



Effects of biomaterial-derived fibroblast conditioned medium on the α -amylase expression of parotid gland acinar cells



Ya-Shuan Chou^a, Tai-Horng Young^{a,*}, Pei-Jen Lou^{b,*}

^aInstitute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei, Taiwan

^bDepartment of Otolaryngology, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan

ARTICLE INFO

Article history:

Received 6 March 2015

Received in revised form 10 August 2015

Accepted 27 August 2015

Available online 29 August 2015

Keywords:

Fibroblasts

Parotid gland acinar cells

α -Amylase

Conditioned medium

Biomaterials

ABSTRACT

Salivary gland cells are surrounded by a complex stromal environment, in which fibroblasts are the main cells in proximity to the gland cells. In this study, the interaction between parotid gland acinar cells (PGACs), fibroblasts, and biomaterials was investigated. We prepared different biomaterials, including chitosan, polyvinyl alcohol (PVA), poly (ethylene-co-vinyl alcohol) (EVAL), polyvinylidene fluoride (PVDF), and tissue culture polystyrene (TCPS) to culture fibroblasts and then collect their conditioned media to culture PGACs. We observed no difference in AQP3, AQP5, and E-cadherin expression among different fibroblast conditioned medium treatments. Interestingly, α -amylase expression was obviously enhanced in PGACs cultured in the presence of conditioned medium from fibroblasts cultured on PVDF. Higher neurotrophin-4 (NT-4) expression was observed in PVDF-derived fibroblast conditioned medium using a growth factor protein array assay. In addition, directly adding NT-4 into the culture medium significantly promoted α -amylase expression by PGACs. Finally, nestin and β III-tubulin expression by fibroblasts cultured on PVDF was also enhanced. Together, these results suggest that PVDF could promote α -amylase expression by PGACs via the NT-4 produced by fibroblasts.

Statement of Significance

To date, there is no effective therapy for patients with dry mouth with persistent salivary hypofunction. The study made use of different biomaterials to culture fibroblasts and then collect their conditioned media to culture PGACs. It was found that the effect of fibroblast conditioned medium from PVDF on the α -amylase expression of PGACs was obviously enhanced and higher neurotrophin-4 (NT-4) expression was found in PVDF-derived fibroblast conditioned medium. In addition, directly adding NT-4 into the culture medium significantly promoted the expression of α -amylase by PGACs and the expression of nestin and β III-tubulin of fibroblasts after being cultured on PVDF was enhanced. Therefore, the present study represents the first description of the role of NT-4 in the expression of α -amylase of PGACs and the role of PVDF in the reprogramming fibroblasts into neural progenitor-like cells, indicating that PVDF could promote the expression of α -amylase by PGACs via the NT-4 produced by fibroblasts.

© 2015 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Understanding the interaction between heterotypic cell–cell combinations is an important issue in the field of tissue engineering. Especially, mesenchymal and epithelial cells have different compartmented structural distribution for establishing functional tissues and organs [1]. For example, epithelial acinar cells are surrounded by mesenchymal cells in a ball-and-socket structure in

the early developmental stage of salivary glands, followed by branching morphogenesis leading to a more complex structure [2]. Salivary glands are responsible for the secretion of saliva which contains digestive enzymes, growth factors, and antimicrobial agents. Irreversible damage to salivary glands is a common side effect caused by radiation therapy for patients suffering from head and neck cancer. To date, patients with dry mouth are treated with saliva substitutes to temporarily relieve their symptoms, but there is no effective therapy for a persistent salivary hypofunction [3,4].

In our previous study, parotid gland acinar cells (PGACs) and fibroblasts have been successfully isolated and expanded from the same human salivary gland [5]. PGACs can express function-related proteins such as α -amylase, E-cadherin, and water trans-

* Corresponding authors at: No. 1, Sec. 1 Jen-Ai Road, Taipei, Taiwan (T.-H. Young), 7, Chung-Shan South Road, Taipei, Taiwan (P.-J. Lou).

E-mail addresses: thyoung@ntu.edu.tw (T.-H. Young), pjlou@ntu.edu.tw (P.-J. Lou).

portation associated proteins aquaporin-3 (AQP-3) and aquaporin-5 (AQP-5), under 2D culture conditions. Moreover, the fibroblast conditioned medium was able to promote the functions of PGACs [6]. Conditioned medium has been extensively used to culture various cells for specific applications [7–9]. Conditioned medium can also be used to test whether a particular cell behavior is dependent on certain cytokines or growth factors released from other cell types. Biomaterials have been demonstrated to affect cell proliferation, differentiation, and function [10,11]. However, the effects of biomaterials on conditioned medium that further regulates target cells are often overlooked.

Therefore, the aim of this study was to investigate whether biomaterials might play an important role in PGAC function by enriching the fibroblast conditioned medium used to stimulate PGACs. We previously observed some interesting fibroblast behaviors during culture on different biomaterials in specified conditions. For example, fibroblasts exhibited poor adhesion on polyvinyl alcohol (PVA), but had better spreading, cytoplasm webbing, and flattening on the PVA/chitosan blended material [12]. Chitosan was able to control fibroblast attachment/detachment in response to medium pH changes to recover cultured cells without additional enzymatic treatment and extensive washing steps [13]. The decrease of the hydrophobic property of poly (ethylene-co-vinyl alcohol) (EVAL) from 44 mol% to 27 mol% ethylene could induce characteristic senescence-associated phenotypic changes such as larger cell shape, re-organized actin cytoskeleton, lower proliferation capacity, and higher levels of senescence-associated β -galactosidase (SA β -gal) activity [14]. In addition, submandibular gland recombinants were able to develop new branches on polyvinylidene fluoride (PVDF) without serum [15]. Therefore, in this study, we cultured fibroblasts on PVA, chitosan, EVAL, and PVDF, and collected their conditioned medium to culture PGACs and observed PGAC functions, with a specific emphasis on α -amylase expression.

