

| Site of Oral Mucosa | DiSabato-Alordarski and Kleinberg (1996)[40] (n = 10) [Right, Left] | Wolff and Kleinberg (1998)[41] (n = 25) | Wolff and Kleinberg (1999)[42] (n = 10) | Won et al (2001)[43] (n = 30) | Lee et al (2002)[44] (n = 20) |
|---|---|---|---|---|---|
| Soft (posterior) palate | 20 , 30 | 15.9 | 32.9 | 21.6 | 26.4 |
| Hard (anterior) palate | 10, 10 | 15.9 | 41.6 | 7.3 | 7.6 |
| Floor of Mouth | | | | | |
| Duct | | 89.7 | 92.2 | | |
| Canine | | 39.3 | 82.1 | | |
| Anterior | 40, 50 | | | | |
| Posterior | 50, 50 | | | | |
| Buccal | | | | 31.4 | 48.8 |
| Cheek | | | | | |
| Anterior | | 38.3 | 77.4 | | |
| Posterior | | 44.1 | 93.0 | | |
| Above parotid duct | 60, 30 | 89.7 | | | |
| Near parotid duct | 60, 60 | 39.3 | | | |
| Anterior tongue | 50, 50 | 77.2 | 99.8 | | 57.2 |
| Posterior tongue | 70, 70 | 90.0 | 115.5 | | |
| Upper lip | 10, 10 | 42.0 | 69.6 | 23.9 | 17.6 |
| Lower lip | 10, 20 | 32.5 | 59.5 | 26.7 | 28.9 |

0.19, and 0.39 ml/min, Wolff et al[14] measured corresponding mucosal film thickness values of 22.4, 27.8 and 41.8 μm, respectively. The group with a salivary flow rate of 0.19 ml/min complained of xerostomia, despite a salivary flow rate higher than the criterion for hyposalivation (0.1 ml/min). However, the average oral mucosal film thickness in this group was 27.8 μm, which was much closer to the 22.4 μm value found in patients with severe hyposalivation (with a corresponding 0.04 ml/min salivary flow rate). Dawes and Odlum[57] hypothesised that dry mouth occurs when the residual saliva level is reduced by more than 29% of normal values. Therefore, the oral mucosal film thickness measured by Wolff et al[14] in their xerostomia group had decreased by 33.5%, compared to healthy controls. This observation indicates that the oral mucosal film thickness correlates with dry mouth and is a more useful index compared to salivary flow rates. However, further studies in this area are needed.

*Significance of minor salivary glands in oral mucosal wetness*

Saliva secreted by minor salivary glands constitutes approximately 7% to 8% of unstimulated and stimulated whole salivary volumes[58]. Despite the estimated minimal contributions from the minor salivary glands, reduced secretion rates from the palatal[59] and labial glands[60-63] are reported amongst individuals with xerostomia. A positive correlation was also observed between soft palatal mucosal wetness and secretion rates from minor salivary glands[43]. Therefore, it is suggested that

the secretions of minor salivary glands may play an important role in protecting the oral mucosa from the sensation of dry mouth[43,64].

Eliasson et al[65] measured the role of minor salivary gland secretion and xerostomia in 142 subjects (18 to 82 years old). Individuals with xerostomia had significantly lower unstimulated and stimulated labial secretion rates compared to secretion rates in individuals without xerostomia. Interestingly, individuals with xerostomia, but normal stimulated whole saliva secretion rates, had lower labial secretion rates compared to individuals without xerostomia. This observation suggests that labial salivary secretion may play an important role in the perception of oral mucosal wetness.

The role of minor salivary gland secretion is likely to be important in maintaining oral mucosal wetness since these glands are broadly distributed in the entire areas of the oral mucosa. Several studies[66,67] have observed that secretory rates of minor salivary glands are unaltered by gustatory stimulation with citric acid, a typical stimulant for increasing salivary flow rates from major glands. Thus, it is proposed that secretory rates of the minor salivary glands may be more accurate in reflecting dry mouth conditions. Satoh-Kuriwada et al[68] measured secretion rates of the lower labial glands and compared whole salivary flow rates in 66 individuals with xerostomia and 30 healthy control subjects. A significantly larger reduction in secretion rates from the labial glands was measured compared to the gum-stimulated whole salivary flow rates. They concluded that measurements of minor salivary gland secretion rates are a more accurate and sensitive method for the assessment of dry mouth[68].

## Oral mucosal absorption

Since the fluid in the oral mucosal film is in constant and close contact with the oral cavity's mucosal epithelia; the role of fluid, ions, nonelectrolytes, and protein exchange between the fluid film and the epithelium on oral health was investigated. Dawes[32] first proposed that absorption of significant fluid volumes is regulated by the oral mucosa. Thelin et al[33] later demonstrated that fluid and ion absorption across the oral mucosa is an important mechanism for fluid clearance. Numerous pharmacological studies have utilised the oral mucosa, specifically the sublingual and buccal mucosa, as a venue for drug delivery[69]. In fact, the oral mucosa has now become an increasingly popular and important mode for drug administration. The rationale for oral mucosal drug delivery is based on the tissue's unique characteristics. For example, the oral cavity has fewer enzymes to potentially interact or interfere with the administered drugs; drugs are absorbed directly into the circulation and the oral mucosa repairs rapidly. A large number of medications have been approved for oral mucosal delivery by the U.S. Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA). Examples are the orally disintegrating or dissolving tablets approved by the FDA and which are available in the U.S. These include alprazolam, aripiprazole, carbidopa, clonazepam, clozapine, desloratadine, donepezil hydrochloride, lamotrigine, lansoprazole, levodopa, loratadine, metoclopramide hydrochloride, mirtazapine, olanzapine, ondansetron hydrochloride, phentermine hydrochloride, prednisolone sodium phosphate, risperidone, rizatriptan benzoate, selegiline hydrochloride, vardenafil hydrochloride and zolmitriptan[70]. Orally disintegrating or thin film delivery of FDA- or EMA-approved drugs include asenapine, buprenorphrine, cannabidiol, fentanyl citrate, miconazole nitrate, midazolam, naloxone, ondansetron, δ-9-tetrahydrocannabinol and zolpidem tartrate[70]. In addition, there are currently wide-scale studies focusing on vaccine delivery via the sublingual mucosa, such as vaccines against the H1N1 influenza or HIV-1 virus[70].

