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Oral Mucosal Absorption: Mechanisms, Methods, and Challenges in Drug Delivery

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Abstract

This review focuses on the mechanisms and methods involved in oral mucosal absorption, with a particular emphasis on drug delivery systems. It begins by discussing the protective role of saliva in the oral cavity, highlighting its importance in maintaining the health of the oral tissues and facilitating drug delivery. The review then delves into the mechanisms of oral mucosal absorption, including passive diffusion, which is the primary mechanism for drug transfer across the oral mucosa. It also explores the use of hydrophilic polymeric matrices as vehicles for oral transmucosal drug delivery systems, which are favored due to the water-rich environment of the oral cavity. The review further discusses the methods used to assess oral mucosal permeation, including both in vivo and in vitro approaches. In vivo methods, such as the buccal absorption test, are used to determine the bioavailability of drugs via this route, while in vitro methods are employed for high-throughput permeability screening. The review concludes by addressing the challenges and limitations associated with oral mucosal drug delivery, such as the difficulty in studying regional variation in drug absorption and the limitations of in vitro permeability models in predicting in vivo situations.

Keywords: Buccal; Diffusion; Epithelial; mucosa; mucoadhesion; polymers.

Introduction

Most drugs are delivered to the systemic circulation by the conventional oral route; however, the systemic absorption of certain drugs can be significantly hindered by the environment of the gastrointestinal tract. Drugs that are susceptible to acid hydrolysis are substrates for the various efflux mechanisms present in the intestinal wall, or have significant intestinal or hepatic metabolism may exhibit poor bioavailability when administered via the oral route [1]. Buccal drug delivery offers several advantages over the peroral route. These advantages include avoidance of presystemic drug elimination within the gastrointestinal tract and/or during the hepatic first-pass metabolism, and independence from the potential variability of absorption caused by the gastric emptying rate, or the presence of food in the upper region of the gastrointestinal tract [2]. In addition, the buccal mucosa is relatively permeable with a rich blood supply and has a substantial resistance to irritation or damage [2-4]. Other important advantage is the facility to include permeation enhancer/enzyme inhibitor or pH modifier in the formulation and versatility in designing as multidirectional or unidirectional release systems for local or systemic actions [5]. The permeability of the buccal mucosa is four to 4,000 times greater than the permeability across skin. As a result, a faster onset of action for several drugs is observed [6]. A shorter turnover time in the oral mucosa (14 days) as opposed to skin (27 days) ensures a faster recovery of the oral mucosa [7].

Absorption through the mucous membranes of the oral cavity was noted as early as 1847 by Sobero, the discoverer of nitroglycerin [8], and the systematic studies of oral cavity absorption were first reported by Walton in 1935 and 1944[9, 10]. Since then, reviews of the subject have been provided by Katz and Barr in 1955[11], Gibaldi and Kanig in 1965[12], Squier and Jhonson in 1975[13], Shojaei in 1998[14] and Junginger, Hoogstraate and Verhoef in 1999[15].

Within the oral mucosal cavity, delivery of drugs is classified into three categories: (i) sublingual delivery, which is systemic delivery of drugs through the mucosal membranes lining the floor of the mouth, (ii) buccal delivery, which is drug administration through the mucosal membranes lining the cheeks (buccal mucosa), and (iii) local delivery, which is drug delivery into the oral cavity.

Structure and Environment of the Oral Mucosa.

Structure:

The oral mucosa is composed of an outermost layer of stratified squamous epithelium (Figure 1). Below this lies a basement membrane, a lamina propria followed by the submucosa as the innermost layer. The epithelium is similar to stratified squamous epithelia found in the rest of the body in that it has a mitotically active basal cell layer, advancing through a number of differentiating intermediate layers to the superficial layers, where cells are shed from the surface of the epithelium[16]. The epithelium of the buccal mucosa is about 40-50 cell layers thick, while that of the sublingual epithelium contains somewhat fewer. The epithelial cells increase in size and become flatter as they travel from the basal layers to the superficial layers.

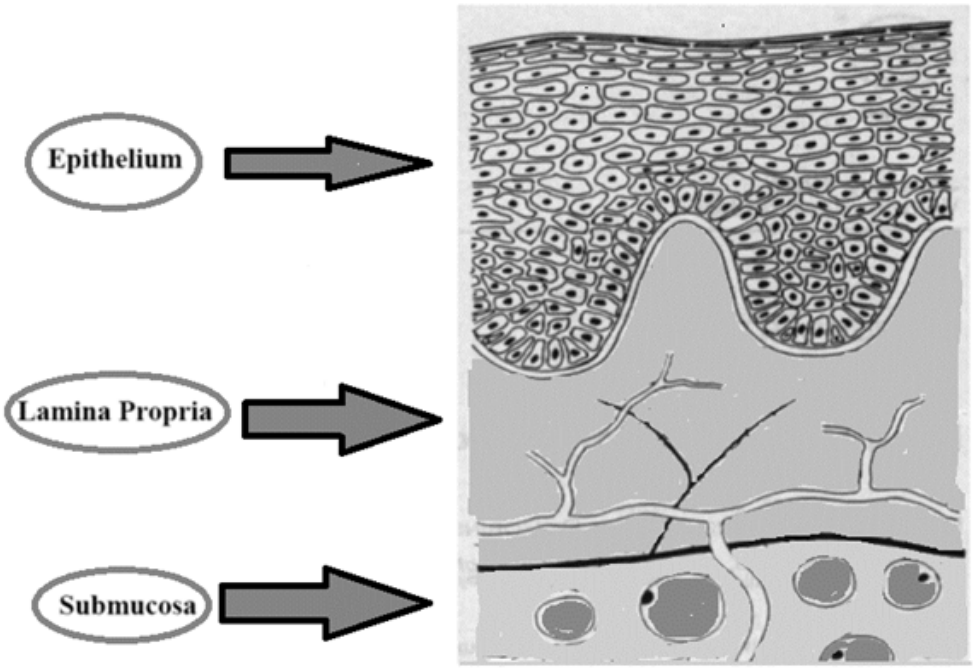

Figure 1. Structure of the oral mucosae. Adapted from reference [17]

