Cannulation of the mouse submandibular salivary gland via the Wharton's duct

Y Kuriki, Y, Liu, D. Xia , E.M. Gjerde, S Khalili, B Mui, C Zheng, S.D. Tran

Faculty of Dentistry, McGill University, CAN

Short Abstract

A protocol for the cannulation of the mouse submandibular salivary gland via the Wharton's duct is described. For this experiment, the trypan blue solution is used as a dyer to demonstrate how this technique effectively delivers infusions into the targeted gland, and to suggest the reliability of this new approach as a potential clinical drug/cell therapy for the regeneration of salivary glands.

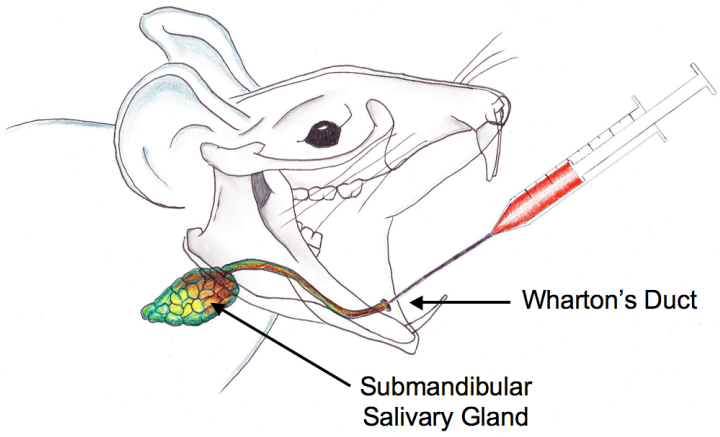
Long Abstract

Hypo-function of the salivary glands is one of the most common symptom of Sjögren's syndrome and side effects of therapeutic irradiation for cancer treatment in the head and neck regions. Malfunction of the salivary gland can often lead to dental diseases such as infection of the oral mucosa and tooth decays et al.

One innovative approach of regenerative medicine for the treatment of salivary gland hypo-function is speculated in RS Redman, E Mezey et al. 2009: stem cells can be directly deposited into the gland as a potent method in reviving the functions of the impaired organ. Presumably, the migrated foreign stem cells will differentiate into glandular cells to function as part of the host salivary gland. Hence, the cannulation technique may be an expedient and effective tool for clinical gene transfer application.

Here we illustrate the steps involved in performing the cannulation procedure on the mouse submandibular salivary gland via the Wharton's duct. C3H mice (Charles River, Montreal, QC, Canada) are used for this experiment, which have been kept under clean conventional conditions at the McGill University animal resource center. All experiments have been approved by the University Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

For this experiment, the trypan blue solution is injected into the gland through the opening of the Wharton's duct with an Ultra Comfort 29-gauge needle and syringe; subsequently, the mouse is dissected to show that the infusions migrated into the gland successfully.



Procedural Description

1. Subcataneously inject 1 µl / g body weight of 0.5 mg / ml atropine sulfate monohydrate (Cat# 11330, Fluka, USA) into each mouse to prevent the salivary secretions from disrupting the movement of the infusions.

2. Anesthetize each mouse with 1 µl / g body weight of a 100 mg / ml ketamine (Phoenix Scientific, AR, USA) and 20 mg/ml xylazine (Phoenix Scientific, AR, USA) solution injected intra-peritoneally (Ip).

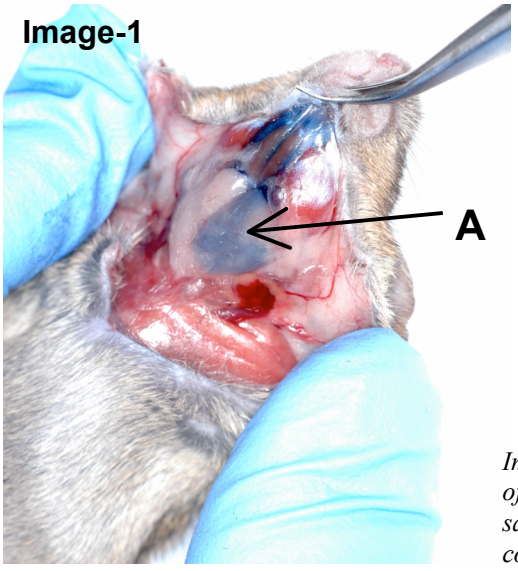
3. Place the mouse on a custom made plastic platform in the ventral position: the maxillary incisor is locked on a metal wire, and the mandibular incisor is hooked on an elastic string in order to hold the mouth open.

4. Using an Ultra Comfort 29-gauge needle (Cat# 600145, Tyco Healthcare, USA) and syringe, inject filtered 0.4% trypan blue stain solution (Cat# 15250, Gibco, USA) into the submandibular salivary gland via the Wharton's duct, which is located right beneath the tongue. The staining solution is used as a dyer to demonstrate the technique.

5. Immediately after the cannulation procedure, the salivary gland is surgically exposed in order to determine how much of the infusions have actually remained in the gland.

Representative Results

After the infusions were injected, the mouse was dissected to surgically expose the post-cannulated salivary gland. As you can see in the image-2, the dark coloration of the Wharton's duct gives strong evidence that the trypan blue solution was infused successful.



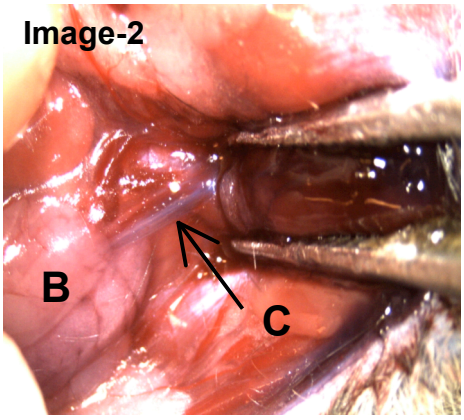


Image-1: The mouse was dissected right after the injection of trypan blue solution into the mouse submandibular salivary gland. A) Submandibular salivary gland containing the trypan blue solution.

Image-2: B) Submandibular Salivary Gland. C) Wharton's duct containing the trypan blue solution.

Discussion

One of the difficulties we have faced in doing the cannulation is preventing the backflow of the injected solution, which in this case is the trypan blue solution. Even if the trypan blue solution has temporarily migrated into the salivary gland successfully, the saliva secretion might interfere with the infusions and cause backflow of the trypan blue solution out of the gland. Although we have used atropine to inhibit the secretion of saliva, just as for precaution, the syringe should remain injected and held in the Wharton's duct for several seconds even after all the infusions has been deposited. This will allow most of the infused solution to remain within the gland.

Conventionally, the systemic application, namely the intravenous injection (IV) of drugs / cells, has been used as a clinical approach for salivary gland regeneration. Intravenously injected infusions will travel through the entire circulatory system, allowing only some of the drugs / cells to migrate into the targeted glands. Compared to this traditional method, the local application, that is the injection of drugs / cells via the cannulation procedure, may be perceived as a more direct and effective way in reviving the functions of the parotid, submandibular, and sublingual salivary glands.

As evident in the representative results, the infusions, which in this case is the trypan blue solution, was successfully deposited into the mouse submandibular salivary gland via the Wharton's duct. Hence, this cannulation technique can be used as a reliable model for the regenerative medicine of mouse salivary gland; and as a future prospect, this procedure may be an expedient method for clinical application.

It would be a great pleasure for this visual-article to be used as an effective tool for students and researchers who are interested in further studies in the regenerative medicine of the salivary gland.