### *Histological structure of the oral mucosa*

The oral mucosa consists of two major tissue constituents, the epithelium and the underlying lamina propria. The former is a stratified squamous tissue with up to 50 layers of cells, as found in the buccal mucosa. The epithelia in the regions of the hard palate, gingiva, and various sites of the dorsum of the tongue are keratinised epithelia. The cells in the keratinised epithelia consist of the basal, prickle, granular and keratinised layers. However, the epithelia in the regions of the lip, buccal, alveolar and soft palatal mucosa, including the underside of the tongue and the floor of mouth, are non-keratinised, consisting of the basal, prickle, intermediate and superficial cell layers[31]. The thickness of the oral mucosa varies considerably in different regions within the oral cavity (Table 2) due to the distinct functions required in different oral locations. The thinnest region within the human oral mucosa is the floor of mouth, with a thickness of 86 to 113 μm. In contrast, the buccal epithelium has a measured thickness up to 500 μm[71-73].

Beneath the epithelium is the lamina propria, a layer of connective tissue containing blood vessels, nerve fibers and a variety of cell populations, including fibroblasts, lymphocytes, plasma cells, macrophages, eosinophilic leukocytes, and mast cells. The function of the lamina propria is to provide nutrition and aid in defense. Underneath the lamina propria is the submu-

**Table 2** The thickness of human oral mucosa (μm).

| Mucosal Site | Klein-Szanto and Schroeder (1977)[71] a) | Prestin et al (2012)[72] b) | Almeida et al (2013)[73] c) |
|---|---|---|---|
| Floor of mouth | 86 ± 13 (6) | | |
| Anterior | | 99 ± 22 | |
| Lateral | | 113 ± 28 | |
| Hard Palate | 248 ± 37 (13) | 239 ± 57 | |
| Anterior palatal arch | | 124 ± 26 | |
| Palatal uvula | | 144 ± 30 | |
| Alveolar mucosa | 260 ± 40 (8) | | |
| Attached gingiva | 255 ± 57 (6) | | 233 (8) |
| Lip | 370 (8) | | |
| Buccal mucosa | 480 ± 90 (23) | 294 ± 68 | 307 (12) |
| Lateral tongue | | 216 ± 59 | 279 (12) |

a) Human oral mucosa samples were fixed and measured with a microscope. The results are expressed in mean ± standard deviation and subject numbers are indicated in parentheses. Data shown in the table[71] summarise results from different reports within the same laboratory[74,75]. b) The thickness of the oral mucosa was measured in the oral cavity in human subjects with optical coherence tomography and the results are expressed in mean ± standard deviation from 143 healthy volunteers. c) Human oral mucosa samples were fixed with paraffin inclusion techniques, stained with hematoxylin-eosin (HE), and mucosa thickness was measured with an Olympus BX41 microscope.

cosa, containing dense connective tissue, blood vessels, lymphatic vessels, nerve fibers, adipose tissue and small salivary glands. It provides support and nutrition to the epithelium. An additional function of the submucosa is to attach to the underlying tissue, muscle or bone. It has been well-documented that the major barrier function of the oral mucosa is found in the top layers of the epithelium. It is important to note that the lamina propria does not provide a substantial barrier to permeations of fluids or other molecules[76,77].

The histological structure of the keratinised oral mucosa is substantially similar to that of skin epidermis. The primary function of the epidermis is to prevent transepidermal water loss (TEWL) from underlying tissues. A major function of the oral mucosa also is to prevent transmucosal water loss (TMWL) and the latter has been proposed as an index for mucosal water retention[78]. In normal skin, the stratum corneum, the outermost epidermal layer, constitutes the main barrier against bidirectional diffusional transport[79]. The significance of this barrier is to preserve hydration of the stratum corneum's outer layer, a critical function for maintaining skin flexibility. The low permeability also generates a water gradient in the stratum corneum, maintaining sufficient cell layer hydration for enzymatic reactions required for metabolic processes, such as desquamation[80,81]. This property is known as stratum corneum moisturisation, which is dependent on the barrier formed by the stratum corneum, the corneocyte envelopes, the intercellular lamellar lipids and natural moisturising factors (NMFs) (see below).

### *Water absorption*

Although a major barrier exists for the movement of various molecules across the oral mucosal epithelia, permeation of fluid, electrolytes, and other macromolecules, especially water flux across the oral epithelia and the entire mucosa, occurs in regulated amounts. Dawes[32] proposed that relatively large volumes of water are absorbed by the oral mucosa. This is based on the osmotic pressure generated by saliva, which is approximately one sixth of extracellular fluid, and creates a driving force for water to traverse across the oral mucosa. Using the average surface area of the oral mucosa at 178 $cm^2$ [52] and a measured water permeability coeffi-

**Table 3** Water permeability in the oral mucosa.

| Mucosal region | $K_p$ ($10^{-5}$ cm $min^{-1}$)* | Investigator (year) |
|---|---|---|
| **Human** | | |
| Buccal | | |
| Isolated | 5.79 | Lesch et al (1989)[87] |
| Isolated | 32.80 | Selvaratnam et al (2001)[88] |
| Cultured | 35.90 | Selvaratnam et al (2001)[88] |
| Hard palatal | | |
| Isolated | 40.40 | Selvaratnam et al (2001)[88] |
| Cultured | 61.50 | Selvaratnam et al (2001)[88] |
| Tongue | | |
| Lateral border | 7.72 | Lesch et al (1989)[87] |
| Ventral | 48.00 | Healy et al (2000)[82] |
| | 49.60 | Howie et al (2001)[83] |
| Floor of Mouth | 9.73 | Lesch et al (1989)[87] |
| **Nonhuman Primate** | | |
| Buccal | 2.40 | Nielsen and Rassing (2000) [89] |
| **Porcine** | | |
| Buccal | 4.51 | Squier and Hall (1985)[84] |
| | 0.82 | Nielsen and Rassing (2000)[89] |
| Floor of Mouth | 7.53 | Squier and Hall (1985)[84] |
| Gingival | 2.79 | Squier and Hall (1985)[83] |
| **Canine** | | |
| Buccal | 306.60 | Galey et al (1976)[90] |

*Water permeability coefficient ($K_p$) was measured with $^3H_2O$ in freshly isolated or cultured human, non-human primate, porcine or canine oral mucosa. $K_p$ was calculated using the equation[91]: $K_p = Q/[A(C_0 - C_i)\ t]$, where $Q$ (mol) is the quantity of $^3H_2O$ crossing the mucosa; $A$ is the area ($cm^2$) of the mucosa; $C_0$ is the concentration (mol/l) of $^3H_2O$ outside of the mucosa; $C_i$ is the concentration (mol/l) of $^3H_2O$ inside of the mucosa; and $t$ is time (min). $K_p$ values were expressed in different units in the original publications (i.e. $10^{-7}$ cm $sec^{-1}$ or $10^{-4}$ cm $min^{-1}$) and have been converted to similar units ($10^{-5}$ cm $min^{-1}$) for direct comparison.

cient ($K_p$) of 4.8 x $10^{-4}$ cm $min^{-1}$ across the human oral mucosa[82,83], the maximum rate of water transfer from the saliva across the oral mucosa is calculated to be 0.19 ml/min[32]. This corresponds to 274 ml/day of fluid absorption, assuming a stable rate of fluid movement. Thelin et al[33], utilising the data observed in healthy volunteers, estimated a total oral cavity fluid absorption rate of 0.564 ml/min. This would be consistent with much larger amounts of fluid volume absorption, again assuming a steady state rate. Although these estimations were not intended to encourage accurate movement of fluid volumes due to multiple uncertainties or assumptions, these novel water absorption models indicate that the absorptive properties of the oral mucosa may contribute significantly to the pathogenesis of dry mouth.

