Common Questions about Salivary Gland and Lacrimal Gland Regeneration

Q1: What are the advantages of your work?
Answer: We have provided a proof-of-concept that exocrine glands, such as salivary glands and lacrimal glands, can be regenerated to be fully functional. This can be achieved by the transplantation of bioengineered salivary gland germ or lacrimal gland germ, which are reconstructed from embryonic epithelial and mesenchymal stem cells using the "Organ Germ Method". These studies showed potential as attractive treatments for xerostomia (dry mouth) and corneal xerosis (dry eye disease).

Q2: What is the difference between your study and previous studies?
Answer: Secretory gland regenerative strategies involving drug therapy, gene therapy and various stem cell transplantation therapies have been proposed and used in attempts to rescue secretory gland impairment in several previous studies. The development of a novel therapeutic treatment to restore secretory gland functions is necessary as an organ replacement regenerative therapy but not for the purpose of tissue repair. In our current study, we achieved fully functional salivary gland and lacrimal gland regeneration in a murine model through the orthotopic transplantation of bioengineered gland germ reconstituted using the Organ Germ Method. We have provided a proof-of-concept for bioengineered secretory organ replacement as a regenerative therapy.

Q3: What are the major breakthroughs in this work?
Answer: The first breakthrough in our study was the demonstration that the Organ Germ Method, as a fundamental technology for organ regeneration, can also be applied to the regeneration of secretory organs, such as the salivary glands and lacrimal glands. The second breakthrough demonstrated that the bioengineered salivary gland established the proper connection to the recipient salivary and lacrimal gland duct. The bioengineered gland germ, including the salivary and lacrimal gland germs, was transplanted using our previously developed inter-epithelial tissue-connecting plastic method with a guide for duct direction inserted into the bioengineered germ. The bioengineered glands developed in vivo with the correct connection to the recipient parotid or lacrimal gland duct, which was confirmed by the transport of saliva and tears without leaking.
Q4: Can you control the size and structure of the bioengineered salivary and lacrimal glands?
Answer: We have demonstrated that bioengineered secretory glands can develop the glandular cell components, including duct cells, acinar cells that produce the secretory fluid and myoepithelial cells that envelop the acinar cells in these bioengineered glands. Furthermore, nerve innervations were detected in the interstitial tissue among the acinar cells, and these nerve fibres connected to the myoepithelial cells. Our study indicated that the engrafted bioengineered gland germ cells successfully formed the correct tissue structures and were potential to secrete saliva and tears in response to neural stimulation. In this study, the bioengineered secretory gland was smaller in size than the natural gland, and size control technology has not been established yet. Such size control technology will be a focus of future studies to enable the practical use of this method.

Q5: Can you regulate the type of secretory fluid?
Answer: Ectodermal organs, such as teeth, hair, salivary glands, and lacrimal glands, develop through reciprocal epithelial and mesenchymal interactions according to the epithelial and mesenchymal cell fates determined during embryogenesis. The bioengineered salivary gland germ develops into the submandibular and sublingual glands with serous- and mucous-type acinar cells, respectively. Furthermore, the bioengineered lacrimal gland germ as a serous gland and the harderian gland germ as a sebaceous gland developed the correct respective acinar structures according to the cell fates of the epithelial and mesenchymal stem cells, which were determined during organogenesis. Our study thus indicates the potential for successful organ regeneration using stem cells.

Q6: How much fluid is secreted by the bioengineered salivary and lacrimal glands?
Answer: The amount of secreted saliva or tears depends on the size of the bioengineered secretory gland. Our studies demonstrated that the amount of saliva or tear secretion was sufficient for cleansing the oral cavity or protecting the cornea, respectively. In addition, we have demonstrated that the secretory function of the bioengineered secretory glands is maintained over the long term (more than 18 months).

Q7: Does this secretory gland regenerative therapy have an effect on dry mouth and dry eye due to autoimmune disease?
We want to examine this issue in future clinical studies. Severe cases of dry mouth or dry eye symptoms due to autoimmune diseases, including Sjögren's syndrome, are thought to be the result of secretory gland disorders caused by autoimmune reactions. Therefore, combining bioengineered organ replacement therapy with the administration of immunosuppressive agents is necessary. In these autoimmune diseases, the presence of a specific antigen is considered in accordance with the respective disease, but the responsible antigens have not been fully elucidated. The identification of the specific antigens will enable the bioengineered secretory gland to be regenerated using cells that suppress the gene expression of that antigen, contributing to the establishment of a radical treatment for dry mouth or dry eye due to autoimmune diseases.

