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ORGANIZATION, BARRIER FUNCTION AND ANTIMICROBIAL LIPIDS OF THE ORAL MUCOSA

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Synopsis

As one moves from the skin across the vermilion region of the lip and into the oral cavity the oral mucosa is encountered. The oral mucosa consists of connective tissue known as the lamina propria covered by a stratified squamous epithelium. In the regions of the hard palate and gingiva the epithelium is keratinized like the epidermis. In the buccal region, the floor of the mouth and the underside of the tongue the epithelium is nonkeratinized. The epithelium on the dorsum of the tongue is a specialized epithelium but can be approximated as a mosaic of keratinized and nonkeratinized epithelia. The nonkeratinized epithelial regions do not produce a stratum corneum. Nuclei with intact DNA are retained in the superficial cells. In all regions the outer portions of the epithelium provides a protective permeability barrier, which varies regionally. Antimicrobial lipids at the surfaces of the oral mucosa are an integral part of innate immunity.

Keywords

mouth mucosa; keratinocytes; keratin; cell envelope; ceramide; permeability

Introduction

The purpose of this review is to point out some of the similarities and differences between skin and oral mucosa, with special emphasis on epidermis and oral epithelia from different regions of the oral cavity. Within the oral cavity, some mucosal regions are covered by a keratinized epithelium resembling epidermis, while other regions are lined by a nonkeratinizing epithelium (1–3). Lipids in the outer portion of oral epithelium determine the permeability barrier function, and certain lipids provide an antimicrobial barrier at the epithelial surfaces (4–6). Emphasis will be placed on epithelial biology, barrier function and antimicrobial lipids.

Lip

The lip is a specialized region that represents the transition from the skin to the oral mucosa. The line where the skin surrounding the red external lip meets the surrounding facial skin is the vermillion border of the lip. The exterior vermillion zone of the lip has a thin keratinized epithelium (1). The underlying connective tissue has a rich blood supply, which contributes to the red coloration (7). The projected area of the corneocytes on the vermillion zone of the lip is slightly larger than that of corneocytes from the cheek, and the TEWL through the lip is greater than TEWL through the cheek (8). Surprisingly, the hydration of the external vermillion zone of the lip is greater than the hydration of the inner lip mucosa (9). Within

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the connective tissue along the vermillion border of the lip are sebaceous follicles, which are sebaceous glands without associated terminal hairs. The composition of the lipid mixture produced by these sebaceous follicles is the same as that produced by the major sebaceous glands of the skin (10). The composition of sebum in the lumen of the gland is 57% triglycerides, 25% wax monoesters, 15% squalene, 2% cholesterol esters and 1% cholesterol (11). As sebum flows to and over the skin surface, the triglycerides undergo partial hydrolysis to release fatty acids, some of which are antimicrobial (3). The most notable antimicrobial fatty acids are lauric acid (C12:0) and sapienic acid (C16:1Δ6). Sebaceous follicles are not only found in the vermillion border of the lip and in the oral mucosa, they encircle every orifice of the human body (3). This distribution strongly suggests a protective function. As one progresses from the external vermillion zone of the lip into the oral cavity there is a transition from keratinized to nonkeratinized epithelium.

Lamina propria

The lamina propria is the mucosal connective tissue (12). In general, the lamina propria consists of two compartments: the papillary layer and the reticular layer. The papillary layer is immediately beneath the epithelium. It contains loosely organized collagen fibers and capillary loops. Nerve endings are found in the papillary layer or sometimes extend into the epithelium. The lower reticular layer contains bundles of collagen fibers arranged parallel to the plane of the mucosa. There are also elastic fibers within the lamina propria. The reticular layer contains sebaceous follicles and major and minor salivary glands. The major salivary glands are the parotid, parietal and sublingual glands, and there is usually one or several lobes that undergo sebaceous differentiation (13–15). The minor salivary glands and sebaceous follicles are numerous and are found throughout the oral mucosa. These sebaceous follicles, if enlarged, are sometimes called Fordyce spots or granules. Fordyce spots on the vermillion border of the lip are sometimes treated for cosmetic reasons (16). The association of sebaceous lobes with major salivary glands and the ubiquitous presence of sebaceous follicles in oral mucosa explain why the lipids at the mucosal surfaces and in saliva have the same composition as lipids at the skin surface (17). The antimicrobial fatty acids, lauric acid and sapienic acid, likely contribute to control of microbial growth within the oral cavity (4, 18).