The turnover time for the buccal epithelium has been estimated at 5-6 days [17], and this is probably representative of the oral mucosa as a whole. The oral mucosal thickness varies depending on the site: the buccal mucosa measures at 500-800 µm, while the mucosal thickness of the hard and soft palates, the floor of the mouth, the ventral tongue, and the gingivae measure at about 100-200 µm. The composition of the epithelium also varies depending on the site in the oral cavity. The mucosae of areas subject to mechanical stress (the gingivae and hard palate) are keratinized similar to the epidermis. The mucosae of the soft palate, the sublingual, and the buccal regions, however, are not keratinized [17]. The keratinized epithelia contain neutral lipids like ceramides and acylceramides which have been associated with the barrier function. These epithelia are relatively impermeable to water. In contrast, non-keratinized epithelia, such as the floor of the mouth and the buccal epithelia do not contain acylceramides and only have small amounts of ceramide [18-20]. They also contain small amounts of neutral but polar lipids, mainly cholesterol sulfate and glucosyl ceramides. These epithelia have been found to be considerably more permeable to water than keratinized epithelia [17-19].

Permeability:

Galey and coworkers showed that the oral mucosae in general are somewhat leaky epithelia intermediate between that of the epidermis and intestinal mucosa. It is estimated that the permeability of the buccal mucosa is 4-4000 times greater than that of the skin [6]. As indicative by the wide range in this reported value, there are considerable differences in permeability between different regions of the oral cavity because of the diverse structures and functions of the different oral mucosae. In general, Harris D and coworkers showed that the permeabilities of the oral mucosae decrease in the order of sublingual greater than buccal, and buccal greater than palatal [17]. This rank order is based on the relative thickness and degree of keratinization of these tissues, with the sublingual mucosa being relatively thin and non-keratinized, the buccal thicker and non-keratinized, and the palatal intermediate in thickness but keratinized.

Gandhi and coworkers stated that the permeability barrier in the oral mucosa is a result of intercellular material derived from the so-called ‘membrane coating granules’ (MCG) [21]. When cells go through differentiation, MCGs start forming and at the apical cell surfaces they fuse with the plasma membrane and their contents are discharged into the intercellular spaces at the upper one third of the epithelium. This barrier exists in the outermost 200µm of the superficial layer.

Permeation studies have been performed using a number of very large molecular weight tracers, such as horseradish peroxidase [22] and lanthanum nitrate [23], When applied to the outer surface of the these tracers penetrate only through epithelium, these tracers penetrate only through outermost layer or two of cells. When applied to the submucosal surface, they permeate up to, but not into, the outermost cell layers of the epithelium. According to these results, it seems apparent that flattened surface cell layers present the main barrier to permeation, while the more isodiametric cell layers are relatively permeable. In both keratinized and non-keratinized epithelia, the limit of penetration coincided with the level where the MCGs could be seen adjacent to the superficial plasma membranes of the epithelial cells. Since the same result was obtained in both keratinized and non-keratinized epithelia, keratinization by itself is not expected to play a significant role in the barrier function [22].

The components of the MCGs in keratinized and non-keratinized epithelia are different, however [18]. The MCGs of keratinized epithelium are composed of lamellar lipid stacks, whereas the non-keratinized epithelium contains MCGs that are non-lamellar. The MCG lipids of keratinized epithelia include sphingomyelin, glucosylceramides, ceramides, and other nonpolar lipids, however for non-keratinized epithelia, the major MCG lipid components are cholesterol esters, cholesterol, and glycosphingolipids [18]. Aside from the MCGs, the basement membrane may present some resistance to permeation as well, however the outer epithelium is still considered to be the rate limiting step to mucosal penetration. The structure of the basement membrane is not dense enough to exclude even relatively large molecules.

The epithelial barrier must be crossed by the drug molecules in order to reach their intended sites of action. The basic drug transport mechanism for buccal epithelium is the same as for other epithelia in the body. There are two major routes involved: transcellular (intracellular) route and paracellular (intercellular) [24] (Fig. 2). In general, for many of the drugs, permeation across the buccal epithelium is thought to be through paracellular route by passive diffusion. **Nevertheless and Kurosaki** [25] suggested the presence of a specialized transport system for cephadroxyl in the human buccal membrane.

Environment

The cells of the oral epithelia are surrounded by an intercellular ground substance, mucus, the principle components of which are complexes made up of proteins and carbohydrates. These complexes may be free of association or some maybe attached to certain regions on the cell surfaces. This matrix may actually play a role in cell-cell adhesion, as well as acting as a lubricant, allowing cells to move relative to one another [26]. Along the same lines, the

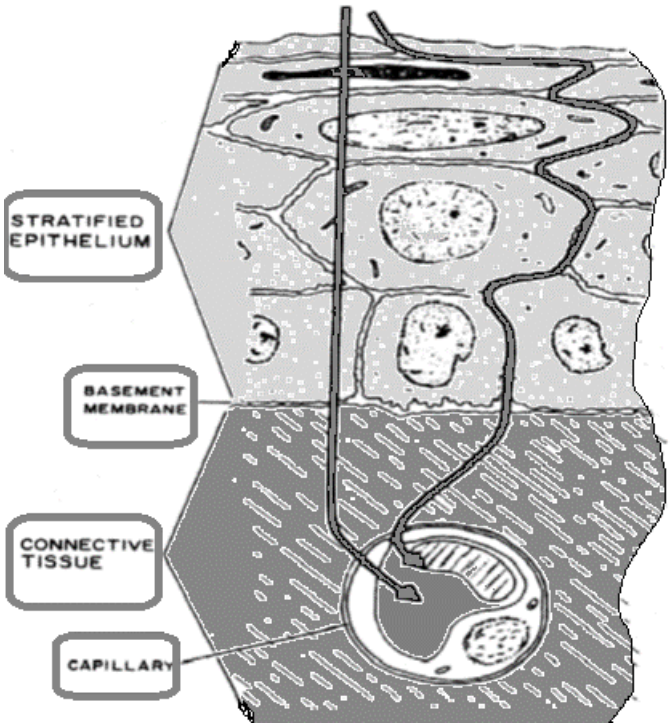

Figure 2. Routes of epithelial penetration: transcellular route and paracellular route; Adapted from reference [27]

mucus is also believed to play a role in bioadhesion of mucoadhesive drug delivery systems [28].