The oral mucosa has a higher permeability to water compared to the skin. Measurements from different oral regions indicate that the buccal mucosa has the highest permeability coefficient (Table 3). The permeability barrier of the oral mucosa is measured from the surface or upper one third of the epithelium[84-86], corresponding to the superficial layer of the whole mucosa.

*Mechanisms mediating water absorption:* although the specific mechanisms mediating oral mucosal water

absorption and fluid flux remain unclear, the process does not require active epithelial transport systems, since inhibiting energy production with potassium cyanide (KCN) does not alter water permeability[84]. The precise locations for transepithelial water movement across the oral mucosal epithelium also remains unclear. However, it has been suggested that there are two routes or pathways, transcellular and paracellular, where the regulated movement of water and other substances cross the oral mucosal barrier. Since oral epithelia have multiple cell layers, movement via these pathways are expected to be complex.

*Transcellular absorption of water via aquaporins:* aquaporins (AQPs) are a family of proteins mediating rapid water flow across cell membranes with low water permeability. AQP functions and isoforms can be divided into three subgroups[92]: 1) water-selective or classic AQPs, including AQPs 1, 2, 4 and 5; 2) aquaglyceroporins, including AQPs 3, 7, 9 and 10, which facilitate passage of water and small uncharged molecules, such as glycerol and other small solutes like urea[93]; and 3) unorthodox AQPs, including AQPs 6, 8, 11 and 12. Currently, it is unclear which AQP isoforms exist in the oral mucosa and there are limited studies characterising the expression of aquaglyceroporins in the oral mucosa.

AQP3 is the most frequently studied aquaglyceroporin isoform. It is the most abundant aquaglyceroporin in the skin, mediating both water and glycerol transport; it plays a vital role in maintaining hydration of the mammalian skin epidermis. In addition, AQP3 regulates lipid metabolism, proliferation and differentiation of keratinocytes, wound healing, migration of water channels within the cells and skin tumorigenesis[94]. AQP3 exists in several cell layers, including the basal[95,96] and the prickle layers of the epidermis[94], but not in the dermis[94]. The subcellular location of AQP3 expression has not been extensively studied, but strong expression has been identified in the plasma membranes, including the prickle and basal cell layers, and in all keratinised cells[94,95]. Recently it has also been shown, with Western blotting, that AQP3 also exists in human skin stratum corneum cells[97] and its proposed role is to act as a barrier against water loss, improving hydration below the cornified layer[98-100]. Measurements of water transport across stripped human skin and reconstructed epidermis confirmed that water transport was sensitive to AQP inhibition with $HgCl_2$ or by lowering the pH, indicating that AQP3 mediates water permeability and is essential for epidermis hydration[95]. AQP3-knockout mice have reduced stratum corneum water content and elasticity in comparison to wild-type mice[101], and are unable to increase hydration in response to high humidity[98,99]. The glycerol content in the epidermis, specifically in the stratum corneum, is reduced in AQP3-knockout mice, presumably from decreased glycerol transport. Although the permeability to water and glycerol in the stratum corneum was significantly reduced in AQP3-knockout mice, the topical or systematic glycerol administration restored all abnormal phenotypes, including the reduced stratum corneum hydration. These results suggest that the functional alterations induced by decreased expression or function of AQP3 are due to the decrease in glycerol transport[98].

Earlier studies found that AQP3, in addition to AQP4 and AQP5, were expressed in the suprabasal layers of the oral epithelium during tooth development in humans and mice[102,103]. However, the function of these AQPs in the developing oral epithelium is unclear. Recently, Poveda et al[104] determined the expression of aquaglyceroporins in rat oral stratified squamous epithelia in the palatal and buccal mucosae, the inferior side of the tongue and the floor of the mouth, by utilising RT-PCR, immunofluorescence and immunogold electron microscopy. They found that AQP3 and AQP9 mRNAs were expressed in these oral epithelia and AQP3 protein was identified by immunostaining in specific epithelia. AQP3 proteins are accumulated and transported to the plasma membrane, where they incorporate into the cornified or surface layers. The location of AQP3 suggests that this aquaglyceroporin may facilitate water flux induced by an osmotic gradient across the plasma membrane, hence regulating the permeability of the mucosal barrier. However, whether AQP3 has a similar function in facilitating glycerol transport and whether glycerol plays a similar role in epithelial hydration in the oral mucosa requires further investigation.

Another aquaglyceroporin, AQP9, is expressed in various tissues, including the liver, brain, skin, epididymis, Leydig cells and leukocytes[105]. AQP9 expression differs and is highly restricted to the upper stratum granulosum in the human epidermis, where AQP3 expression is limited[96]. AQP9 also co-exists with occludin, suggesting that AQP9 and the tight junction may co-regulate transport of glycerol, urea or other small solutes[96].

AQP9 appears to be capable of mediating permeation of a broad spectrum of uncharged solutes, including glycerol, mannitol, sorbitol, urea, thiourea, adenine, pyrimidines (uracil and fluorouracil [5-FU]) and monocarboxylates (lactate and β-hydroxybutyrate)[105,106]. However, contradictory results exist in terms of AQP9's role in water and urea permeability. The expression of AQP9 in normal human epidermal keratinocytes did not alter osmotic water permeability and is consistent

with the lack of change in water and urea permeability measured in erythrocytes from AQP9-null and wild-type mice[107]. AQP9 also mediates methylarsonic acid and arsenite transport[108-110]. Although the function of AQP9 in the epidermis is not well characterised, it is hypothesised that this aquaglyceroporin isoform is also required for normal skin hydration and elasticity since its expression is in the outermost layer of the stratum granulosum. However, further evidence is required to identify distinct physiological role(s).

AQP9 is expressed in the marginal areas of the basal and suprabasal layers of rat oral stratified squamous epithelia in the palate, buccal mucosa, the inferior side of the tongue and the floor of the mouth[104]. Therefore, it was proposed that AQP9 may act as a channel for glycerol uptake from general circulation. Nonetheless, the role of glycerol in oral mucosal hydration remains unclear.