In the regions of the hard palate and gingival, the lamina propria is anchored to the periostium of the underlying bone. This arrangement allows these regions to withstand the shearing forces associated with chewing food (3). In the nonkeratinizing regions there is a submucosa beneath the reticular layer that attaches to muscle. This, in combination with some regional differences in collagen and elastin, allow these regions of the oral mucosa to be more flexible, which is essential for speech (3). The lamina propria contains fibroblasts that make collagen. There are also mast cells, macrophages and other cellular components of the immune system (19).

Keratinized oral epithelium

Most of the cells in the keratinizing oral epithelia are keratinocytes. Keratin gene expression varies as a function of differentiation (20). Genes are expressed pair-wise so that a type I, or acidic, keratin is paired with a type II, or basic/neutral, keratin (20, 21). In the basal cells of the keratinizing oral epithelia keratin 5 and keratin 14 are expressed. As keratinocytes undergo differentiation production of keratins 5 and 14 stop and keratins 6 and 16 are co-expressed followed by keratins 1 and 10. Approximately 85 percent of the protein in the stratum corneum is keratin (22).

In addition to keratins, differentiating keratinocytes in the regions of the gingiva and hard palate accumulate proteins in the form of keratohyalin granules (20). One of these proteins is

profilaggrin (23). This is a high molecular weight, histidine-rich highly phosphorylated protein. At the end of the differentiation program, profilaggrin is dephosphorylated and proteolytically cleaved to produce 10 to 12 filaggrin units (24). Filaggrin causes aggregation of the keratin filaments into dense bundles of keratin that lie parallel to the plane of the epithelium. This collapse of the cytoskeletal network is associated with the extreme flattening of the cells in the stratum corneum. After the aggregation of the keratin, filaggrin is broken down into its component amino acids (25). This results in an extremely high osmolarity (approximately 2 M) within the cornified cells. In addition to amino acids, the cornified cells also contain a significant amount of lactate, a product of anaerobic glycolysis, that contributes to the high osmotic strength and is important in maintaining a low pH (26). This high osmotic strength of the interior of the corneocytes essentially removes all free water out of the intercellular spaces of the stratum corneum.

Just prior to the time that the cytoskeletal system collapses, the nucleus, mitochondria and other internal organelles are degraded (27). This results in release of ionic calcium that had been sequestered by the membrane system (28).

In addition to accumulating proteins, differentiating keratinocytes in the keratinizing regions accumulate lipids (29). Much of this lipid accumulates in the form of lamellar granules (27). In transmission electron micrographs, these organelles appear round to ovoid in shape and are about 0.2 μm in diameter (30). They have a unit bounding membrane surrounding one or sometimes several stacks of internal lamellae. In the uppermost granular layer the bounding membrane of the lamellar granule fuses into the cell plasma membrane and the contents are extruded into the intercellular space. The initially extruded lipid is largely a mixture of phospholipids, glucosylceramides and cholesterol. Hydrolytic enzymes, also delivered by the lamellar granules (31–33), act on the initially extruded phospholipids and glucosylceramides to produce ceramides and fatty acids. In contrast to the epidermis, small amounts of phospholipid and glucosylceramide pass into the stratum corneum (34).

At about the same time that the cytoskeletal system collapses, the cell plasma membrane is replaced by a cornified envelope (20, 35). This is a thick band of protein, some of which is derived largely from involucrin from the keratohyalin granules. This band of protein is held together by isopeptide linkages formed through the action of a transglutaminase and by disulfide linkages (27, 35). The outer surface of this polymerized protein contains only small amounts of covalently bound lipid compared to what is found in epidermal stratum corneum (36). The main components of this covalently bound lipid are ω-hydroxyceramides (37, 38). The ω-hydroxyacid components of these ceramides are 30- through 34-carbons in length. These hydroxyceramides are ester-linked to acidic side chains on the surface of the protein envelope through ester-linkages involving the ω-hydroxyl group (39).

The end product of the keratinization product is the stratum corneum (27). This consists of flat, hexagonal cells embedded in a lipid matrix. The cells are filled with keratin and a high concentration of amino acids and other low molecular weight substances and are bounded by a thick band of polymerized protein with covalently bound lipid on the outer surface. The lipids filling the intercellular spaces throughout the stratum corneum consist mostly of ceramides, cholesterol, fatty acids and cholesterol sulfate. There are small proportions of phospholipids, glucosylceramides and cholesterol esters (34). The stratum corneum provides a permeability barrier, but it is not as impermeable as the barrier of the skin (40).