In stratified squamous epithelia found elsewhere in the body, mucus is synthesized by specialized mucus secreting cells like the goblet cells, however in the oral mucosa; mucus is secreted by the major and minor salivary glands as part of saliva [2, 26]. Up to 70% of the total mucin found in saliva is contributed by the minor salivary glands

[2, 26]. At physiological pH the mucus network carries a negative charge (due to the sialic acid and sulfate residues) which may play a role in mucoadhesion. At this pH mucus can form a strongly cohesive gel structure that will bind to the epithelial cell surface as a gelatinous layer [16]. Another feature of the environment of the oral cavity is the presence of saliva produced by the salivary glands. Saliva is the protective fluid for all tissues of the oral cavity. It protects the soft tissues from abrasion by rough materials and from chemicals. It allows for the continuous mineralisation of the tooth enamel after eruption and helps in remineralisation of the enamel in the early stages of dental caries [29].

Saliva is an aqueous fluid with 1% organic and inorganic materials. The major determinant of the salivary composition is the flow rate which in turn depends upon three factors: the time of day, the type of stimulus, and the degree of stimulation [2, 26]. The salivary pH ranges from 5.5 to 7 depending on the flow rate. At high flow rates, the sodium and bicarbonate concentrations increase leading to an increase in the pH. The daily salivary volume is between 0.5 to 2 liters and it is this amount of fluid that is available to hydrate oral mucosal dosage forms. A main reason behind the selection of hydrophilic polymeric matrices as vehicles for oral transmucosal drug delivery systems is this water rich environment of the oral cavity.

Mechanisms Involved in Oral Mucosal Absorption

Passive Diffusion

JC**.** postulated that the major mechanism involved in the transfer of a drug across the oral mucosa is described by simple Fickian or passive diffusion of the unionized form of the drug in accordance with the pH-partition hypothesis [30, 31]. This was first demonstrated for buccal absorption with a series of amphetamines done by Beckett and coworkers [32]. In this study, drug transport appeared to be a passive diffusion process since optical isomers of a drug were absorbed to the same extent; absorption was dependent on the concentration of un-ionized lipid-soluble form of the drug; and no difference in the percentage absorption of the drug at any given pH value was observed when the drug was given separately or as a mixture with other drugs. Since this initial finding, there have been many studies demonstrating the passive nature of transfer across the oral mucosa [33-39]. Under these conditions of passive diffusion, the physicochemical properties of the membrane and the drug dictate the transport rate across the biological membrane [2].

Carrier-Mediated Transport

Although passive diffusion is the major transport mechanism for drug permeation across the buccal mucosa, the absorption of certain nutrients from the oral cavity; done by Manning and coworkers*;* has been shown to involve carrier systems. The absorption of D-glucose and L-arabinose across the buccal mucosa was shown to be both saturable and stereospecific [40]. This indicated the presence of a carrier-mediated transport system for these sugars, since saturation and stereospecificity are not characteristics of a passive diffusion process. Additionally, the absorption of D-glucose, galactose, and 3-O-methyl-D-glucose was at least partly dependent on the presence of sodium ions, and the transport of D-glucose was inhibited by galactose and 3-O-methyl-D-glucose, suggesting that there was at least one common carrier system in the buccal mucosa [40]. Such a specialized mechanism for D-glucose transport was also observed by Kimura and coworkers in a cultured stratified cell layer of human oral mucosal cells [41]. In another study by Kurosaki Y et al.; assessing the absorption of D-glucose at various sites in the oral cavity, absorption was found to be saturable only in the dorsum of the tongue, and these authors suggested that a specialized transport system for D-glucose existed only at this site [42]. However, using Western blot analysis by Oyama and coworkers, various glucose transporters have been identified in cells of the buccal mucosa as well as the dorsum of the tongue and the buccal mucosa [43]. Therefore, greater clarification is required in this area.

In addition to sugars, the absorption studies ;done by Sadooghabasian and coworkers, and Evered and coworkers*;* of various vitamins, including L-ascorbic acid, nicotinic acid, and nicotinamide, have been shown to be dependent on the presence of sodium ions, indicating absorption from the oral cavity by carrier-mediated processes [44, 45]. When the absorption of thiamine was investigated by Evered and coworkers in vivo, absorption rates showed saturation at high concentrations of the vitamin [46], giving further support to the finding that carrier-mediated processes are involved in the oral mucosal absorption of some nutrients.

Recent investigations; done by Utoguchi and coworkers; have also indicated the existence of an energy-dependent carrier- mediated monocarboxylic acid transporter system in primary cultures of rabbit and hamster oral mucosal cells, and in hamsters in vivo [47, 48]. Such carrier-mediated systems may be important in the transport of certain drugs, such as salicylic acid. Kurosaki and coworkers has also been shown that the absorption of cefadroxil, an aminocephalosporin antibiotic, is absorbed in the human oral cavity via a specialized transport mechanism, since its absorption demonstrated saturation phenomena and was inhibited in the presence of another aminocephalosporin, cephalexin [25]. Therefore, evidence is building to suggest that passive diffusion of compounds may not be the only mechanism by which compounds permeate the buccal mucosa.