Q8: What are the future issues for the clinical application of this method?
Answer: Several problems must be solved before the use of bioengineered secretory glands becomes feasible. To fully realise the practical clinical application of secretory gland regeneration, suitable cell sources must be identified. Our research group is currently attempting to induce bioengineered organ germ to develop a fully functioning organ using embryonic organ germ-derived epithelial and mesenchymal cells via the Organ Germ Method. In the future, we will need to identify cell sources from various tissue-derived stem cell populations isolated from patients who have the organ-inducible ability to reproduce the epithelial and mesenchymal interactions for organogenesis. Further investigation of the clinical application of these methods, including engraftment and recipient niches for organ regeneration, will contribute to the development of salivary gland regeneration therapy in humans.

Q9: Do you think that you can apply your studies to any field in the future?
Answer: We provided novel evidence of the successful replacement of a fully functional salivary gland and lacrimal gland through the transplantation of bioengineered germ. Our current study indicates that bioengineered organ replacement, which can achieve the full functional restoration of organ function, is widely applicable to not only ectodermal placode organs but also endodermal endocrine organs, including the liver and pancreas.

Q10: What is the difference between your study and previous tooth and hair regeneration studies?
Answer: We previously reported a novel bioengineering technique for reconstituting regenerated organ germs from teeth and hair follicles using a three-dimensional cell
processing method (Nature Methods 2007). Recently, we reported fully functional ectodermal organ regeneration involving teeth (PNAS 2009, PLoS ONE 2011) and hair follicles (Nature communication 2012, Scientific Reports 2012). In our current study, we achieved fully functional salivary gland regeneration through the orthotopic transplantation of a bioengineered salivary gland germ that was reconstituted using the Organ Germ Method. We have provided a proof-of-concept for bioengineered secretory organ replacement as a regenerative therapy.

Q11: How long will it take to implement the practical application of your secretory gland regeneration?
Answer: To fully realise the practical clinical application of secretory gland regeneration, suitable cell sources must be identified. We aim to achieve the practical use of secretory gland regeneration through various studies with the support of the national research project.

● Questions about salivary gland regeneration

Q1: What is the role of the salivary gland?
Answer: Salivary glands play essential roles in normal upper gastrointestinal tract function and oral health, including the digestion of starch by salivary amylase, swallowing, and the maintenance of tooth hard tissues through the production of saliva. Salivary glands, such as the parotid, submandibular, and sublingual glands, produce the serous-type saliva that includes many digestive enzymes and the mucous-type saliva that protects the oral viscosity. The combination of serous- and mucous-type saliva plays essential roles in homeostasis and oral functions.

Q2: What are the major symptoms of xerostomia?
Answer: Salivary gland impairment, which results from various physiological conditions including radiation therapy for head and neck cancer, Sjögren's syndrome, and aging, leads to acinar cell damage and salivary gland hypofunction, including xerostomia (dry mouth syndrome). Xerostomia causes various clinical problems in oral health, including dental decay, bacterial infection, mastication dysfunction, swallowing dysfunction, and dysgeusia, and a general reduction in the quality of life. Xerostomia patients account for one-quarter of the total population in North America (Crossley H. et al., Gen Dent., 55(4), 288-296, 2007), and xerostomia is recognised as an important issue in oral and general health.

Q3: What are the problems with the current treatments for xerostomia?
Answer: The current therapies for xerostomia involve the administration of artificial saliva substitutes and sialogogues, which are drugs or substances that increase the salivary flow rate. However, these saliva substitutes and drugs cannot restore salivary gland function. Therefore, a novel therapeutic treatment for the restoration of salivary gland function is needed.

Q4: Do the bioengineered salivary glands function normally?
Answer: Saliva secretion is an essential function of salivary glands that is critical for maintaining oral and general homeostasis and should be restored by salivary gland regeneration. The central nervous system controls saliva secretion. We analysed biological salivary secretion using gustatory stimulation with citrate. The engrafted bioengineered salivary glands secreted significant quantities of saliva in response to citrate stimulation via afferent and efferent neural networks. Our study indicates that the bioengineered salivary gland secreted saliva via the proper innervations and via neurotransmission under the control of the central nervous system.