In epidermal stratum corneum, the free fatty acids as well as the amide-linked fatty acids in the ceramides are predominantly long (20–28 carbons) and saturated (41, 42). The ester-linked fatty acid in the acylceramide, EOS, is linoleic acid. In contrast, in oral stratum corneum the amide-linked ω-hydroxyacids cover the same range of chain lengths, but they

are a mixture of saturated and monounsaturated species (43). Also, the major fatty acids ester-linked to the acylceramide are palmitic, stearic and eicosanoic acids. Linoleate is only 9% of the total.

In epidermal and oral stratum corneum, ceramides, fatty acids and cholesterol are the primary lipids that determine the permeability barriers. The epidermal fatty acids and ceramides are rod or cylindrical in shape. This is ideal for formation of highly ordered, and thereby highly impermeable, membrane domains. The cholesterol would provide a degree of flexibility to avoid brittleness. In oral stratum corneum, some of the fatty acids and ceramides, by virtue of the presence of cis double bonds, are no longer cylindrical in shape. In addition, oral stratum corneum contains small amounts of phospholipids and glucosylceramides that are not present in epidermal stratum corneum. These lipids are more conical in shape and suited for the formation of fluid membranes. These geometric factors contribute to the greater permeability of the keratinized oral regions compared to skin.

Nonkeratinized epithelium

The inside of the lips and cheeks, the floor of the mouth and the underside of the tongue are covered by a nonkeratinized epithelium (6). Again, most of the cells in these epithelial regions are keratinocytes. In contrast to the keratinizing epithelia, internal cellular organelles are not degraded in nonkeratinizing epithelia. Intact nuclei with high molecular weight DNA survive to the surface layers. The basal keratinocytes in these regions express K5 and K14/K19 (20). Other keratinocytes expressed at later times during differentiation include K4, K13 and low levels of K1 and K10. Keratohyalin granules are not produced in the nonkeratinizing epithelia.

The nonkeratinizing epithelia do not produce lamellar granules, but they do produce two similar secretory organelles: a cored granule (44) and a lamellate granule (34). Both of these organelles are about the same size and shape (spherical and approximately 0.2 micrometer diameter) as the lamellar granules. They secrete their contents into the intercellular spaces about 2/3 of the way from the basal layer to the surface. This corresponds to a change in permeability so the outer third of the epithelium provides the permeability barrier (3). This superficial barrier, although it contains internal organelles including mitochondria, is metabolically inactive. The barrier function is less effective than that of the keratinized regions (40).

The superficial layers of the nonkeratinizing oral epithelia contain abundant phospholipids, which reflects the fact the mitochondria, nuclei, plasma membranes, etc. are not degraded in these epithelial regions. These lipids probably have nothing to do with barrier function. There are small amounts of ceramides in addition to saturated fatty acids and cholesterol. In fact, a strong correlation ($r^2 = 0.9$) has been found for ceramide content and barrier function across all epithelial regions (34).

Other cell types

In addition to keratinocytes, the oral epithelia contain three other cell types (19). The basal layer includes Merkel cells, which are usually adjacent to nerve endings. These cells are involved in sensory function. The suprababasal layer contains two populations of dendritic cells. The Langerhans cells are antigen presenting cells and part of the immune system. Melanocytes are pigment producing cells.

Conclusion

The pattern of epithelial terminal differentiation varies regionally within the oral cavity. Epithelial thickness, structural proteins and permeability barrier function vary between skin and oral mucosa and among different oral mucosal regions. Certain lipids, mainly ceramides saturated fatty acids and cholesterol, determine the permeability barrier functions of skin and different regions of oral mucosa. Other lipids (sphingosine, sapienic acid & lauric acid) provide for antimicrobial barriers.