There has also been a report by Brayton and coworkers regarding the active transport of antibacterial agents in oral mucosa. In a cell line derived from oral epithelium, the uptake of ciprofloxacin and minocycline was not only saturable and inhibited in the presence of other compounds, but the intracellular levels of both antibiotics were 8–40-fold higher than the extracellular levels as well, demonstrating an active transport process [49]. Whether the permeability of these compounds across the entire oral mucosa occurs via an active transport process, however, remains to be determined.

Estimating the ability of a drug to move through the oral mucosal membrane.

At present, there is no simple model capable of predicting buccal permeability of a wide range of small molecular drugs. This has led to the use of existing transdermal models such as the Potts–Guy (PG) model [50] for predicting buccal permeability due to the greater structural and biochemical proximity of buccal mucosa to skin than other tissues such as intestine. However, use of such models is inadequate as the permeation of ionized drug species is significant in the buccal mucosa and distribution coefficient (logD) was found to correlate better to the buccal permeability as opposed to logP that was proposed in the existing models. Drug permeability across a biological membrane depends on the properties of the barrier and permeant. Various structural and physicochemical parameters such as, size, charge, lipophilicity, and hydrogen-bonding capacity influence the permeability of a molecule across a membrane [51].

Kokate, Amit and coworkers have designed a simple model to predict the buccal drug permeability, based on the work done by Mälkiä and coworkers [51]. A computational model to predict buccal permeability of drugs based on their structural and physicochemical properties was developed. Molecular volume, $logD_{6.8}$, number of hydrogen bond donors, and number of rotatable bonds were the most important parameters describing $logK_p$, briefly in the following equation:

$$\log K_p\left(\frac{cm}{s}\right) = -3.13(\pm 0.95) - 0.012(\pm 0.0051) \times MV - 0.617(\pm 0.170) \times HBD + 0.263(\pm 0.110) \times nRotB + 0.654(\pm 0.200) \times \log D_{6.8}$$

Where $\log K_p$ is the logarithm of the permeation coefficient, MV is the molecular volume, nRot is number of rotatable bonds, HBD is no. of hydrogen bond doners and $\log D_{6.8}$ is logarithm of distribution coefficient at pH 6.8, which corresponds to salivary pH.

Methods for assessment of oral mucosal permeation:

There have been a range of models used in the preclinical setting to assess the permeability of compounds across the buccal mucosa. While in vivo methods are often more appropriate in terms of assessing bioavailability via this route, in vitro and in situ methods have been instrumental for preclinical compound screening, elucidating mechanisms of transport across the buccal mucosa, and assessing the potential of chemical penetration enhancers for improvement of buccal transport.

***In-vivo* methods;**

One of the most common in vivo methods used to assess the permeability of the buccal mucosa is the buccal absorption test of Beckett and Triggs [32]. In this test, a known volume of a drug solution is introduced into the oral cavity of a subject, who swirls it around for a specified period of time and then expels it. The subject then rinses his or her mouth with an aliquot of distilled water or buffer solution, and the expelled drug solution and rinse are combined and analyzed for drug content. The difference between the initial and final drug concentration in the solution is assumed to be the amount of drug taken up into the oral mucosa. The buccal absorption test of Beckett and Triggs has been modified slightly by various investigators.

To account for the production of saliva throughout the test, a correction factor was included by Dearden and Tomlinson [52]. Arbab and coworkers**;** Schurmann and coworkers**,** and Tucker; have added a marker compound into the swirling solution, such as phenol red or polyethylene glycol, to account for salivary dilution and accidental swallowing of the solution [33, 53, 54]. Since kinetic profiles cannot be determined using the original buccal absorption test, Tucker modified the test by taking small samples of the swirled solution from the oral cavity every few minutes without removing the entire test solution [54]. The major benefit of this is that the absorption kinetics of a drug may be studied in a single subject in a simple 15–20-min test.

Although the original and modified buccal absorption tests are easy to perform, do not require blood sampling, and allow for both the rate and the extent of drug loss from the oral cavity to be determined, there are some drawbacks to the method. One of the major disadvantages of this technique is that only the concentration of drug remaining in the oral cavity (swirling solution) is measured, and blood samples are not determined. The amount of drug which disappears from the swirling solution cannot be equated to the amount entering the systemic circulation, due to other factors including membrane storage, potential metabolism, and swallowing of the drug [55, 56]. Since the solution is swirled around the oral cavity, absorption of compound may also occur through all surfaces within the oral cavity, and so the degree to which absorption occurs across a specific site (e.g., buccal and sublingual) remains unknown.

To overcome the limitation of nonspecific absorption across all surfaces of the oral cavity and to study regional variation in drug absorption, various absorption or perfusion cells have been designed (by Kurosaki and coworkers, Barsuhn and coworkers; Rathbone and coworkers; and Yamahara and coworkers); which can be clamped or attached to particular mucosae within the oral cavity of both animals and humans [42, 57-64]. In this method, a drug solution is perfused through the cell and the drug absorption is again calculated by drug disappearance from the perfusate. Gandhi and coworkers stated that the major drawback with the perfusion cell technique is leakage and large intersubject variation [21]; however, these devices are a major advance in assessing the absorption characteristics of a particular region within the oral cavity, and would be most informative if the appearance of drug in the plasma was simultaneously monitored. In particular cases where plasma cannot be simultaneously assayed,

and there is information available relating the concentration of drug in saliva to the concentration in plasma, it is possible to collect saliva as a surrogate for plasma.

Following collection and analysis, an appropriate multiplication factor is incorporated for extrapolation to plasma concentrations. Such a method was recently used by Adrian and coworkers to assess the buccal absorption of nicotine in humans [65]. In this study, the disappearance of nicotine from the perfusion solution was used to determine the rate of nicotine absorption, and saliva was simultaneously collected directly from the parotid gland using a modified Carlson-Crittenden cup [66] as a surrogate for plasma. While salivary concentrations increased as perfusion concentrations decreased, it is unknown whether this actually related to an increase in plasma concentrations. Consequently, it is important to determine whether there is a correlation between plasma concentrations and salivary concentrations, if this technique is to be successfully used as a predictor of systemic buccal absorption. However, as informative as such techniques may be, they may not be suitable in the preclinical setting, where high throughput permeability screening is required.

