Press Release

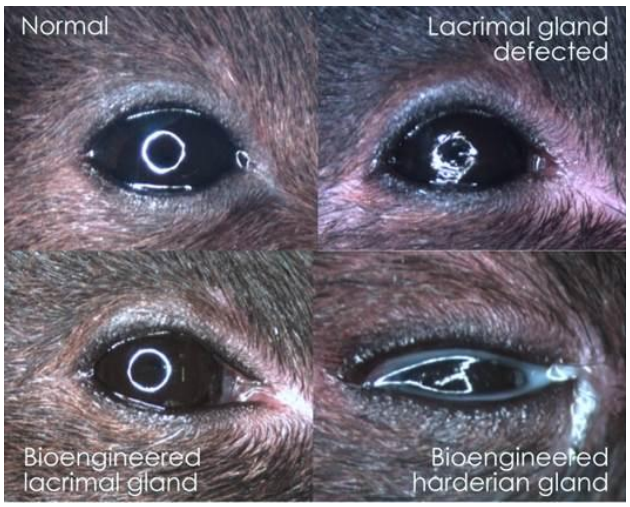
Research group headed by Professor Takashi Tsuji regenerates a "fully functional bioengineered lacrimal gland"

Substantial advance in the development of next-generation "organ replacement regenerative therapies" for dry eye disorders

— Announced in the online version of the UK scientific journal "*Nature Communications*" at 16:00 hrs (London Time), October 1st, 2013 —

Organ replacement regenerative therapy has been proposed as having the potential to enable the replacement of organs that have been damaged by disease, injury or ageing. A research group led by Professor Takashi Tsuji (Professor in the Research Institute for Science and Technology, Tokyo University of Science, and Director of Organ Technologies Inc.) has provided a proof-of-concept for bioengineered organ replacement as the next step for regenerative therapy.

Dr. Tsuji's research group (M. Hirayama *et al*.,) reports the successful orthotopic transplantation of a bioengineered lacrimal gland germ into an adult extra-orbital lacrimal gland defect model mouse, which mimics the corneal epithelial damage caused by lacrimal gland dysfunction. The bioengineered lacrimal gland germ and harderian gland germ both developed *in vivo* and achieved sufficient physiological functionality, including tear production in response to nervous stimulation and ocular surface protection. This study demonstrates the potential for bioengineered organ replacement to functionally restore the lacrimal gland.



This research was performed in collaboration with Professor Kazuo Tsubota (Department of Ophthalmology, Keio University School of Medicine, Japan).

The findings were announced in an Advance Online Publication in "*Nature communications* (http://www.nature.com/ncomms/index.html) (doi: 10.1038/ncomms3498)" journal with the press embargo ending at 0:00 Japan on 2nd October, 2013 (16:00 London, 11:00 East coast in US on 1st October, 2013).

The paper will be available online at http: //www.nature.com/ncomms/index.html

* See separate sheet for an outline of the research outcome.

BACKGROUND TO THE RESEARCH

1. The role of lacrimal gland.

The lacrimal gland, which develops from its organ germ induced by an epithelial-mesenchymal interactions during embryogenesis, plays important roles in maintaining a healthy ocular surface via tear secretion. The mature lacrimal glands, which consist of acini enveloped by myoepithelial cells and a duct, form effective tear secretory systems under the control of the central nervous systems. Aqueous tear, which is secreted from the lacrimal glands, are composed of water and proteins. Tear lipids, which prevent the tear evaporation, are secreted by meibomian glands in humans and harderian glands in mice. Tears are indispensable for lid lubrication, protection of the epithelial surface and visual function (Figure 1a, 1b).