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References

1. Wertz PW, Swartzendruber DC, Squier CA. Regional variation in the structure and permeability of oral mucosa and skin. Advanced Drug Deliv Rev. 1993; 12:1–12.
2. Durdiakova J, Kamodyova N, Ostatnikova D, Vikova B, Celec P. Comparison of different collection procedures and two methods for DNA isolation from saliva. Clin Chem Lab Med. 2012; 50:643–647. [PubMed: 22149741]
3. Wertz PW, Squier CA. Cellular and molecular basis of barrier function in oral epithelium. Crit Rev Therap Drug Carrier Systems. 1991; 8:237–269.
4. Brogden, KA.; Drake, DR.; Dawson, DV.; Hill, JR.; Bratt, CL.; Wertz, PW. Antimicrobial lipids of skin and oral mucosa. In: Dayan, N.; Wertz, PW., editors. INNATE IMMUNE SYSTEM OF SKIN AND ORAL MUCOSA. Hoboken: Wiley; 2011. p. 75-82.
5. Brogden NK, Mehalick L, Fischer CL, Wertz PW, Brogden KA. The emerging roles of peptides and lipids as antimicrobial epidermal barriers and modulators of local inflammation. Skin Pharmacol Physiol. 2012; 25:167–181. [PubMed: 22538862]
6. Walker DM. Oral mucosal immunology: an overview. Ann Acad Med Singapore. 2004; 33S:27S–30S.
7. Walker, WB. The oral cavity and associated structures. In: Walker, HK.; Hall, WD.; Hurst, JW., editors. CLINICAL METHODS: THE HISTORY, PHYSICAL AND LABORATORY EXAMINATIONS. 3rd Ed.. Vol. Chapter 129. Boston: Butterworths; 1990.
8. Kobayashi H, Tagami H. Functional properties of the surface of the vermillion border of the lips are distinct from those of the facial skin. Brit J Dermatol. 2004; 150:563–567. [PubMed: 15030342]
9. Caisey L, Gubanova E, Baras D, Leveque JL. Unexpected distribution of surface hydration level of the lip. J Euro Acad Dermatol Venereol. 2008; 22:1159–1162.
10. Nordstrom KM, McGinley KJ, Lessin SR, Leyden JJ. Neutral lipid-composition of Fordyce granules. Brit J Dermatol. 1989; 121:669–670. [PubMed: 2597641]
11. Wertz, PW. Sebum secretion and acne. In: Webster, GF.; Rawlings, AV., editors. ACNE AND ITS THERAPY. New York: informa healthcare; p. 37-44.
12. Squier CA, Kremer MJ. Biology of oral mucosa and esophagus. J Natl Inst Monogr. 2001; 29:7–15.
13. Meza-Chavez L. Sebaceous glands in normal and neoplastic parotid glands: possible significance of sebaceous glands in respect to the origins of tumors of the salivary glands. Am J Pathol. 1949; 25:627–645. [PubMed: 18152859]
14. Linhartova A. Sebaceous glands in salivary gland tissue. Arch Pathol. 1974; 98:320–324. [PubMed: 4415696]
15. Martinez-Madrigal F, Micheau C. Histology of the major salivary glands. Am J Surg Pathol. 1989; 13:879–899. [PubMed: 2675654]
16. Kim YJ, Kang HY, Lee E-S, Kim YC. Treatment for Fordyce spots with 5-aminolaevulinic acid-photodynamic therapy. Brit J Dermatol. 2007; 156:399–400. [PubMed: 17223899]