Q5: Can you use adult tissue-derived stem cells for the construction of bioengineered salivary glands?
Answer: Our research group is currently attempting to induce bioengineered salivary gland germ to develop a fully functioning organ using embryonic salivary gland germ-derived epithelial and mesenchymal cells via the Organ Germ Method. In the future, the identification of cell sources from various adult tissue-derived stem cell populations isolated from patients who have the organ-inducible ability to reproduce the epithelial and mesenchymal interactions for organogenesis will be required.

Q6: What types of clinical cases can be treated with regenerative salivary gland replacement therapy?
Answer: The number of xerostomia patients is rapidly increasing in accordance with an aging society. Xerostomia causes various clinical problems in oral health, including dental decay, bacterial infection, mastication dysfunction, swallowing dysfunction, and dysgeusia, and a general reduction in the quality of life. Therefore, a novel therapeutic treatment for the restoration of salivary gland function is required for xerostomia treatment.
Questions about lacrimal gland regeneration

Q1: What is the role of the lacrimal gland?
Answer: The lacrimal gland maintains a healthy ocular surface via tear secretion. The mature lacrimal glands, which consist of acini enveloped by myoepithelial cells and ducts, make effective tear secretory systems by working under the control of the central nervous system. Aqueous tears are composed of water and proteins that are secreted from the lacrimal glands. Tear lipids, which prevent tear evaporation, are secreted by the meibomian gland in humans and by harderian glands in mice. Tears are indispensable for lid lubrication, the protection of the epithelial surface, and visual function.

Q2: What are the major symptoms of corneal xerosis (dry eye disease)?
Answer: Dry eye disease (DED), which is caused by insufficient tear production, is the result of lacrimal gland dysfunction, as observed in Sjögren’s syndrome and ocular cicatricial pemphigoid; however, there are many other causes, including aging and long-term work with a visual display. More than 22 million individuals in Japan and approximately 12% of American people >50 years old are estimated to have DED. DED leads to corneal epithelial damage, which is characterised by the loss of individual cells from the superficial cell layer of the corneal epithelium. The irregularity of the ocular surface, which is caused by corneal epithelial damage, results in ocular discomfort, a significant loss of vision, and a decreased quality of life.

Q3: What are the problems with the current treatments for dry eye disease?
Answer: The current therapies for DED, including artificial tear solutions, are transient and do not completely replicate the normal tear complex, which is composed of water, salts, hydrocarbons, proteins and lipids. Several therapeutic approaches have been developed to restore lacrimal gland function, including heterotopic salivary gland transplantation and regenerative medicine.

Q4: Do the bioengineered lacrimal glands function normally?
Answer: The appropriate nervous control of tear fluid secretion is essential for the full function of the bioengineered lacrimal glands, and tears are required to protect the ocular surface. In our study, the bioengineered lacrimal and harderian glands secreted transparent tears and turbid tears, respectively, in response to cool cell activation on the ocular surface. In addition, the tear flow of the bioengineered lacrimal gland was equivalent to that of the glands of normal control mice. The lipid
concentration of the tears from the bioengineered harderian gland was increased compared with that of the tears produced by the normal or bioengineered lacrimal gland-engrafted mice. Our study demonstrates that the bioengineered lacrimal and harderian glands displayed a functional secretory ability under appropriate neural control.

Q5: What type of clinical cases can be treated with regenerative lacrimal gland replacement therapy?
Answer: Dry eye disease (DED), which is caused by a tear shortage, results from lacrimal gland dysfunction caused by systemic diseases, such as Sjögren's syndrome, Stevens-Johnson syndrome and ocular cicatricial pemphigoid, or by other causes, including aging, long-term work with a visual display, dry room environments, the use of contact lenses or refractive surgery, or environmental exposures. DED is one of the most prevalent eye diseases leading to corneal epithelial damage, which is characterised by the loss of individual cells from the superficial cell layer of the corneal epithelium. Therefore, a novel therapeutic treatment for the restoration of lacrimal gland function is required to treat DED.

Q6: Can you regenerate a meibomian gland in humans?
Answer: The meibomian gland is an organ that secretes lipid tears onto the eye surface. In this study, we have successfully regenerated the harderian gland as an equivalent organ in mice. These results suggest that regeneration of the meibomian gland using our Organ Germ Method is feasible because the meibomian gland is similar to the ectodermal sebaceous glands associated with hair organ development.

Thank you for your interesting questions.