*Hyperosmotic stress increases AQP3 and AQP9 expression:* the expression of AQP3 and AQP9 is significantly increased in response to hyperosmotic stress in several specific cell types. Incubation of human keratinocytes in hypertonic medium through the addition of 200 mM sorbitol significantly increased AQP3 mRNA expression. This effect is the direct result of changes in medium osmolality and not by the type of solute added to the culture medium, since 100 mM NaCl, 200 mM mannitol, 200 mM glucose or 200 mM sucrose produced similar effects. However, hypertonic challenges did not alter AQP9 expression[111]. Arima et al[112] observed that in rat brain cortical astrocytes, hyperosmotic stress by addition of mannitol or sorbitol increased the expression of AQP4 and AQP9 mRNA and the increased expression was inhibited by 10 μM SB203580, a p38 mitogen-activated protein kinase (MAPK) inhibitor. Yang et al[113] also exposed rat brain astrocytes to hyperosmotic medium and observed an increase in the expressions of AQP isoforms 3, 4, 8, and 9, and p38 MAPK inhibition blocked the increase in AQP4 and AQP9 expression.

It is still unclear which other AQP isoforms are expressed in the oral mucosa; however, AQP3 and AQP9 may facilitate both transcellular osmotic water flow[104] and glycerol transport in oral epithelial cells. Since their expression appears to be regulated by the osmotic status of oral fluids, these AQPs may play a key role in the hydration of the oral mucosa. Considering that the oral mucosa is a multilayer structure, how water and ions traverse across multiple cell layers to reach the circulatory blood vessels needs further investigation. More studies are also needed to elucidate the AQP isoform expression and their localisation within the cell. Further delineation of these AQP functions, including their roles in oral mucosal fluid transport, will contribute to a better understanding of the mucosal hydration processes.

*Paracellular water absorption through tight junctions:* tight junctions (TJs) are major intercellular barriers with a primary function, which form a sealing complex between cells, establishing not only barriers, but channels for water, ions and to a much lesser magnitude, large molecules. TJs are essential in salivary secretory function (see review by Zhang et al)[114], but whether they also significantly contribute to oral mucosal hydration or dehydration has not been extensively studied. In measuring the permeability of porcine oral mucosa to polyethylene glycol (PEGs), Goswami et al[115] found the pore radius of aqueous pathways were 18 to 22 Å in the buccal mucosa and 30 to 53 Å in the sublingual mucosa. However, these pore sizes are much larger than reported radii ( < 9 Å) of aqueous pathways in other epithelia, such as the kidney, intestine, lung and salivary glands[114]. This discrepancy requires further investigation.

Although their distribution varies considerably, a number of TJ mRNAs and proteins are expressed in the mammalian epidermis, a tissue with similar characteristics as oral mucosa, including claudins 1, 4, 5, 6, 7, 10, 11, 12, 17 and 18, occludin, JAM-A, ZO-1, MUPP-1 and cingulin[116]. TJs are present in the uppermost epidermal living cell layers of the stratum granulosum, including claudin-1, claudin-4, occludin, ZO-1, ZO-2 and cingulin. These major TJ proteins are expressed in stratified epithelia, and are also expressed in the gingival, lingual and other oral mucosa[117]. Measurements of occludin in the oral mucosa revealed that TJs exist in the upper spinous and granular keratinocytes[118]. Studies using TJ protein-knockout animal models suggest that TJ proteins are essential for regulating normal TEWL. Claudin-1 is critical in retaining water, since claudin-1-knockout mice die within 1 day after birth due to increased TEWL[119]. However, Ouban and Ahmed[120] observed that normal squamous mucosae from the tongue, gingiva and palate have only low levels of claudin-1 expression.

Since TJ formation is dependent on established adherens junctions (AJs), paracellular water permeability is also affected by the stability of AJs. Epidermal E-cadherin-knockout mice showed increased TEWL and died shortly after birth due to the leaky TJs[121]. E-cadherin knockout prevented TJ formation since TJ assembly is dependent on AJs[122]. However, in oral mucosal epithelia, the major intercellular junctions are primarily desmosomes, TJs and gap junctions[31].

Whether desmosomes influence TJ barrier and channel function remains unclear. In cultured human gingival epithelial cell monolayers, the formation of intercellular desmosomes appears to be dependent on $Ca^{2+}$ concentration in the culture medium; low $Ca^{2+}$ (0.3 mM) medium abolishes desmosome formation[123]. Given that desmosomes are strong intercellular connections, with a critical role in maintaining cell-to-cell distance, it is likely that disturbances in desmosome formation may impair TJ function. Studies focusing on the properties of TJs should prove invaluable in providing insights on the mechanisms regulating water transport in the oral mucosa.

Although TJs are likely to be critical for fluid transport in the oral mucosa, the characteristics of water movement and its regulation as it passes through the barriers are undefined. Further investigations are needed in characterising which TJ proteins are expressed in oral mucosal cells, what roles these proteins play in water absorption and secretion, and how the function of these proteins are regulated.

*Water absorption coupled with glucose transport:* in the small intestinal mucosa, $Na^+$ and glucose transport through the $Na^+$/glucose cotransporter (SGLUT1) creates dilation of the tight junctions, mediating bulk absorption of water and other nutrients through the paracellular pathway[124,125]. It has been reported that the stoichiometry of SGLUT1 transport involves two $Na^+$ ions, one glucose molecule and 249 water molecules[126], which would provide a high-capacity water transport mechanism. It has been shown that oral mucosal cells express SGLUT1 and glucose transporter (GLUT)-1, -2 and -3 (see below). Thus, it is highly likely that the oral mucosal cells possess a similar water transport system as identified in the intestinal epithelium. However, studies to explore this unique system in oral mucosal cells are needed.