17. Brasser AJ, Barwacz CA, Dawson DV, Brogden KA, Drake DR, Wertz PW. Presence of wax esters and squalene in human saliva. Arch Oral Biol. 2011; 56:588–591. [PubMed: 21247555]
18. Fischer CL, Drake DR, Dawson DV, Blanchette DR, Brogden KA, Wertz PW. Antibacterial activity of sphingoid bases and fatty acids against Gram-positive and Gram-negative bacteria. Antimicrob Agents Chemother. 2012; 56:1157–1161. [PubMed: 22155833]
19. Squier, CA.; Brogden, KA. Non-Keratinocytes in the Oral Epithelium. In: Squier, CA.; Brogden, KA., editors. Human Oral Mucosa, Development, Structure & Function. West Sussex, UK: Wiley-Blackwell; 2011. p. 41-50.
20. Presland RB, Dale BA. Epithelial structural proteins of the skin and oral cavity: Function in health and disease. Crit Rev Oral Biol Med. 2000; 11:383–408. [PubMed: 11132762]
21. Steinert PM. Structure, function, and dynamics of keratin intermediate filaments. J Invest Dermatol. 1993; 100:729–734. [PubMed: 7684423]
22. Scheuplein RJ, Blank IH. Permeability of the Skin. Physiol Rev. 1971; 51:702–747. [PubMed: 4940637]
23. Brown SJ, McLean WHI. One remarkable molecule: filaggrin. J Invest Dermatol. 2012; 132:751–762. [PubMed: 22158554]
24. Dale BA, Resing KA, Lonsdale-Eccles JD. Filaggrin: A keratin filament associated protein. Ann NY Acad Sci. 1985; 455:330–342. [PubMed: 2417519]
25. Rawlings AV, Scott IR, Harding CR, Bowser PA. Stratum corneum moisturization at the molecular level. J Invest Dermatol. 1994; 103:731–741. [PubMed: 7963664]
26. Rawlings AV, Matts PJ. Stratum corneum moisturization at the molecular level: an update in relation to the dry skin cycle. J Invest Dermatol. 2005; 124:1099–1110. [PubMed: 15955083]
27. Matoltsy AG. Keratinization. J Invest Dermatol. 1976; 67:20–25. [PubMed: 778293]
28. Forslind B, Lindberg M, Roomans GM, Pallon J, Werner-Linde Y. Aspects on the physiology of human skin: studies using particle probe analysis. Micros Res Tech. 1997; 38:373–386.
29. Squier CA, Wertz PW, Cox P. Thin-layer chromatographic analyses of lipids in different layers of epidermis and oral epithelium. Archs Oral Biol. 1991; 36:647–653.
30. Landmann L. The epidermal permeability barrier. Anat Embryol. 1988; 178:1–13. [PubMed: 3288004]
31. Freinkel RK, Traczyk TN. Lipid composition and acid hydrolase content of lamellar granules of fetal rat epidermis. J Invest Dermatol. 1985; 85:295–298. [PubMed: 4045218]
32. Grayson S, Johnson-Winegar AG, Wintroub BU, Isseroff RR, Epstein EH Jr, Elias PM. Lamellar body-enriched fractions from neonatal mice: preparative techniques and partial characterization. J Invest Dermatol. 1985; 85:289–294. [PubMed: 4045217]
33. Madison KC, Sando GN, Howard EJ, True CA, Gilbert D, Swartzendruber DC, Wertz PW. Lamellar granule biogenesis: a role for ceramide glucosyltransferase, lysosomal enzyme transport and the Golgi. J Invest Dermatol Symp Proc. 1998; 3:80–86.
34. Law S, Wertz PW, Swartzendruber DC, Squier CA. Regional variation in content, composition and organization of porcine epithelial barrier lipids revealed by thin-layer chromatography and transmission electron microscopy. Arch Oral Biol. 1995; 40:1085–1091. [PubMed: 8850646]
35. Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. Bioessays. 2002; 24:789–800. [PubMed: 12210515]
36. Chang F, Swartzendruber DC, Wertz PW, Squier CA. Covalently bound lipids in keratinizing epithelia. Biochem Biophys Acta. 1993; 1150:98–102. [PubMed: 8334143]
37. Wertz, PW.; Downing, DT. Covalently bound omega-hydroxyacylsphingosine in the stratum corneum, 1987. Wertz, Madison & Downing; 1989.
38. Wertz PW, Madison KC, Downing DT. Covalently bound lipids of human stratum corneum. J Invest Dermatol. 1989; 91:109–111. [PubMed: 2909622]
39. Stewart ME, Downing DT. The omega-hydroxyceramides of pig epidermis are attached to the corneocytes solely through omega-hydroxyl groups. J Lipid Res. 2001; 42:1105–1110. [PubMed: 11441138]
40. Squier CA, Cox P, Wertz PW. Lipid content and water permeability of skin and oral mucosa. J Invest Dermatol. 1991; 96:123–126. [PubMed: 1987287]

41. Wertz PW. Composition and morphology of epidermal cyst lipids. J Invest Dermatol. 1987; 89:419–425. [PubMed: 3668284]

42. Ponec M, Weerheim A, Lankhorst P, Wertz P. New acylceramide in native and reconstructed epidermis. J Invest Dermatol. 2003; 120:581–588. [PubMed: 12648220]

43. Hill JR, Wertz PW. Structures of the ceramides from porcine palatal stratum corneum. Lipids. 2009; 44:291–295. [PubMed: 19184160]

44. Squier CA. Membrane coating granules in nonkeratinizing oral epithelium. J Ultrastruc Res. 1977; 60:212–220.