***In-vitro* Methods:**

In vitro permeability models are often employed to determine the barrier nature of a particular biological tissue because the diffusion of drugs can be studied in an environment where variables such as temperature, pH, and osmolarity can be easily controlled [67]. When using an in vitro method to predict the absorption of compounds across the human buccal mucosa, an appropriate animal model must be chosen on the basis of its similarity in structure and permeability to the human buccal mucosa. Using the buccal mucosa of an appropriate animal model, in vitro permeability studies are then commonly conducted in diffusion cells. The advantage of in vitro diffusion cells is that the amount of drug that has actually diffused across the tissue can be determined over time, and thus the kinetics of tissue transport may be assessed. There are various diffusion cells that are used in the preclinical screening of compound permeability, including Franz-type diffusion cells, flow-through cells, and modified Ussing chambers.

Animals Models:

Because of the limited availability of the human buccal mucosa, it is often necessary to use freshly excised mucosa from an alternative animal species. If the role of in vitro studies is to assess the potential of the buccal mucosa as an alternative route for drug delivery in humans, then the buccal mucosa from the animal species chosen should be similar to the human buccal mucosa in terms of permeability, biochemistry, and morphology.

Aungst; and Siegel have used the oral mucosa of rats [68, 69] ,while Coutelegros.; Garren; Kitano; Kurosaki; Sveinsson; Tsutsumi, and Ungphaiboon and coworkers have used the oral mucosa of hamsters [70-79], but these surfaces are keratinized, and so may not be an appropriate model of the non-keratinized human buccal mucosa.

Rabbit buccal mucosa is non-keratinized and has been used by Dowty**;** Gandhi**;** Nair**;** and Siegel; in many in vitro studies assessing the mucosal permeability of compounds [80-83]; however, the small area of available non-keratinized tissue often limits its use [20].

The buccal mucosa of dogs and monkeys is non-keratinized and therefore may be used by Squier**;** Addy**;** Mehta**;** Nielsen**;** and Siegel and coworkers; as a model for the human buccal mucosa; however, the epithelium of the mucosa in these animals is much thinner, and consequently, more permeable than that of humans [20, 84-87].

The comparative permeability of tritiated water through various animal species and humans is shown in Table 1. In addition, the thickness of the buccal epithelium in each species is shown. Because of the physiologic, anatomic, nutritional, and metabolic similarities between humans and pigs [88], Koh and Squier; suggested that the pig has become a widely used and important animal model for research on human disease. The buccal mucosa of pigs is non-keratinized and has a similar structure, morphology, and composition to the human buccal mucosa [19, 89, 90]. Additionally, as shown in Table 1, the thickness of the epithelia in human and porcine buccal mucosa is fairly similar. While structure and morphology are important determinants in the comparative process, the permeability characteristics of the model tissue must reflect the barrier nature of the human buccal mucosa.

Table 1. Epithelial thickness and permeability coefficient (P) for tritiated water through the buccal mucosa of different species together with epithelial thickness. [20], data are ± SD

Species	P × 10^{-7} (cm/min)	Epithelial thickness (µm)
Human	579 ± 122	580 ± 90
Pig	634 ± 60	772 ± 150
Monkey	1,025 ± 154	271 ± 50
Dog	1,045 ± 37	126 20

The permeability of tritiated water through porcine buccal mucosa has been shown by Lesch and coworkers to be very similar to that of the human buccal mucosa [91], and more recently, no significant differences were observed by Nielsen and coworkers in the permeability of mannitol or testosterone through the porcine and the human buccal mucosa [86]. Because of the large amounts of pig oral mucosa available from slaughterhouses and

the similar structure and permeability to human tissue, most laboratories use porcine buccal mucosa when assessing mucosal permeability and the effect of various chemical penetration enhancers on mucosal drug delivery [38, 92-115]. It is therefore recommended that preclinical evaluation of compound permeability across the buccal mucosa be performed with porcine buccal mucosa, as a result of its similar structure and permeability characteristics to that seen in the human buccal mucosa.

Issues Associated With In Vitro Permeability Assessment:

While the transport of compounds across the buccal mucosa may be assessed in a controlled environment with in vitro techniques, the ability to correlate the results obtained from the in vitro to the in vivo situation is often limited. It is thus necessary to consider the in vitro conditions and minimize, where possible, artifacts that may not be representative of the in vivo situation. This becomes particularly important when deciding on the thickness of the tissue to use in in vitro permeability experiments. When excising the buccal mucosa (as detailed in the appendix to this chapter), it is possible to use either full- thickness tissue (containing the buccal epithelium and underlying connective tissue) or buccal epithelium alone.

In studies in by Nicolazzo and coworkers using both full- thickness and epithelial tissue, the presence of connective tissue significantly reduced the buccal permeation of the hydrophilic marker caffeine and the lipophilic marker estradiol [102].

However, Devries and coworkers observed that the difference in permeability was greater for estradiol than for caffeine, which may have been a result of the more hydrophilic nature of the connective tissue, which acts as a greater barrier for lipophilic compounds than for hydrophilic compounds [93]. Given the blood vessels are located directly beneath the epithelial surface in vivo, Nicolazzo and coworkers suggested using epithelial tissue in place of full-thickness tissue, avoiding the artificial barrier created by connective tissue in the absence of circulation.

Integrity markers are often used in in vitro permeability experiments to ensure that the model membrane is intact and that the observed permeability profiles of model compounds are not a result of compromised tissue integrity. One common method of assessing tissue integrity is to include a nonabsorbable marker at the completion of a permeability experiment.