### *Ion absorption*

It was long thought that the oral epithelium did not have absorptive properties since its function was to form an impermeable barrier[31]; whether this concept was valid was the subject of investigations in the 1960s. These early studies confirmed that $Na^+$ and $K^+$ were transported across the oral mucosa (see review by Thelin et al)[33]. In 1988, Orlando et al[127] measured transmucosal electrical potential differences (PD) and short-circuit currents ($I_{sc}$) using Ussing chambers from isolated hamster, rabbit, dog, and human buccal mucosae, and measured PD values from -18 to -39 mV across these mucosal samples. It was also found that a component of the $I_{sc}$ across the mucosae was generated by $Na^+$ flux, since 18% and 32% inhibition was measured following application of $10^{-6}$ M and $10^{-4}$ M amiloride, respectively, a $Na^+$ channel and $Na^+/H^+$ exchanger inhibitor. In addition the $I_{sc}$ was dependent on extracellular $Na^+$ concentrations and complete replacement of $Na^+$ by choline in the bathing solution reduced $I_{sc}$ by 60%. Studies with $^{14}$C-mannitol flux also showed that the paracellular pathway contributed approximately 21% to the conductance. Similar results were obtained by altering ion transport properties and barrier function in the buccal mucosa, using smokeless tobacco[128]. It was concluded that the buccal mucosa PD was generated by $Na^+$ absorption via epithelial $Na^+$ channels (ENaC) and by $Na^+/K^+$ pumps. Specifically, $Na^+$ enters oral mucosal cells through ENaC and is extruded out of the basolateral aspect of the cell by the $Na^+/K^+$-pump. This would be consistent with the baseline mean $Na^+$ concentration of 20 mM in saliva[129,130] and only 0.3 to 0.5 mM measured within buccal mucosal cells[131]. This gradient provides a strong driving force for transcellular $Na^+$ transport, whereas, the $Na^+$ concentration in the interstitial space is much higher and the gradient is reversed. Therefore, $Na^+$ traversing across TJs seems less likely. Based on the studies by Orlando et al[127] and others, therapies utilising ENaC inhibitors would potentially treat the symptoms of dry mouth by inhibiting $Na^+$ transport, thereby blocking water absorption and increasing mucosal hydration[33].

The study by Orlando et al[127] raises important issues. Firstly, although transepithelial $Na^+$ absorption has been thought to mediate oral mucosal hydration through ENaC, only 60% of the $I_{sc}$ was dependent on $Na^+$ containing solutions. In addition, amiloride at low and high concentrations ($10^{-6}$ M and $10^{-4}$ M, respectively) inhibited only small portions of basal $I_{sc}$ (18% and 32%). Therefore, other ion transport mechanisms are likely to be involved. Secondly, the evidence indicates that ion flux via paracellular pathways may play a more significant role. Finally, as discussed by Orlando et al[127], it will be important to further investigate the role of anions, such as $Cl^-$ or $HCO_3^-$ efflux, on transepithelial movement and their contribution to oral hydration.

McMurchie et al[132] measured $^{22}Na^+$ uptake in isolated human cheek (buccal mucosal) cells and found a Michaelis constant ($K_m$) for $Na^+$ uptake from 5.7 mM of extracellular $Na^+$ and a maximal uptake rate of 4.3 nM $Na^+$/mg protein/30 s. The $Na^+$ uptake was dependent on a $H^+$ gradient with a $K_m$ for 0.17 µM of intracellular $H^+$. This $Na^+$ uptake was inhibited by amiloride ($10^{-4}$ M), 5-(N-methyl-N-isobutyl)-amiloride (MIA) and 5-(N,N-hexamethylene)-amiloride (NNHA), but not by $Na^+/K^+$-ATPase or $Na^+/K^+/2Cl^-$ cotransporter inhibitor oua-

bain or bumetanide, respectively. This suggests that $Na^+$ uptake is mediated by the $Na^+/H^+$ exchanger.

Dysfunctional $H^+$ gradient-dependent $Na^+$ uptake in cells has been associated with hypertension. Uptake of $^{22}Na^+$ in buccal mucosal cells, isolated from hypertensive adult human subjects, was 45% lower than in cells obtained from control subjects[133]. Similarly, the activity of the $Na^+/H^+$ exchanger in buccal mucosal cells isolated from hypertensive adolescents was 50% lower compared with adolescents with lower or normal blood pressures[134]. Also in buccal mucosal cells, alterations in the cellular ionic concentrations also alter $Na^+$ uptake. Utilising the $K^+$ or $Na^+$ ionophores, valinomycin or gramicidin, $Na^+$ uptake was increased by 177% and 227%, respectively. Treatment with a dual $K^+$ and $H^+$ ionophore enhanced $Na^+$ uptake by 654%[135]. These results demonstrated that by increasing cytosolic $H^+$, thereby increasing the $H^+$ gradient, this significantly potentiates $Na^+$ uptake. Interestingly, incubation of these same cells with saliva[135] or low molecular weight salivary components[135,136] for 2 h significantly stimulated $Na^+$ uptake. $Na^+/H^+$ could also be stimulated with pre-incubation with 25 mM potassium phosphate buffer. In these studies, the $V_{max}$ of the $Na^+/H^+$ exchanger activity was increased, whereas $K_m$ for extracellular $Na^+$ was unchanged[135].

Since salivary concentrations of $K^+$ and phosphate are high, it is possible that the stimulation of $Na^+/H^+$ exchange is by the K salts of $PO_4^{3-}$, $HPO_4^{2-}$ or $H_2PO_4^-$. Physiologically, in vivo pH values of oral fluids are likely to be similar to the cytosolic pH in oral mucosal cells and large proton gradients may not exist. It is also questionable if $Na^+$ transport mechanisms are designed to mediate significant $Na^+$ absorption and ionic components or whether substances in the saliva play a more significant role stimulating $Na^+$ absorption through transcellular pathways. Further research is needed to address such issues and what role higher salivary $K^+$ and phosphate content, and its association with $Na^+$ absorption, may have on symptoms of dry mouth or overall oral health.

The relationship between $Na^+$ transport and water absorption in the oral mucosa has not been established. As $Na^+$ concentration is higher in oral fluids than in mucosal cells, $Na^+$ is likely to be absorbed by ENaC or other mechanisms, into the cells and then extruded by the $Na^+/K^+$-pump. It is unlikely that significant amounts of $Na^+$ traverse across the paracellular pathway considering that TJs may lack a substantial $Na^+$ gradient as a driving force. On the other hand, water may move through the paracellular pathway more easily since osmotic gradients seem to exist across the oral mucosa. Investigation of these issues is of high significance in revealing the mechanisms associated with dry mouth.

*Nonelectrolyte absorption*

The oral mucosal absorption of endogenous nonelectrolytes produced by the body and secreted into saliva is unclear. This is partly due to a limited number of studies, which identify and characterise nonelectrolytes secreted by the salivary glands. Urea and sugars, such as glucose, are secreted into saliva in relatively large amounts, but their absorption by the oral mucosa is difficult to quantify.

Exogenous or foreign nonelectrolytes, such as sugar alcohols, including maltitol, erythritol, mannitol, sorbitol, xylitol and isomalt, are widely used as sweeteners in oral health products, including toothpastes, chewing gums and mouthwashes. Mannitol has also been used as a tracer to examine paracellular permeability. Absorption of most of these nonelectrolytes, especially various types of sugars, appears to be coupled with water movement[125,126]. The influence of these nonelectrolytes on the function of oral mucosa has received increasing attention in recent years. The transport of exogenous nonelectrolytes and their impact on the oral mucosa will be discussed separately.