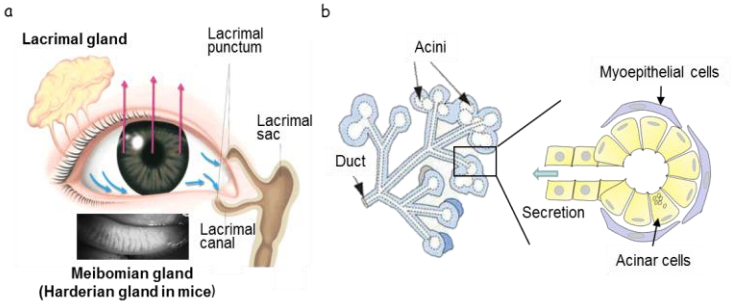


Figure 1: (a) The lacrimal gland. (b) Histology of the lacrimal gland.

2. The dry eye disease.

Dry eye disease (DED), which is caused by tear shortage by the lacrimal gland dysfunction, is observed in Sjogren's syndrome and Stevens-Jonson syndrome, as well as other causes, including aging and long-term work with a visual display. DED, which causes corneal epithelial damages, results in a significant loss of vision, and decreased quality of life. It is estimated that more than 22 million in Japan and approximately 12% of American people >50 years old have DED. Current therapies for DED, including artificial tear solutions, are conservative. Several therapeutic approaches have been developed to restore lacrimal gland function, including heterotopic salivary gland transplantation and regenerative medicine.

3. Regeneration of lacrimal gland and harderian gland.

We have developed an *ex vivo* cell manipulation method called "the organ germ method", which regenerates a bioengineered organ germ through the induction of the epithelial-mesenchymal interactions using epithelial cells and mesenchymal cells (*Nature Methods* **4**, 227-30, 2007). Moreover, demonstrated the proof of concept for the regeneration of ectodermal organs such as teeth and hair follicles using this method (*PNAS* **106**, 13475-13430, 2009, *Nat.Commun.* **3**:784, 2012). These reports verified the concept of organ regeneration by the transplantation of bioengineered organ germ regeneration (Figure 2).

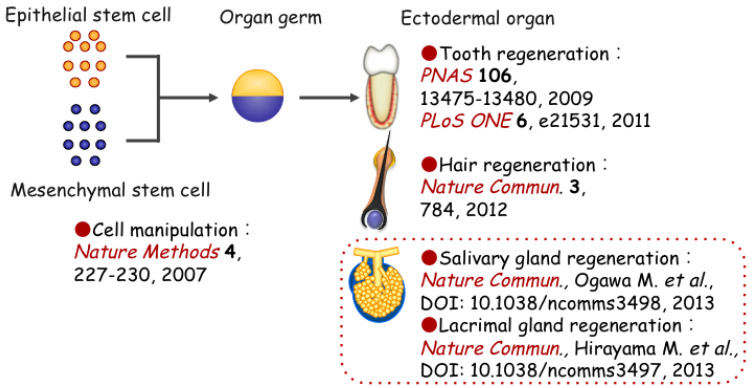


Figure 2: Development of bioengineered organ regeneration.

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OUTLINE OF THE RESEARCH OUTCOME

During embryogenesis, the lacrimal gland develops into a functional, mature lacrimal gland with complete secretory structure, including acini, ducts and the nerve fibres from the lacrimal gland germ. In this study, we sought to achieve functional bioengineered lacrimal gland replacement via orthotopic engraftment of a bioengineered lacrimal gland germ into an extra-orbital lacrimal gland defect mouse model that mimics ocular surface damage by lacrimal gland dysfunction. In addition, we investigated whether we can regenerate the bioengineered harderian gland, which secretes the tear lipids to the ocular surface (Figure 3).