Nicolazzo and coworkers used the high molecular weight fluorescein isothiocyanate (FITC)-labeled dextran with a molecular weight of 20 kDa as a marker of tissue integrity [102], on the basis that studies by Junginger and Hoogstraate and coworkers investigating the permeability of FITC-dextrans have revealed that passage of such hydrophilic compounds through porcine buccal epithelium is restricted to permeants with a molecular weight less than 20 kDa [15, 97, 98].

Nicolazzo and coworkers have demonstrated that such a high molecular weight dextran appears only in the receptor chamber following intentional tissue damage, and this was accompanied by an increase in the permeability of caffeine [102].

Wertz and Squier and coworkers stated that because of the possible effects of active and carrier-mediated processes and metabolic biotransformation, the issue of tissue viability is important for in vitro buccal mucosal experiments. The barrier nature of the buccal mucosa resides in the upper layers of the epithelium, where unlike in the stratum corneum, the cells contain a variety of functional organelles [18, 20, 116, 117], and so tissue viability may be an important component of the barrier function of the tissue.

Various methods have been employed to assess the viability of excised buccal mucosa, including measurement of biochemical markers, microscopic methods, and linearity of transport data [17]. Dowty and coworkers used a biochemical methods, including measurement of adenosine 5'-triphosphate (ATP) levels and utilization of glucose, provide information on the metabolic activity of the tissue, this does not necessarily relate to the barrier function of the tissue. In excised rabbit buccal mucosa, levels of ATP were measured and found to decline by 40% in 6 h, and this correlated well with transmission electron microscopic evaluation of the tissue (intact superficial cells) [80]. In addition, the permeability of a model peptide was unaltered up to 6 h postmortem, but at 8 h, a significant change in permeability was observed [80]. These investigators therefore claimed that excised rabbit buccal mucosa could be used for diffusion studies for ~6h.

Recently Shojaei and Zhang and coworkers have claimed that the tissue can be considered viable if the drug permeability does not change over the course of the experiment, and thus the actual permeability experiments themselves may provide insight into the viability of the tissue [14, 67]. This method was employed in permeation experiments using porcine buccal mucosa, where the permeability of compounds was assessed in two consecutive permeability experiments to ensure the nature of the barrier was not compromised [111, 118].

While this demonstrates that the barrier nature of the tissue was unaltered between the permeation experiments, the tissue may have already undergone tissue death in the time between the excision and the commencement of the initial permeation experiment, and thus the permeability rate obtained in vitro may not be representative of the in vivo situation. Therefore, more studies assessing the dependence of the barrier nature of the buccal mucosa on tissue viability are required, especially since the role of specialized transport processes in oral mucosal permeability is becoming more appreciated.

Imbert and coworkers, assessed the viability of excised porcine buccal mucosa using histological evaluation and a 3-[4,5-di methylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) biochemical assay which has previously been used in assessing the viability of excised buccal mucosa and cornea [119, 120]. Histological evaluation

of tissue done by Nicolazzo and coworkers demonstrated that the buccal epithelium appeared viable up to 9 h postmortem, and this was supported by the MTT biochemical assay, which indicated that viability was maintained for up to 12 h [102]. Therefore, it is recommended that all permeation experiments using porcine buccal mucosa be performed within 9–12 h of animal death. While there are limitations associated with the use of an in vitro permeability model for assessing the transport of compounds across the buccal mucosa, it can still be useful in assessing and comparing the permeability of compounds under different conditions, such as pH, temperature, and osmolarity, which pro- vide valuable information on the mechanisms involved in drug transport. Additionally, the preliminary effects of potential chemical penetration enhancers or formulation excipients may be assessed, and these may provide a substantial rationale for subsequently assessing the effect of these agents in man.

Buccal Cell Cultures

The use of buccal cell cultures for assessing the permeability of the buccal mucosa has attracted recent attention. In order to culture buccal epithelial cells, the cells must be harvested from an appropriate source and cultured under specific conditions using an appropriate growth medium, temperature, and humidity [56]. Cell cultures have been successfully grown by Tavakolisaberi and coworkers from hamster cheek pouch. These cultured cells, however, did not differentiate to form a complete keratinized surface as seen in the normal hamster cheek pouch, and they consequently displayed a greater permeability to compounds when compared with keratinized hamster cheek pouch mucosa [121]. Therefore, the cultured hamster cheek cells more closely mimicked the human buccal mucosa due to their lack of keratinization, and so this may be an appropriate model for predicting permeability through the human buccal mucosa.

Another cell culture model which has been proposed as a model of the human buccal epithelium is the TR146 cell line by Nielsen and coworkers [86, 122-125]. Rupniak and coworkers stated that the TR146 cells originate from a human buccal carcinoma [126], and when cultured, Jacobsen and coworkers showed that they form an epithelium resembling that of the buccal mucosa [123], with the appropriate differentiation patterns seen in human non-keratinized epithelium [127]. However, Nielsen and coworkers proved that the TR146 cell culture model has less of a barrier nature when compared to human and porcine buccal epithelium, as demonstrated by a significantly greater permeability to tritiated water, mannitol, testosterone, dextrans, and nicotine [86, 124, 128, 129], and this may be due to the cancerous nature of the original cells.

Recently, a cell culture derived from biopsies of healthy human buccal mucosa has been developed by Selvaratnam and coworkers with remarkably similar morphology, membrane- coating granule structure and appearance, and lipid composition to intact buccal tissue [130]. The barrier nature of this cell culture model is similar to intact buccal mucosa, and so this cell culture may be an alternative model to the TR146 cell culture. With the development of tissue culture techniques, it is anticipated that various cell culture models may be developed with similar morphological and barrier properties to normal intact buccal mucosa. Such models may be very useful in assessing the buccal permeability and metabolism of many compounds.