*Sugars:* saliva contains low concentrations of glucose[137-139] and other forms of sugar, such as sucrose. The glucose concentration in saliva collected from healthy human subjects has been reported to be 0.03 to 0.13 mM with an average level of 0.07 mM[137-140]. Sugars are absorbed by the oral mucosa, including D-glucose and sucrose[141-143], although the physiological significance of sugar absorption by the oral mucosa remains undefined. Manning and Evered[144] observed that sugars were transported across the buccal mucosa by a carrier-mediated mechanism and the absorption of D-glucose, galactose and 3-O-methyl-D-glucose were partly dependent on $Na^+$. Subsequently, several studies demonstrated that oral mucosal epithelial cells expressed sugar transport mechanisms, including glucose transporter (GLUT)-1, -2, and -3 and $Na^+$/glucose cotransporter (SGLUT1). GLUT1 is extensively expressed in all epithelial cell layers of the oral mucosa, reflecting the high turnover rates[145]. Studies carried out *in vitro*, elucidating the mechanisms of glucose uptake in isolated human oral mucosal cells from the buccal mucosa and the dorsum of the tongue by Oyama et al[146], found that D-glucose uptake was greater in cells from the tongue's dorsum compared to the buccal mucosa. Glucose uptake was inhibited more extensively by 2-deoxy-D-glucose, a substrate of the facilitative glu-

cose transporters compared to α-methyl-D-glucoside, a specific substrate of SGLT1. This suggests that a significant portion of glucose transport is via facilitative transporters compared to transport through SGLUT1[146]. Furthermore, it has been demonstrated that other glucose transporters, GLUT1 and GLUT3, also participate in glucose uptake in oral mucosal cells[146]. However, not all glucose is transported via glucose transporters. In isolated and cultured human oral mucosal cells, approximately 40% of $^{14}$C-D-glucose movement is via transporters and the remainder is likely to be through paracellular pathways, potentially via tight junctions[147].

In enterocytes, glucose and fructose uptake is mediated by SGLUT1 and GLUT5, respectively, and these monosaccharides are transported out of the cell through GLUT2 located on the basolateral membrane[148]. It is unclear if glucose transport in oral mucosal cells is mediated by a similar mechanism. Since glucose transport appears to be associated with water transport in small intestinal mucosal cells, delineation of oral mucosal glucose transport via a similar system and a possible association with water movement would be of importance.

*Urea:* urea is synthesised in the body by the urea cycle in the liver, transported through blood, and excreted by the kidney. Urea is a water-soluble and non-toxic polar nonelectrolyte and is involved in many metabolic processes, most importantly with nitrogen excretion. The discovery of a cellular urea transporter (UT) ended the long-held assumption that urea moved freely across cell membranes. There are two types of urea transporters, UT-A and UT-B. The former consists of a group of subtypes, UT-A1 to UT-A5, with additional mRNA isoforms; UT-B consists of UT-B1 and UT-B2 (see review by Sands[149] and Klein et al[150]). UT-A isoforms are expressed mainly in kidneys and mediate urea reabsorption. In addition, the other UT-A isoforms have been isolated in the liver (A2), heart (A2b) and testis (A5)[149], but their function in these organs remains poorly understood. UT-B has been isolated in a number of tissues, including red blood cells and endothelial cells[149], as well as the human bladder[151], rat urothelia[152], the rat gastrointestinal tract[153] and the human colon[154]. It is important to note that UT-B is also expressed in bovine parotid glands[155]. However, whether oral mucosal cells express urea transporters remains unclear.

The role of urea transporters in tissues other than kidney and red blood cells is still poorly understood. It has been hypothesised that these tissues require urea removal following endogenous intracellular production (ureagenesis). For example, urea is likely to be produced by active polyamine synthesis since urea is a by-product of polyamine metabolism. The oral mucosal cells have a fast turnover; therefore, it is possible that high urea synthesis reflects increased polyamine metabolism. It is well known that saliva contains urea. Salivary urea appears to play a critical role in maintaining oral pH, and as a result, prevents dental caries. For example, salivary urea concentrations were significantly lower (3.4 to 5.5 mM) in adolescents with high decayed, missed and filled teeth (DMFT) indices compared to the levels (5.5 to 9.1 mM) found in adolescents with low DMFT indices[156]. Urea has been widely used in oral health products, such as toothpastes, chewing gums, and mouth rinse liquids, to improve the oral fluid pH and to prevent caries. Urea can also enhance stratum corneum hydration and has been used topically to improve barrier function and increase hydration in the skin.

There is very little information available regarding urea transport in the oral mucosa. Nonetheless, the oral mucosa has been proposed to absorb urea[141-143,157]. Early studies[141] demonstrated urea absorption by the buccal mucosa in rats. Dawes and Dibdin[158] measured the levels of urea in the saliva of human subjects chewing urea-containing gum and found that the measured residual urea was 81.5%. To explore the possibility that urea was absorbed by the oral mucosa, Dawes[157] measured urea recovery in 10 individuals after chewing gums containing 27.3 mg urea and 0.5 mg Phenol red as a recovery marker. After 10 min, the urea recovery was 85.7%, whereas the Phenol red recovery was 96.7%. It was concluded that urea was partly absorbed by the oral mucosa. Therefore, investigations examining the expression of urea transporters in oral mucosal cells and the characteristics of urea absorption will be important.

*Amino acids:* human saliva contains a considerable level of free L-amino acids[159-161], although the significance of their existence in saliva remains poorly delineated. Measurements of amino acid levels in saliva have been utilised as biomarkers for physiological conditions or diseases, such as migraine headaches[162]; however, oral biological studies have focused on amino acid effects on ammonium production and incidences of caries[159,160].

Few studies have been conducted, which measure amino acid absorption by the oral mucosa. Vadgama and Evered[163] confirmed that amino acids were absorbed by the oral mucosa. A solution containing amino acids was administered to three subjects, with the solution remaining in the subjects' mouth for 5 min and then being subsequently collected. The remaining amino acid concentration measured was standardised with inulin, an insoluble and non-absorbable polysaccharide. The recovery of inulin was > 99%, whereas the absorp-

tion of all 15 tested L-amino acids was concentration-dependent. Absorption rates correlated with the amino acid concentrations in the solution. The long-chain neutral amino acids were absorbed rapidly, whereas the short-chain neutral amino acids and glycine, serine, and threonine were absorbed slowly. Overall, if amino acid concentrations were similar, the rapidly absorbing amino acid group included phenylalanine, arginine, methionine, leucine, and isoleucine; the intermediate absorbing group included proline, glycine, histidine, lysine, alanine and serine; and the slower absorbing group included aspartic acid, threonine, glutamic acid and valine. Replacing $Na^+$ with $K^+$ in the buffering solution reduced the uptake rates significantly when amino acid concentrations were low. With increasing amino acid concentrations, the influence by $K^+$ in the solution was weakened. These results indicate that specific amino acid transport is $Na^+$-dependent and is consistent with the current understanding of amino acid transport in multiple organ systems[164].