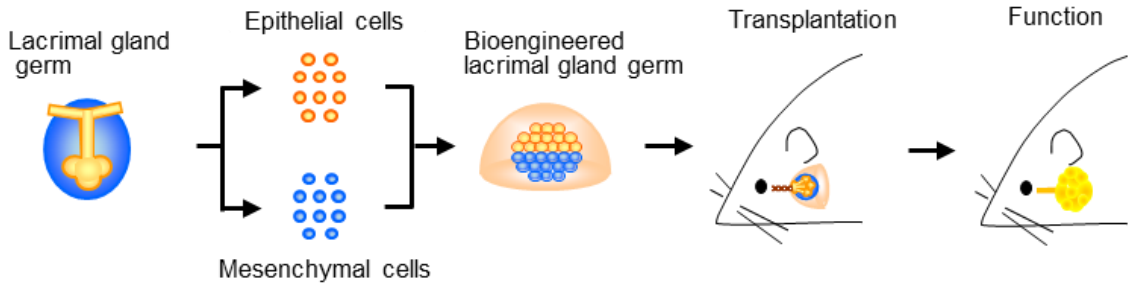

Figure 3: Strategy for functional lacrimal gland regeneration via transplantation of a bioengineered organ germ.

1. Generation and transplantation of the bioengineered lacrimal and harderian gland germs

We successfully regenerated the bioengineered lacrimal and harderian gland germs, which could develop into secretory gland structure through branching morphogenesis, using the organ germ method (Figure 4a). We also demonstrated that the bioengineered lacrimal and harderian gland germs could be engrafted *in vivo* at high frequency by transplanting them into an extra-orbital lacrimal gland defect mouse model (Figure 4b). We confirmed the duct connection between the bioengineered gland and the recipients by injecting Evans blue and showing that the dye transferred from the recipient's lacrimal excretory duct to the bioengineered gland without leaking (Figure 4c).

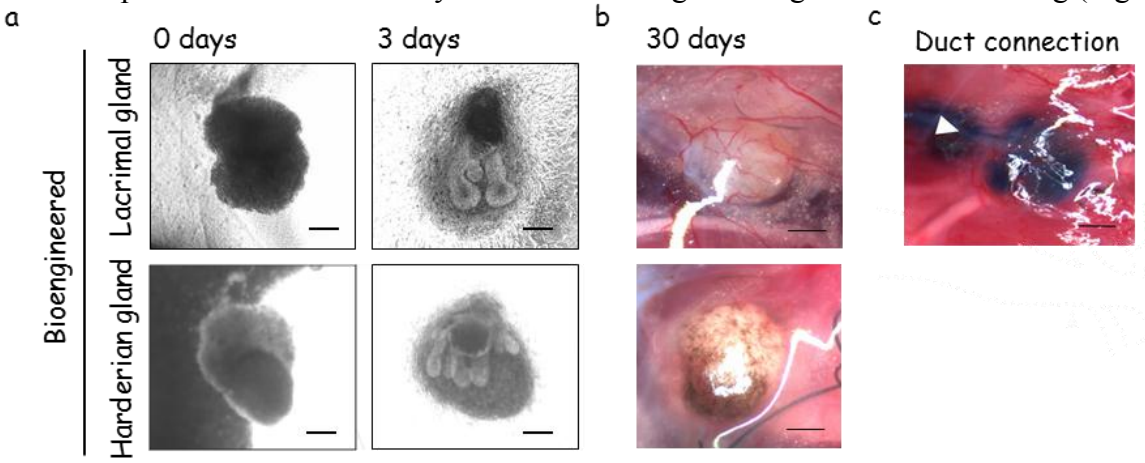

Figure 4: Generation and transplantation of the bioengineered lacrimal and harderian gland germs.
(a) Development of the bioengineered lacrimal and harderian gland germs by organ culture. Scale bar, 100 µm. (b) The bioengineered lacrimal and harderian gland at 30 days after transplantation. Scale bar, 500 µm. (c) Analysis of the duct connection between the bioengineered gland and the recipient using the Evans blue dye injection. Scale bar, 500 µm.

2. Histology of the bioengineered lacrimal and harderian glands

For effective tear secretion, coordination between the lacrimal gland structures, such as acini and myoepithelial cells, and nerve innervations is important. The engrafted bioengineered lacrimal and harderian glands achieve a three-dimensional histology with nerve innervations (Figure 5a). Moreover, the expression of lactoferrin, a protein secreted by the lacrimal gland, was found in the acini of bioengineered lacrimal glands (Figure 5b). We also confirmed the lipids, which are secreted mainly by the harderian gland, were present in the acini of the bioengineered harderian glands (Figure 5c). These results indicate that the bioengineered lacrimal and harderian glands are able to secrete the appropriate tear contents in response to neural stimulus.

