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Histological features of oral epithelium in seven animal species: As a reference for selecting animal models

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ABSTRACT

Several animals have been used as models for basic and clinical research on oral mucosa. Few studies have focused on the selection of an appropriate animal model. This study aimed to provide histological references for selecting a potential model. Histological features were assessed by exploring 6 morphological characteristics and 2 immunohistochemical markers. The morphological characteristics included keratinization, basal membrane appearance, epithelial thickness, rete ridge length, adjacent rete ridge distance, and regional variation; the immunohistochemical markers included Ki67 (a proliferative marker) and Cytokeratin 19 (CK19; a stemness marker). The histological similarity of each species compared to humans was calculated according to the designated scoring criteria. The results showed that the buccal mucosae from dog and pig were non-keratinized, with similar rete ridge length and distance, compared to that of humans. The dog, rat, and cavy mucosae had analogous gross appearances in the basal membrane. The dog oral mucosae shared similar epithelial thickness with human oral mucosae. Compared to the human mucosa, the dog, pig, rat, and rabbit mucosae exhibited corresponding regional variations. The Ki67-positive cells in human and canine mucosae were predominantly localized in the suprabasal layers, whereas most of the proliferative cells were in the basal layer in other species. CK19 immunoreactivities were detected only in human and canine mucosae. The canine mucosae gained the highest point value (14), whereas the scores for the pig, rat, rabbit, cavy, sheep, and buffalo mucosae were 8, 6, 5, 5, 5, and 2, respectively. The histological variations in the oral epithelium of diverse animal species are considerable; the mucosae from dogs are most similar to human mucosae, implicating its histological basis as an animal model.

1. Introduction

Animal models have significantly prompted the development and progress of medical research. In recent decades, some recognized animal species, including beagle dogs, pigs, rats, rabbits, and cavies, have offered novel insights into physiological and pathological processes within the human body and allowed for the preclinical testing of new pharmaceutical targets for various diseases. In addition, these animal species have played a prominent role in basic and clinical research related to oral mucosa, predominantly including oral delivery (Kasarello et al., 2015) and the molecular biology of oral cancer (Chen and Lin, 2010), aphthous stomatitis (Al-Azri et al., 2014; Fernandes Teixeira et al., 2014; Johnson et al., 2012), and transplantation of engineered oral mucosa (Moharamzadeh et al., 2012). Few researchers have focused on the selection of animal models in studies of diseases and functions of oral mucosa.

Rat, rabbit, and cavy species are most commonly employed to investigate oral administration (Kasarello et al., 2015; Nerkar and Gattani, 2013), the pathogenesis of oral squamous cell cancer, oral mucosa wound healing (Kara et al., 2013; Kilic et al., 2013), and therapeutic methods for oral mucositis (Li et al., 2014). Beagle dogs and pigs are predominantly used in research related to preclinical testing of *ex vivo*-produced oral mucosa equivalents (Ohki et al., 2006; Ophof et al., 2008; Ophof et al., 2002; Wei et al., 2009). These animal models could be divided into small and large animals according to their distinct advantages; the former include rat, rabbit, and cavy species, whereas the latter include beagles and pigs. Small animal species are inexpensive and easy to care for, and they can be investigated in collective numbers (Talac et al., 2004); these advantages predominantly account for their selection as animal models by researchers. Large animal species resemble humans in size, physiology, development, and disease progression (Wendler and Wehling, 2010). In addition, canine and porcine buccal mucosa is non-keratinized and more similar to human mucosa than the buccal mucosa of other species, except for that of nonhuman

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primates (Collins et al., 1981; Kinikoglu et al., 2012; Shabana et al., 1989). These advantages suggest the selection of large animal species for animal models for the evaluation of cell-based devices (Moharamzadeh et al., 2012).

Because of the analogous advantages, individual researchers could select different species in the abovementioned experiments. Rat, rabbit, and cavy species are alternatively employed to investigate the causes and therapeutic effects of oral mucositis (Fernandes Teixeira et al., 2014; Johnson et al., 2012; Li et al., 2014), oral delivery (Kasarello et al., 2015; Kilic et al., 2013; Nerkar and Gattani, 2013), and oral mucosa wound healing (Kara et al., 2013; Ryu et al., 2012; Zhu et al., 2015). Because of intrinsic interspecies variations, the experimental outcomes might be subjected to bias because of the selection of different animal species (Chen and Lin, 2010). An appropriate and established animal species with oral mucosa that mimics that of humans is required (Wendler and Wehling, 2010).

Form follows function, which is particularly true of oral mucosa, whose structure reflects various functional adaptations (Winning and Townsend, 2000). Each animal species has distinct eating patterns and chewing habits, suggesting morphological variations. Morphological features exert a tremendous influence on the translatability of the experimental outcome of the animal model (Campisi et al., 2008). Keratinized oral epithelium has low permeability, which compromises investigations related to oral delivery; keratinized oral epithelium presents less aphthous mucositis, which is inappropriate as an ulcer animal model. In this context, we are confident that the suitability of different animal models varies.