Buccal Permeation Enhancement:

To overcome the permeability issue of the buccal mucosa, there is a simple approach to include chemical compounds into the buccal delivery systems that promote the penetration of the active drug ingredients into the across it. So, these compounds are termed “buccal penetration enhancers or promoters”.

Aungst stated that the chemicals used as penetration enhancers ideally should be safe and non-toxic, pharmacologically and chemically inert, non-irritant, and non-allergenic [131]. In addition, the tissue should revert to its normal integrity and barrier properties upon removal of the chemical.

Mechanisms of action of buccal penetration enhancers:

Ganem-Quintanar and coworkers stated that mechanisms by which penetration enhancers are thought to improve mucosal absorption include the following [132]:

(i) Changing mucus rheology: Mucus covers the respiratory, gastrointestinal and genitourinary tracts, and forms a viscoelastic layer of varying thickness which affects drug absorption [132]. In the mouth, it is covered by a layer of saliva. There is evidence that, for certain drugs, saliva can hinder absorption, but usually it is insignificant compared to the other barriers during passage through the oral mucosa [2]. Therefore, saliva may have only a transient contribution to the oral barrier function, for example, decreasing the initial passage of water through the buccal epithelium [133].

(ii) Increasing the fluidity of membrane lipid bilayers: As the intercellular pathway is the most generally accepted route for drug absorption [56, 116, 117, 134, 135] the disruption of inter- cellular lipid packing, by interaction with either lipid or protein components, is thought to increase permeability. Biophysical techniques, e.g. differential scanning calorimetry (DSC) and infrared spectroscopy (IR), have demonstrated that, there is indeed, a correlation between increased lipid fluidity and enhanced membrane permeability. Varying degrees of insult may occur in tissues that are in intimate contact with the enhancer [132], therefore, a transient increase in the fluidity of the intercellular lipids may be thought of as a relatively nontoxic effect, whereas extraction of the intercellular lipids or denaturation of cellular proteins may be viewed as being somewhat more drastic. Therefore, an important consideration is to ensure that the effect of the enhancer on membrane permeability is reversible [136].

(iii) Affecting the components involved in the formation of intercellular junctions: This could be particularly important in the case of intestinal membranes, where the barriers to paracellular diffusion of molecules and ions are the tight junctions or 'zona occludens'. Although in oral mucosa tight junctions are almost absent, desmosomes, which are thought confer added structural integrity, occupy ≈47% of the intercellular space of the palatal stratum corneum and a further ≈10% is occupied by saccules [137]. This suggests that disruption of these structures may provide a permeability pathway through the oral stratum corneum, but this has not been entirely confirmed [137, 138].

(iv) Overcoming the enzymatic barrier: Protease inhibitors for endo- and exo-peptidases are potential penetration enhancers. Although various peptidases and proteases are present within the oral mucosa, and it is possible that metabolism may act as an enzymatic barrier, the intercellular pathway is thought to be deficient in proteolytic activity [132, 136]. However, changes in membrane fluidity induced by penetration enhancers may indirectly alter enzymatic activity [132].

(v) Increasing the thermodynamic activity of drugs: This may be affected by the vehicle composition, which will influence drug solubility [139, 140] and also by ion-pair formation between the enhancer and the drug [70, 141].

Cyclodextrins as penetration Enhancer:

Senel and Hincal showed that cyclodextrins have been classified as a new class of penetration enhancers.[142]. Irie and coworkers showed that cyclodextrins can enhance drug permeation by increasing drug availability and stability at the surface of the biological barriers[143]

Mucoadhesion and Mucoadhesive Polymers used in Oral Mucosal Delivery:

Bio adhesion is defined as the state in which two bodies' one or both of adherents are of a biological nature and are held together for extended periods of time by interfacial forces. A bio adhesive can therefore be defined as a substance, which has an ability to interact with biological materials, and is capable of being retained on the biological substrate for a period of time. One distinctive feature of bioadhesion is that adhesion almost always occurs in the presence of water.

Mucus is a gel like structure that covers the oral cavity. Mucus is secreted by the goblet cells lining the epithelia or by the special exocrine glands with mucus cells acini [144, 145]. Any bioadhesive material must first interact with the mucus for bioadhesion. Thus, mucus serves as a link between the bioadhesive material and the membrane. The composition and thickness of the mucus layer vary with location, sex, and state of health [145].

Mucus is a hydrated glycoprotein network. These glycoproteins consist of a protein core with oligosaccaharide pendant chains most of them end with sialic acid or sulfonic acid [146, 147], or L-fucose [148]. These oligosaccharide chains are covalently attached to the hydroxy amino acids, serine, and threonine along the polypeptide backbone [149]. Figure 2 shows a schematic representation of the glycoprotein mucin. Each of the side chains composes anywhere from 2-20 sugars in length and terminates in sialic or fucose. About 25% of the polypeptide chain lacks sugars and is referred to as "naked" protein region, which is especially prone to enzymatic cleavage [150]. The remaining 75% of the backbone is heavily glycosylated. The terminal sialic acid groups have a pKa of 2.6 [149]. At physiological pH, the mucus network is negatively charged because of the presence of sialic acid and sulfate residues, which are responsible for bioadhesion.

Mechanisms of adhesions:

The mechanisms of bioadhesion are not completely clear. To develop a good adhesive system, it is important to understand and elaborate the forces responsible for adhesive bond formation. Two steps have been described for an adhesive bond formation: 1. intimate contact of the polymer by wetting and swelling. 2. interpenetration and diffusion [151]. Bioadhesive bonds formed can be either mechanical or chemical in nature.

Mechanical or Physical bonds: These bonds form as a result of physical entanglement between the polymer and the mucin chains and interpenetration of the mucin strands into the porous polymer. The ate of bond formation depends on the rate of interpenetration of the polymer chains and the mucin strands, which in turn depends on the diffusion coefficients, and flexibility of the chains. The bonding strength of the adhesive bond is directly proportional to the depth of penetration of the polymer and mucin chains [152].