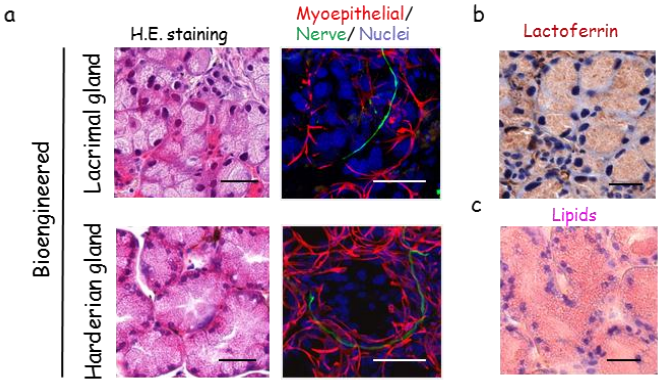

Figure 5: Histology of the bioengineered gland
(a) Histological staining of the bioengineered glands. Red; myoepithelial cells enveloping the acini, Green; nerve fibres
(b) Lactoferrin staining (brown).
(c) Oil staining (pink).

3. Secretion of bioengineered tears and lipids

Appropriate nervous control of tear fluid secretion is essential for the full function of the bioengineered lacrimal glands, and it is required to protect the ocular surface (Figure 6a). In our study, the bioengineered lacrimal and harderian glands secreted transparent tears and turbid tears in response to cool cell activation on the ocular surface (Figure 6b). In addition, the tear flow of the bioengineered lacrimal gland was equivalent to that of the glands of normal control mice (Figure 6c). The lipid concentration of the tears from the bioengineered harderian gland was increased compared with that of the normal or bioengineered lacrimal gland engrafted mice (Figure 6d). These results demonstrate that the bioengineered lacrimal and harderian glands displayed functional secretory ability under appropriate neural control.

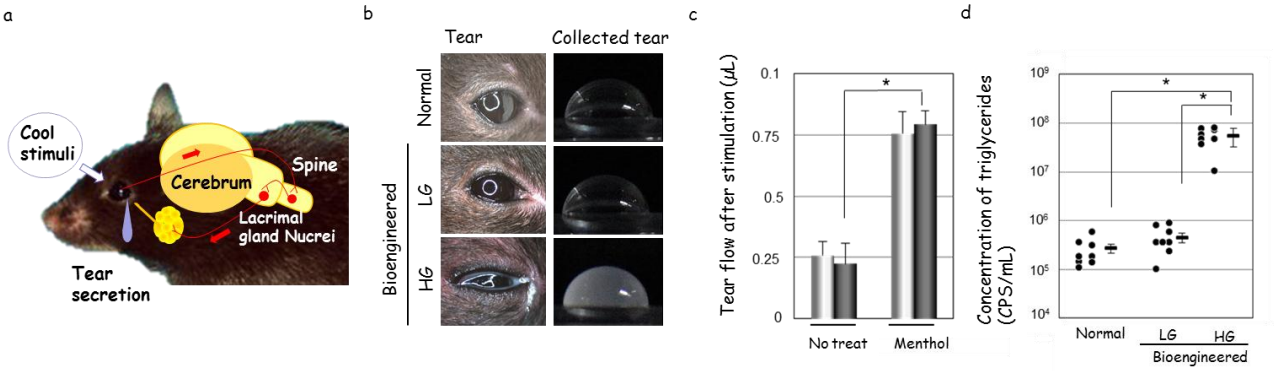

Figure 6: Secretion of bioengineered tear and lipids.
(a) The pathway of tear secretion reflex by the ocular surface stimulation. (b) Secreted tears after the secretory stimulation. (c) Analysis of the tear flow of the bioengineered lacrimal gland. (Gray; normal, Black; bioengineered lacrimal gland). (d) Analysis of the tear lipid concentration.