With the limited data provided since Vadgama and Evered's report[163], further investigations focusing on the significance of amino acid transport mechanisms in the oral mucosa are needed. Specifically, the following issues need to be examined: the significance of amino acid absorption and secretion by the oral mucosa; the amino acid transport systems expressed in the oral mucosa; the mechanisms by which amino acids are transported by oral mucosal epithelial cells; the regulation of amino acid transport, typically which factors play a role in controlling the transport systems; and the role of amino acid transport in dry mouth and oral mucosal disorders as well as other dental disorders in the oral cavity.

**Oral mucosal secretion**

Although it has been shown that water, ions, and nonelectrolytes are absorbed by oral mucosal epithelia, the question as to whether the oral mucosa also secretes fluid and ions into the oral cavity remains unaddressed. In the airway mucosa, $Cl^-$ secretion by epithelial cells is critical for maintenance of normal airway mucosal hydration and for appropriate fluid levels; mucosal dehydration is a functional derangement in cystic fibrosis and results from decreased $Cl^-$ secretion via the cystic fibrosis transmembrane conductance regulator (CFTR) and increased $Na^+$ absorption through ENaC[33,165,166]. Based on airway mucosal models and since the oral mucosa is an absorptive epithelium, Thelin et al[33] proposed that the oral mucosal epithelium share similar mechanisms regulating oral mucosal hydration and dry mouth was the result of increased $Na^+$ absorption and decreased $Cl^-$ secretion. Since $Na^+$ absorption through ENaC or $Na^+/H^+$ exchanger has been identified in oral mucosal epithelia, it was reasonable to hypothesise that inhibiting ENaC and/or $Na^+/H^+$ exchanger may improve oral mucosal hydration. There have been attempts to change mucosal hydration by reducing $Na^+$ absorption, but currently no studies have been performed attempting to increase $Cl^-$ secretion. This may reflect the issue of whether oral mucosal cells express $Cl^-$ channels or anion exchanger. In addition, the role of $Cl^-$ efflux in oral mucosal hydration remains to be established. Singh et al[167] conducted a Phase I clinical study evaluating the effects of blocking $Na^+$ absorption utilising an ENaC inhibitor in patients with primary Sjögren's syndrome and observed that dry mouth symptoms significantly improved. Although the significance of $Cl^-$ secretion in oral mucosal hydration and dry mouth is uncertain, the similarities between $Cl^-$ secretory dysfunction in the airway epithelium and mucosal dehydration[165,166] make it an intriguing subject.

$Cl^-$ efflux in other similar epithelia, such as epidermal keratinocytes and intestinal mucosa, is mediated by the $Cl^-$ channel or anion exchanger. Mastrocola et al[168] observed that human keratinocytes had two $Cl^-$ transport mechanisms with the anion exchanger accounting for 50% and the $Cl^-$ channel contributing 40% of $Cl^-$ efflux. Similarly, studies of $Cl^-$ transport in cultured human keratinocytes using patch-clamps, Ussing chambers and isotope efflux analysis confirmed that $Cl^-$ efflux is mediated by a $Ca^{2+}$-activated $Cl^-$ channel, not sensitive to protein kinase A or C activation[169]. In mouse intestinal cells and human Caco-2 cells, $Cl^-$ and water secretion are coupled[170]. $Cl^-$ secretion can be activated by application of glucose to the apical (luminal) side, but not the basolateral (serosal) side. Glucose also induces an increase in intracellular $Ca^{2+}$, consistent with electrogenic $Cl^-$ efflux mediated by a specific type of $Cl^-$ channel. The addition of a $Ca^{2+}$-activated $Cl^-$ channel blocker niflumic acid or intracellular $Ca^{2+}$ chelators partially inhibited the $Cl^-$ efflux. The glucose-stimulated $Cl^-$ efflux is also partly inhibited by $Cl^-$/anion exchange inhibitors, suggesting that $Cl^-$/anion exchange plays a significant role in mediating $Cl^-$ efflux. Interestingly, low concentrations (0.6 mM) of glucose induce a significant increase in $Cl^-$ secretion[170]. It is possible that oral mucosal epithelial cells possess various $Cl^-$ channels and/or $Cl^-$/anion exchangers, similar to other epithelial cells. Further studies in this area could have a tremendous impact on oral mucosal hydration models and the pathogenesis of dry mouth.

In summary, there are very few, if any, studies conducted to examine fluid and ion secretion from the oral mucosa. This is partly due to the presumption that the oral mucosal epithelia are considered absorptive and not secretory. However, the histological characteristics of the oral mucosal epithelia make water secretion possible, since oral epithelia have similar components, including TJs between cells, commonly found in other secretory epithelia. The oral mucosa has a medium level of permeability to water, i.e., less than gastrointestinal epithelia, but greater than skin. If a substantial proportion of water flux is mediated by TJs, the dry mouth condition is likely to establish a water gradient between the interstitial space and the mucosal surface, providing a driving force for water efflux via TJs. Future investigations would address the following questions: whether there is a difference in water absorption between healthy subjects and patients with dry mouth, including the dryness induced by Sjögren's syndrome, radiation therapy, and aging; how fluid efflux occurs in experimental conditions, such as in isolated mucosa using Ussing chamber; whether ENaC activity is altered in dry mouth; whether oral mucosal cells express $Cl^-$ channels or $Cl^-$/anion exchanger; and what role TJs and aquaporins play in fluid absorption and secretion.

## Factors influencing mucosal fluid and ion transport

### *Intercellular lipids*

The oral mucosa has a strong barrier function, highly similar to that in the epidermis. In the last four decades, researchers have conducted a series of investigations characterising the barrier function and permeability of the oral mucosa, concluding that the barrier function in the oral mucosal epithelium is similar to that found in the epidermis[86]. The oral mucosa is capable of blocking entry by foreign substances, such as chemicals, toxins and microbial agents. It also prevents water loss. This unique property is mediated by intercellular lipids released from membrane-coating granules (MCGs)[171,172], also termed lamellar bodies, specifically within the epidermis.

