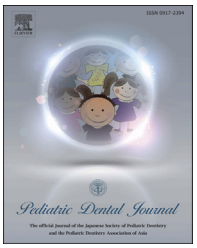
Available online at www.sciencedirect.com

Pediatric Dental Journal

journal homepage: www.elsevier.com/locate/pdj



Original Article

Establishment of *ex vivo* mucocele model using salivary gland organ culture

Ryoko Hino [1], *Masaharu Futagi* [1], *Aya Yamada*, *Makiko Arakaki*, *Kan Saito*, *Yu Sugawara*, *Mariko Ono*, *Emiko Fukumoto*, *Takashi Nakamura*, *Satoshi Fukumoto**

Division of Pediatric Dentistry, Department of Oral Health and Development Sciences, Tohoku University Graduate School of Dentistry, Sendai 980-8575, Japan

ARTICLE INFO

Article history:
Received 27 December 2013
Accepted 7 March 2014
Available online 2 June 2014

Keywords:
Mucocele
Salivary gland
Organ culture

ABSTRACT

A mucocele is a common lesion originating from the minor salivary glands and frequently seen in children. For this study, we established an *ex vivo* mucocele model using a mouse salivary gland organ culture method.

Results: First, medium from the upper portion of salivary gland organ cultures was either removed or not, then culturing was continued for 10 days. After that 10-day period, 13 of 21 specimens (61.9%) in the medium removed group showed mucocele-like mucus restoration, while only 1 of 15 (6.7%) in the non-medium removed group showed restoration. Next, we examined mucocele type in the *ex vivo* salivary gland organ cultures and found mucous retention type mucocele formation in only the main duct of most of the cultures. Other types were observed in the main excretory and intercalated ducts, but not exclusively in the intercalated duct. We also investigated the effect of duct cutting on mucocele recrudescence. Mucoceles that developed in the cultures were cut with stainless needles under a stereomicroscope and mucus was promptly discharged. At 7 days after duct cutting, 5 of 19 specimens (26.3%) showed recrudescence mucus retention.

Conclusions: This is the first report of establishment of an *ex vivo* pathogenic condition for examination of salivary glands. Our model may be useful to establish mucocele treatment options including drug screening and surgical procedures.

1. Introduction

Minor salivary glands are found in most parts of the oral cavity except the gingiva. Mucoceles, which are generally asymptomatic, are a common oral mucosal lesion that originate from those glands, and are seen more frequently in children and adolescents [1,2]. The term mucocele describes the accumulation of mucus within a salivary gland, and they are typically subdivided into two types based on histopathological findings. One is a mucous extravasation cyst, which is generally regarded as being the result of trauma, such as lip

* *Corresponding author.*
E-mail address: fukumoto@dent.tohoku.ac.jp (S. Fukumoto).
[1] These two authors contributed equally to this work.
http://dx.doi.org/10.1016/j.pdj.2014.03.003

biting, while the other is a mucus retention cyst caused by the obstruction of the duct of a minor or accessory salivary gland [3,4]. In the retention type (also called salivary duct cyst), trapped mucus is lined by columnar or cuboidal ductal epithelium [3,5].

The clinical features of mucoceles have been well documented and vary depending on depth within soft tissue and the degree of keratinization of the overlying mucosa. Superficial lesions are presented as raised soft-tissue swelling with a bluish color, while deeper lesions are more nodular, lack a vesicular appearance, and have a normal mucosal color. Size can vary from 1 mm to several centimeters [4]. The lower labial mucosa is the most frequently affected site, though mucoceles can also develop in the cheek, tongue, palate, and floor of the mouth, where it is called a ranula [3,6]. Mucoceles often arise within a few days after minor trauma, but then plateau in size and can persist unchanged for months unless treated [7,8]. Although most cases are asymptomatic, functional disturbances such as difficulty with eating and changed dietary habits may require immediate surgical intervention. Different techniques have been described for the treatment of mucoceles, though they usually require surgical excision [8,9]. On the other hand, no evidence showing the mechanism of formation or healing of a mucocele has been reported.

