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### Data Article

# Data supporting chitosan facilitates structure formation of the salivary gland by regulating the basement membrane components



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#### ABSTRACT

To investigate the role of basement membrane (BM) in chitosan-mediated morphogenesis of the salivary glands, the embryonic submandibular gland (SMG) experimental model was used. Chitosan promotes branching at distinct stages in SMG morphogenesis. When enzymes such as type IV collagenase, dispase, and cathepsin B were used to digest the BM components, the morphogenetic effect mediated by chitosan disappeared. Immunofluorescence revealed that the corresponding receptors for BM components, including CD49c, CD49f, CD29, and dystroglycan, were locally enriched at the epithelial–mesenchymal junction around BM areas. The functional roles of laminin  $\alpha 1$  and  $\alpha 5$  in SMG branching were explored via siRNA knockdown, and suppression was confirmed at both the RNA and protein levels (Yang and Hsiao, *Biomaterials*, <http://dx.doi.org/10.1016/j.biomaterials.2015.06.028>, 2015). This data article demonstrates the experimental approaches to investigate the role of basement membrane in the structure formation of the salivary gland engineered by biomaterials.

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## Specifications Table

Subject area	Biology
More specific subject area	Chitosan mediated morphogenetic effect of the salivary gland structure formation
Type of data	Figures and Charts
How data was acquired	An <i>ex vivo</i> culture of submandibular gland (SMG) explants was used. Enzymes were applied to digest away basement membrane (BM) components. The spatial distribution of BM receptors was demonstrated and analyzed via immunofluorescence staining. siRNA-mediated suppression of BM components was confirmed by qPCR and Western blotting
Data format	Raw and analyzed Data
Experimental factors	SMG explants were cultured in a chitosan-containing system to induce structure formation. Explants were then treated with specific reagents and harvested for molecular and cytological analyses
Experimental features	Image recordings were used for quantitative analyses, including branching numbers and immunofluorescence intensity. Quantitative PCR and Western blotting were performed and analyzed
Data source location	The National Taiwan University, Taipei, Taiwan
Data accessibility	Data is available with this article

## Value of the data

- This data demonstrated the potential of using a standard experimental model of embryonic submandibular glands (SMGs) to investigate the morphogenetic effects of biomaterials
- Treatments of digesting enzyme and siRNA toward BM components render the investigation of BM function in salivary gland morphogenesis feasible.
- The spatial and temporal change of the BM components and corresponding receptors during the structure formation of the salivary glands could be explored by immunofluorescence settings and analyses.

## 1. Data, experimental design, materials and methods

The information and data presented in this data article demonstrated the experimental approaches to investigate the role of basement membrane in chitosan-mediated morphogenesis of the salivary gland.

## 2. Materials and methods

### 2.1. Preparation of the chitosan-containing system

The chitosan-containing system was established using a water-soluble form of chitosan. A 2 wt% (w/v) chitosan solution was prepared by dissolving chitosan (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) in 1 M acetic acid. The solution was subsequently mixed with the standard culture medium used for SMG explant culture, neutralized with sodium hydroxide, and prepared at the indicated concentrations as previously described [2,3]. Mock medium was prepared in the same way as the chitosan-containing medium by adding the same amount of acetic acid and sodium hydroxide without chitosan. The mock and control media had similar effects on SMG without significant differences [4]. The control medium was therefore used for comparison in following assays.

### 2.2. *Ex vivo* explant culture of the submandibular gland (SMG)

Animal protocols were approved by the Animal Care and Use Committee of the National Taiwan University and were in accordance with the guidelines. E12.5 and E13 submandibular glands (SMGs) retrieved from ICR mice were used for explant culture, and the protocol followed the methods described previously [5]. Cultured SMG explants were photographed and measured at the indicated

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