In this study, we aimed to compare the morphological features and expression of critical molecules associated with cell proliferation and the stemness of the oral epithelium in seven animal species. We used hematoxylin–eosin staining to assess the morphological similarity using 6 morphological indicators, including keratinization, gross appearance of the basal membrane area, epithelial thickness, length of rete ridge, distance of adjacent rete ridge, and regional variation; we performed immunohistochemistry staining to evaluate the immunohistochemical similarity, including the expression of the proliferative marker Ki67 and the stemness marker CK19 (Larouche et al., 2005; Wu et al., 2013).

2. Materials and methods

2.1. Specimen collections

Human mucosae ($n = 20$) derived from redundant tissues during trauma or plastic surgeries were obtained from the Hospital of Stomatology, Wuhan University; a pathological biopsy confirmed that the samples were normal oral mucosae. The protocol for harvesting human mucosal tissues was approved by the review board of the Ethics Committee of the Hospital of Stomatology, Wuhan University. The participants were fully informed and provided written consent. The animal mucosal tissues were taken from the respective species ($n = 6$) at the precise age that corresponds to the age of a human between 20 and 30 years old (an equal number of males and females).

Oral mucosal epithelium displays considerable structural variation in different regions (Winning and Townsend, 2000). In this study, we selected hard palatal and buccal mucosae as representative samples of masticatory mucosae and lining mucosae, respectively. The palatal oral mucosae were taken from corresponding sites opposite from the upper molar of each animal, whereas the buccal mucosae were obtained from similar areas opposite the first upper molar. Canine and rodent mucosal tissues were collected when the animals were sacrificed for other experimental purposes. Porcine, ovine, and bovine tissues were collected from the respective meat processing houses when the animals were killed for edible meat. The use of animals was in compliance with the institutional animal care protocols of the Hospital of Stomatology, Wuhan University.

2.2. Histological processes and immunohistochemistry

The oral mucosal tissues were fixed in 4% paraformaldehyde in 0.1 M PBS, pH 7.4, at 4 °C overnight after the collections and then dissected into 4-μm segments. The sections were deparaffinized in xylene twice, rehydrated through graded alcohol series, and subjected to HE staining and immunohistochemistry using the following antibodies: Ki67 (ab15580, 1:200) and CK19 (ab84632, 1:300). For the first antibody, we used heat-induced antigen retrieval (EDTA, pH9), with developing at 4 °C overnight. Secondary antibody kits (Maixim, Beijing, China) and DAB (ZSGQ, Beijing, China) were used as the chromogen according to the respective protocols. Skin samples were used as positive controls, whereas negative controls were obtained by utilizing PBS as a substitute for the primary antibodies.

2.3. Score indicators

Eight indicators were employed to evaluate the similarity, including the keratinization, gross appearance of the basement membrane zone, epithelial thickness, length of the rete ridges, distance of adjacent rete ridges, regional variations, and expression pattern of the Ki67- and CK19-positive cells. 1) The keratinization demonstrated whether the epithelium was keratinized, non-keratinized, or para-keratinized. 2) The gross appearance of the basement membrane zone referred to whether the dominant type of rete ridges was single, complex, or latticed. Simple and complex rete ridges have a single cusp or multiple cusps, respectively, whereas latticed rete ridges are cross-linked (Fig. 1A–C) (Moore et al., 1992; Wu et al., 2013). 3) Epithelial thickness referred to the vertical distance from the superficial layer to the connection area between the epithelium and lamina propria (Fig. 1B). 4) The length of the rete ridges was equivalent to the vertical distance from the top to the basal part of rete ridges (Fig. 1A). 5) The distance of the rete ridges corresponded to the horizontal length of the adjacent rete ridges (Fig. 1C). 6) The regional variations were reflected by the relative horizontal distance of the adjacent rete ridges between the palatal and buccal epithelia. 7) The expression of the Ki67-positive cells was assessed by the percentage of Ki67-positive cells in the most basal layer. 8) The CK19 expression was evaluated based on whether the epithelial cells expressed CK19. To complete the morphometric analysis, 4 high-resolution fields of each specimen were randomly selected at 200× magnification utilizing a light microscope (Leica).

2.4. Scoring criteria

Each indicator received 1 point if it could be comparable to that of humans. Keratinization and CK19 expression were scored by visual observation. If the keratinization corresponded to that of humans, the species were scored, and if the basal cells expressed CK19, the species were scored. For the other indicators, the statistical results determined whether the species were scored. If the differences were not statistically significant, the species were scored. Otherwise, the corresponding index was scored as 0 (Wu et al., 2014). Because of the double comparison of the palatal and buccal mucosae, some indicators were scored as 2, as is shown in Table 1. A total score, typically ranging from 0 to 14, was assigned to each animal species.

2.5. Statistical analysis

One-way ANOVA testing was used for the multiple inter-group comparisons, and the unpaired *t*-test (one-tailed) was employed for the two-group comparisons utilizing Graphpad Prism software (GraphPad Software, Inc., San Diego, CA, USA). A p value <0.05 was considered statistically significant.