Lamellae of MCGs, formed in the stratum spinosum, move to the stratum granulosum and their contents are discharged into the intercellular space between the granular and keratinised layers through exocytosis[173-175]. In non-keratinised oral mucosal epithelia, MCG content are not lamellar but amorphous and are released into the intercellular spaces between the intermediate and superficial layers[86]. The regulation of MCG release remains poorly understood. It is assumed that any factors altering exocytosis have an impact on barrier function. Unfortunately, direct evidence supporting this association is still needed. Nevertheless, studies have observed that some factors influence MCG lipid release. For example, MCG release by the buccal mucosal epithelium is reduced in Zinc-deficient rats[176,177]. Long-term (24 months) application of smokeless tobacco applied to the cheek pouches of Syrian hamsters also abolished the release of MCGs, leading to lipid accumulation within the granular cells[178]. However, whether these changes in lipid secretion altered the water and electrolyte permeability was not specifically examined.

The shape difference of MCGs between the keratinised and non-keratinised oral epithelia results from their distinct lipid compositions. Accordingly, the permeability differences between keratinised and non-keratinised mucosae are also related to their intercellular lipid compositions[86]. Similar intercellular lipids released from MCGs in both keratinised and non-keratinised epithelia include phospholipids (38% to 44%), cholesterol (14% to 34%), and triglycerides (11% to 17%). However, the keratinised mucosa, closely resembling the epidermis, has more acylceramides and ceramides (6.3% to 9.1%) compared to the non-keratinised mucosa (less than 1%), whereas, the content of glycosylceramides is much higher in non-keratinised mucosa (6% to 17%) compared to keratinised mucosa (about 2%). In addition, the content of cholesteryl esters is significantly higher in non-keratinised mucosa (6.0% to 15.0%) than in keratinised mucosa (0.2% to 1.1%). Compared with the epidermis, the amount of acylceramides and ceramides is 25% to 50% less, a primary factor for greater water permeability in keratinised oral mucosa[86,179-181]. Although the neutral lipid components in non-keratinised epithelia have been proposed to effectively restrict the penetration of pathogens through the mucosa, including toxins and enzymes, the barrier to fluids and small molecules such as nonelectrolytes is not high.

Although the role of MCGs in reducing oral mucosal permeability to water, electrolytes and nonelectrolytes has been documented for decades, it remains unclear whether there are differences in the quality and quantity of MCGs in normal versus dry mouth oral mucosae. Additional well-designed trials are needed to quantify the impact of autoimmune disorders (Sjögren's syndrome) and other disorders associated with dry mouth on the expression and composition of MCGs.

### *Natural moisturising factors (NMFs)*

Although intercellular lipids are considered a major barrier in epidermal and oral mucosal epithelia, it has been

proposed that NMFs may also play a vital role in barrier function. NMFs include low-molecular-weight, water-soluble compounds, such as free amino acids and their derivatives, pyrrolidone carboxylic acid, lactate, citrate, urea, sugars and certain types of inorganic ions[80]. The amino acids and derivatives are derived primarily from filaggrin, a special protein synthesised in the keratohyalin granules within cells of the stratum granulosum. Recent studies have proposed that lactate and potassium are more important for maintaining water content in the epidermis[56,182]. These low-molecular-weight substances have a strong capacity for water retention[80]. They account for 5% to 30% of the total dry weight of stratum corneum[80,183], which is comparable to their intercellular lipid content; the latter accounting for 10% to 25% of the total dry weight of the stratum corneum[184]. While NMFs are known to play a substantial role in binding and retaining water, and are able to produce a softer and more flexible skin surface[80,81], their importance in oral mucosal barrier function remains unclear.

*Potential regulators of mucosal transport*

Limited studies have been performed elucidating the regulation of oral mucosal fluid and ion absorption and secretion. It is well-documented that abnormal ENaC activity plays a critical role in the development of serious disorders, such as cystic fibrosis and salt-sensitive hypertension[185]. In lung epithelia, the lack of CFTR can lead to increased ENaC activity, resulting in over-absorption of $Na^+$ and airway epithelial dehydration[186].

Recent studies demonstrate that airway ENaC activity can be altered by the short palate lung and nasal epithelial clone 1 (SPLUNC1)[187], extracellular nucleotides ATP, UTP and nucleosides such as adenosine[188,189], as well as the pH of the airway surface fluid[165]. SPLUNC1 is a water-soluble ENaC inhibitor[190-192]. Extracellular nucleotides and adenosine are released by epithelial cells in response to mechanical stress in the airways[193,194]. Similarly to oral mucosal fluid, the airway surface fluid within the lungs covers the respiratory epithelium, playing an essential role in normal lung function by maintaining optimal mucus concentrations, a necessary component for airway defense mechanisms. The height of the airway surface fluid is controlled by $Na^+$ absorption through ENaC and $Cl^-$ secretion via both $Ca^{2+}$-activated $Cl^-$ and CFTR $Cl^-$ channels[190]. Since saliva contains ATP, ADP and AMP[195] as well as SPLUNC1[196], it is highly likely that these extracellular nucleotides activate, whilst SPLUNC1 inhibits $Na^+$ absorption, as observed in the airway epithelia.

Saliva contains multiple metabolic hormones, such as insulin, glucagon, glucagon-like peptide-1 (GLP-1), cholecystokinin, vasoactive intestinal peptide, ghrelin, and obestatin[197]. Although their major function in saliva appears to be related to modulation of taste perception and/or stimulation of wound healing through mucosal regeneration, it may be possible that many of these hormones and proteins act as regulatory factors on oral mucosal fluid and ion transport. A comparison of the levels of hormones, nucleotides, nucleosides and SPLUNC1 in saliva from healthy subjects and patients with dry mouth will most likely provide important information and lead to further exploration in oral mucosal fluid and electrolyte transport regulation.

## Conclusion and future directions

Although it has been confirmed that hyposalivation is the major aetiological factor for dry mouth, increasing evidence indicates that abnormal or dysfunctional fluid and electrolyte absorption and secretion by oral mucosal epithelia may be another major factor. Since the majority of current research projects focus on salivary gland dysfunction, more research on oral mucosal transport physiology is needed and is of great significance. Many issues in this area need to be clarified, such as to characterise water and ion absorption, to further confirm ENaC and/or $Na^+/H^+$ exchange expression in oral mucosal epithelial cells, to characterise the roles of these ion transporters, to elucidate the regulatory mechanisms by which these transporters are controlled, to examine whether $Cl^-$ channels and/or whether the $Cl^-/HCO3^-$ exchangers are expressed in oral mucosal cells, to compare whether TJ proteins and aquaporins are expressed differently between healthy subjects and patients with dry mouth, and to measure the differences of intercellular lipids between healthy subjects and dry mouth patients. Further information on these important topics will provide a solid basis for developing novel preventive and therapeutic interventions for dry mouth.

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