Recently, salivary gland organ cultures have been used to analyze the molecular mechanism of branching morphogenesis. In vivo, mouse submandibular gland (SMG) development is initiated as a thickening of oral epithelium on about embryonic day 12 (E12), when an initial epithelial bud on a stalk grows into condensing neural crest-derived mesenchyme. Clefts in the epithelium result in 3–5 epithelial buds by E13.5, and then branching morphogenesis occurs with continued proliferation, along with successive rounds of cleft formation, duct elongation, and lumen formation, resulting in a highly branched gland by E14. [10] *Ex vivo* organ cultures of SMG tissues from E13 show a similar developmental pattern of branching morphogenesis on the filter as compared with in vivo development. Previously, our group identified the role of platelet-derived growth factors (PDGFs) and their receptors in SMG branching morphogenesis [11]. PDGFs induced fibroblast growth factor 7 (FGF7) and FGF10 expression in SMG mesenchyme, which in turn enhanced the branching of organ-cultured SMG tissues [11,12], indicating that epithelial–mesenchymal interaction via PDGF–FGF signaling is important for salivary gland development and further that this culture system is useful to identify the mechanism of salivary gland morphogenesis. Based on the results of those experiments, we considered that the pathogenic condition of salivary glands could be reproducible using this organ culture system. For the present study, we established a mucocele pathogenic model using SMG organ cultures.

2. Materials and methods

2.1. *Animals and reagents*

Pregnant ICR mice were used in this study. Dulbecco's modified Eagle's medium/F-12 (D-MEM/F-12, Gibco), penicillin and streptomycin (Pen Strep, Gibco), vitamin C (Sigma–Aldrich), and a transferrin agent (Sigma–Aldrich) were used for the organ cultures.

2.2. Ex vivo *submandibular organ cultures*

Submandibular and sublingual salivary gland rudiments (referred to as SMGs in this study) were dissected from E13 mice and cultured on cell culture inserts (0.4 μm pore size, Falcon) at the air–medium interface. Each insert was placed in 2 ml of D-MEM/F-12 in six-well dishes (Greiner Bio-one). The medium was supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 150 μg/ml vitamin C, and 50 μg/ml transferrin. Five SMGs were cultured on each filter at 37 °C in an atmosphere of humidified 5% CO_2 and 95% air. The SMGs were photographed using a phase-contrast microscope (SZX121, OLYMPUS) and end buds were counted at each time point.

3. Results

3.1. *Establishment of mucocele by removal of upper medium*

Submandibular and sublingual salivary gland organs from E13 mouse embryos (Fig. 1A) were digested and cultured on filter

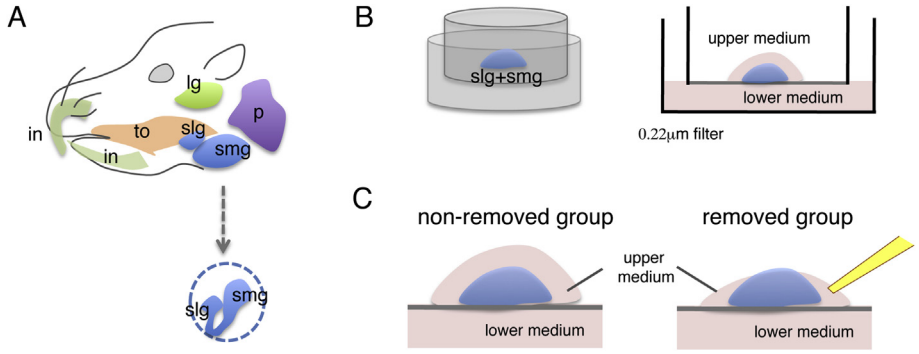


Fig. 1 – *Ex vivo* organ culture system using mouse salivary glands. (A) Sublingual and submandibular glands were dissected from the neck region of mice. (B) Salivary gland tissues were cultured on 0.22-mm pore filters with both upper and lower medium. (C) In the removed group, the upper medium was partially removed with a pipette.