Chemical Bonds: These may include strong primary bonds (i.e., covalent bonds and ionic bonds), van der walls interactions, and hydrogen bonds. Mucoadhesive delivery system with primary bonds as the driving force for mucoadhesion is not sought due to irreversible damage of tissue/mucosa surface. Researchers have focused on developing mucoadhesive systems that bond through van der waals interactions and hydrogens bonding. Although these forces are weak, strong bioadhesion can be produced through numerous interaction sites. Therefore, polymers with high molecular weights and greater concentrations of polar groups (such as -COOH, and -OH) tend to develop more intense mucoadhesive bonds [152].

Theories of bioadhesion:

Several theories of bioadhesion have been proposed for the adhesiveness of the polymer with the mucus. These bioadhesion theories include adsorption, wetting, electronic, diffusion, and fracture.

Adsorption theory: This theory states that the adhesive bond is formed after an initial contact between the polymer and the substrate. Van der waals interactions, hydrogen bonds, and other related weak interaction bonds are thought to play an important role. This theory was best explained by Kinloch and Huntsberger [32].

Wetting theory: This theory explains bioadhesion based on the ability of the polymer or the mucus to spread and develop an intimate contact with each other. Work of adhesion, which is the energy required to break the unlike molecules can be expressed in terms of surface and interfacial tension (y) defined as the energy per cm^2 . Work of adhesion is given by Dupre's Equation [33].

$$W_a = \gamma_m + \gamma_p - \gamma_{mp} \quad \text{Eq. 2}$$

Where, the subscripts m and p refers to mucosa and bioadhesive polymer respectively. The work of cohesion, required to separate the two layers of the spreading liquid so that one of the layer flows on the other layer. Work of cohesion is given by

$$W_c = 2\gamma_m \; or \; 2\gamma_p \quad \text{Eq. 3}$$

Spreading occurs if the work of adhesion is greater than the work of cohesion. The term (W_a - W_c) is known as the spreading coefficient (S). For the bioadhesive polymer p to spread on the mucous membrane m, the spreading coefficient S is given by

$$s_{p/m} = \gamma_m - \gamma_{mp} + \gamma_p \quad \text{Eq. 4}$$

if $S_{p/m}$ is positive, the bioadhesive polymer will adhere to the mucous membrane. For a liquid adhering to the mucous membrane m, the contact angle is given by

$$\cos\theta = \left(\gamma_m - \left({}^{\gamma_{mp}}/_{\gamma_p}\right)\right) \quad \text{Eq.5}$$

Mutual spreading of the mutual systems i.e., pig intestinal mucosa and bioadhesive polymer, is essential for the mucoadhesion [151, 153].

Diffusion theory: This theory states that the intermingling and interpenetration of the mucus chains and the bioadhesive polymer produces a semi-permanent bond [154]. The bioadhesive polymer and the mucus glycoproteins come in close contact with each other. The bond strength depends on the depth of the penetration of these polymer chains. The following equation can be used to estimate the depth of the penetration:

$$L = (tD_b)^{0.5} \quad \text{Eq. 6}$$

Where t is the contact time and D_b is the diffusion coefficient of the biomaterial in mucus. The depth of penetration (L) depends upon the diffusion coefficient, time of contact and other experimental variables [155].

Electronic theory: According to this theory, electron transfer occurs on contact of an adhesive polymer with a mucus glycoprotein network due to their differences in electronic structures.

Fracture theory: This is the most useful theory for the study of bioadhesion through tensile experiments. This theory is related to the forces required for the separation of the two layers after adhesion. The fracture strength σ , which is the adhesive strength, is given by

$$\sigma = \left({}^{E\varepsilon}/_{l}\right)^{0.5} \quad \text{Eq. 7}$$

Where E is the fracture energy, ε is the Young's modulus, and *l* is the critical crack length [156].

From a bioadhesive delivery point of view, understanding the mechanism of bioadhesion, theories, which best explain these phenomena, are a combination of wetting, diffusion, and electronic theory, although other mechanisms may be operative for a given system.

Salamat-Miller and coworkers wrote a full comprehensive review about the mucoadhesion and the use of mucoadhesive polymers in the buccal delivery[157].

Conclusion:

The study of oral mucosal absorption and its application in drug delivery systems has gained significant attention due to its potential to improve the bioavailability of drugs and reduce the side effects associated with systemic administration. The review has highlighted the importance of saliva in maintaining the health of the oral tissues and facilitating drug delivery. The mechanisms of oral mucosal absorption, particularly passive diffusion, have been discussed, and the use of hydrophilic polymeric matrices as vehicles for oral transmucosal drug delivery systems has been emphasized. The review has also explored the methods used to assess oral mucosal permeation, including both in vivo and in vitro approaches. In vivo methods, such as the buccal absorption test, allow for the bioavailability of drugs via this route to be determined, while in vitro methods, such as diffusion cells, enable the kinetics of tissue transport to be assessed. The use of appropriate animal models in in vitro permeability studies has been emphasized to ensure that the model membrane is intact and that the observed permeability profiles of model compounds are not a result of compromised tissue integrity. The review has also addressed the challenges and limitations associated with oral mucosal drug delivery, such as the difficulty in studying regional variation in drug absorption and the limitations of in vitro permeability models in predicting in vivo situations. To overcome these limitations, various absorption or perfusion cells have been designed to study regional variation in drug absorption. Future studies should focus on the buccal routes and permeability, as these areas have significant potential for improving drug delivery systems. The correlation between plasma concentrations and salivary concentrations should be further investigated to determine the validity of using saliva as a surrogate for plasma in assessing systemic buccal absorption. Additionally, high-throughput permeability screening is required in the preclinical setting, and in vitro permeability models can be useful in assessing and comparing the permeability of compounds under different conditions, providing valuable information on the mechanisms involved in drug transport.

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