4. Bioengineered lacrimal gland protects the ocular surface

The goal of lacrimal gland regenerative therapy is to restore the damaged ocular surface caused by DED. Therefore, we analysed the corneal epithelial damage of the bioengineered lacrimal gland engrafted mice. The impaired area of the corneal epithelial surface in the bioengineered lacrimal gland engrafted mice was reduced compared to the lacrimal gland defect mice at 30 days after surgery (Figure 7a). Chronic DED results in corneal epithelial thinning. In our study, the corneal thickness of the bioengineered lacrimal gland engrafted mice was maintained at the level of normal mice at 60 days after surgery (Figure 7b). These results indicate that our bioengineered lacrimal gland successfully developed, achieved full lacrimal gland function and maintained a healthy ocular surface.

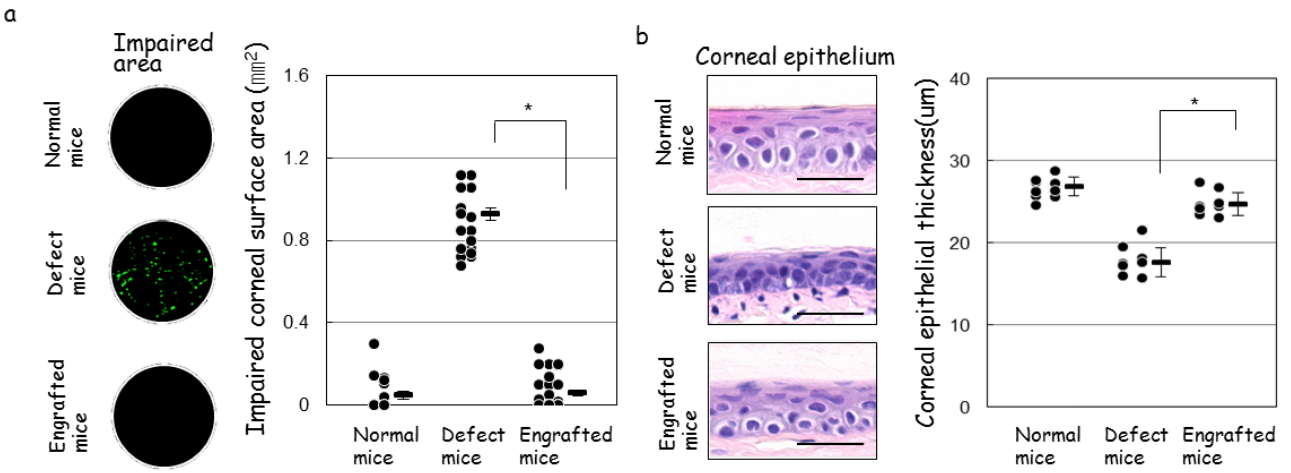

Figure 7: Bioengineered lacrimal gland protects the ocular surface.
(a) Analysis of the area of impaired corneal surface (green dotted area). Scale bar, 1 mm. (b) Histology of the corneal epithelial layer. Scale bar, 25 µm.

CONCLUSION

In conclusion, the bioengineered lacrimal gland germ and harderian gland germ both developed in vivo and achieved sufficient physiological functionality, including tear production, in response to nervous stimulation and ocular surface protection. This study is the first to demonstrate the potential for bioengineered organ replacement to functionally restore the lacrimal gland. In addition, we provided proof-of-concept for lipid secretory organ regeneration by regenerating the harderian gland, which secretes lipids that are critical for tear function.

Further studies on the identification of stem cells, including adult tissue stem cells, embryonic stem cells and inductive pluripotent stem cells, as sources for bioengineered lacrimal and harderian gland germs will contribute to the development of lacrimal gland organ regeneration.

Reference Sheet

● **Article details;**

Functional lacrial gland regeneration by transplantation of a bioengineered organ germ

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