2. Materials and methods

2.1. Cell isolation and culture

Human parotid glands were obtained from 20 patients during parotid tumor surgery according to a protocol approved by the Institutional Review Board of the National Taiwan University Hospital. The methods for cell isolation and culture have been described elsewhere [5]. The specimens from the non-tumor portion of the parotid gland were washed with ice cold phosphate buffered saline (PBS) containing 2% antibiotics (penicillin 200 mg/mL, streptomycin 200 mg/mL, and amphotericin B 0.5 mg/mL). After removing the connective tissue from each specimen, the remaining shallow-yellow acinar tissue was cut into small fragments of about 0.5 mm³ in size and treated with collagenase B (Roche, 2.5 mg/mL) and DNase I (Roche, 1 mg/mL) at 37 °C for 30 min. Subsequently, the digested tissue was filtered and centrifuged to prepare different cells. Cells were cultured with selective medium at 37 °C with 5% CO₂ atmosphere in a humidified incubator. The isolated fibroblasts from parotid glands were cultured in Dulbecco's Modified Eagle's Medium (DMEM)-F12 (Invitrogen) with 10% fetal bovine serum (FBS). PGACs, on the other hand, were cultured in Keratinocyte Serum Free Medium (K-SFM; Invitrogen) containing 5 ng/mL epidermal growth factor (Sigma) and 50 μ g/mL bovine pituitary extract (BD). Unless otherwise specified, neurotrophin-4 (NT-4, ProSpec) was not added into the medium to culture PGACs.

2.2. Preparation of fibroblast conditioned medium

First, an appropriate amount of polymer was dissolved in solvent to form a polymer solution. The solvent for PVA (Chang Chun,

BF-17) was water, for chitosan (Sigma C-3646) was 0.5 M acetic acid, for EVAL (Kuraray E105A, containing ca. 56 mole% vinyl alcohol) and for PVDF (Elf Ato Chem Kynar 740 type) was dimethyl sulfoxide. The polymer concentration for chitosan was 1%, and others were 2.5%. The detailed procedure for coating polymer solution on tissue culture polystyrene wells (TCPS, Corning) has been described elsewhere [16]. The coated biomaterials were sterilized in 70% alcohol overnight and rinsed extensively with PBS, followed by treatment under ultraviolet light overnight. Uncoated TCPS wells were treated similarly and used as controls. Subsequently, fibroblasts were seeded on various biomaterials at the density of 1.25×10^4 cells/cm² for 3 days. The conditioned medium was then centrifuged, diluted 1:1 with fresh DMEM-F12 medium with FBS to culture PGACs (5×10^4 cells/cm²) for another 3 days.

2.3. Cell proliferation

The AlamarBlue Cell viability assay was modified from a previous study for cell proliferation measurement [17]. AlamarBlue solution (10%, AbD Serotec) was directly added into each well and incubated for 4 h at 37 °C. Following incubation, 100 μ L medium from each well was transferred to a 96-well black polystyrene microplates and fluorescence (excitation 530 nm, emission 590 nm) was measured with a fluorescence microplate reader (SpectraMax M2e, Molecular Devices).

2.4. Western blot analysis

Western blot analysis for protein expression was carried out by using standard protocols. In brief, cells were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer containing Complete Protease Inhibitor Cocktail and Phosstop Phosphatase Inhibitor Cocktail Tablets (Roche Diagnostics GmbH). Proteins from cell extracts were denatured and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) on 10% polyacrylamide gels and transferred onto nitrocellulose membranes (Millipore). Protein concentration was measured by using the commercial protein assay reagent (Bio-Rad). After blocking in 2.5% bovine serum albumin (BSA) at room temperature for 2 h, the membranes were probed with primary antibody at 4 °C overnight, washed, incubated in horseradish peroxidase (HRP)-conjugated secondary antibodies, and, finally, visualized by enhanced chemiluminescence (ECL; Millipore). The following antibodies were used in this experiment: anti- α -amylase, anti-nestin, anti- β III-tubulin, and anti-GAPDH (Abcam).

2.5. Immunofluorescence microscopy

Cells were washed with PBS 3 times, fixed with 4% paraformaldehyde, and then permeabilized with 0.1% Triton X-100 for 20 min at room temperature. After blocking with 2.5% BSA in 0.1% Tween-20/PBS, the cells were stained with the primary antibody of interest at 4 °C overnight. The following antibodies were used in this study: anti- α -amylase (Abcam), anti-aquaporin 3 (AQP3, Abcam), anti-AQP5 (Abcam), anti-E-cadherin (BD), anti-vimentin (Abcam), anti-nestin (Abcam), anti- β III-tubulin (Abcam), and rhodamine-phalloidin (Millipore). The cells were then washed and incubated with Alexa Fluor 488- and Alexa Fluor 568-labeled secondary antibodies (Invitrogen), and counterstained with DAPI (Millipore). Immunofluorescent images were obtained using a Leica DMI 6000 inverted microscope.

2.6. Growth factor protein analysis of fibroblast conditioned medium

The growth factor proteins secreted by fibroblasts in the conditioned medium were analyzed by protein array according to the

ID	Title	Pages
226	Effects of biomaterial-derived fibroblast conditioned medium on the α -amylase expression of parotid gland acinar cells	10

Download Full-Text Now



<http://fulltext.study/article/226>



-  Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search
-  Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price
-  Free Downloadable Articles
In each journal some of the articles are available to download for free
-  Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

<http://FullText.Study>