

Short communication

Development of an oral mucosa equivalent using a porcine dermal matrix[☆]

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Abstract

We evaluated the suitability of a porcine acellular dermal matrix for the development of a 3-dimensional oral mucosal equivalent using an ex vivo-produced oral mucosal equivalent (EVPOME). Oral keratinocytes were seeded in a submerged model, and then in an air-liquid interphase model, using Transwell[®] inserts. EVPOME showed good cell viability and increased glucose consumption over time. Histological evaluation showed that stratified differentiated epithelium had formed in all matrices.

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Introduction

EVPOME, an ex vivo-produced oral mucosal equivalent,^{1,2} was developed mainly for use in regeneration and has had good clinical outcomes, but it can also be used in *in vitro* studies.^{1,3} It is produced using a non-immunogenic human acellular dermal matrix (AlloDerm[®], Acelity (LifeCell Corp), Branchburg, NJ, USA). To our knowledge, however, use of a porcine-derived matrix has not been fully evaluated. We have therefore investigated the suitability of a porcine-sourced dermal matrix to form a 3-dimensional oral mucosa.

Material and methods

Cell culture

Oral keratinocytes, which we obtained from healthy patients (Institutional Review Board of the University of Michigan, Ann Arbor, USA), were isolated by enzymatic dissociation (Gibco[™] Trypsin/EDTA 1X, Thermo Fisher Scientific, Waltham, MA, USA) and maintained in EpiLife[®] cell culture medium supplemented with epidermal growth factor (Gibco[™]) and calcium chloride 0.06 mmol (Sigma-Aldrich, St Louis, MO, USA).

EVPOME

Thick (1.04–2.28 mm) and medium (0.53–1.02 mm) human matrices (AlloDerm[®], Acelity (LifeCell Corp), Branchburg, NJ, USA), and a porcine matrix (0.53–1.02 mm) (Strattice[®], Acelity) (n = 3) were hydrated in phosphate-buffered saline

[☆] Procedures were done following approved protocols by Institutional Review Board of the University of Michigan.

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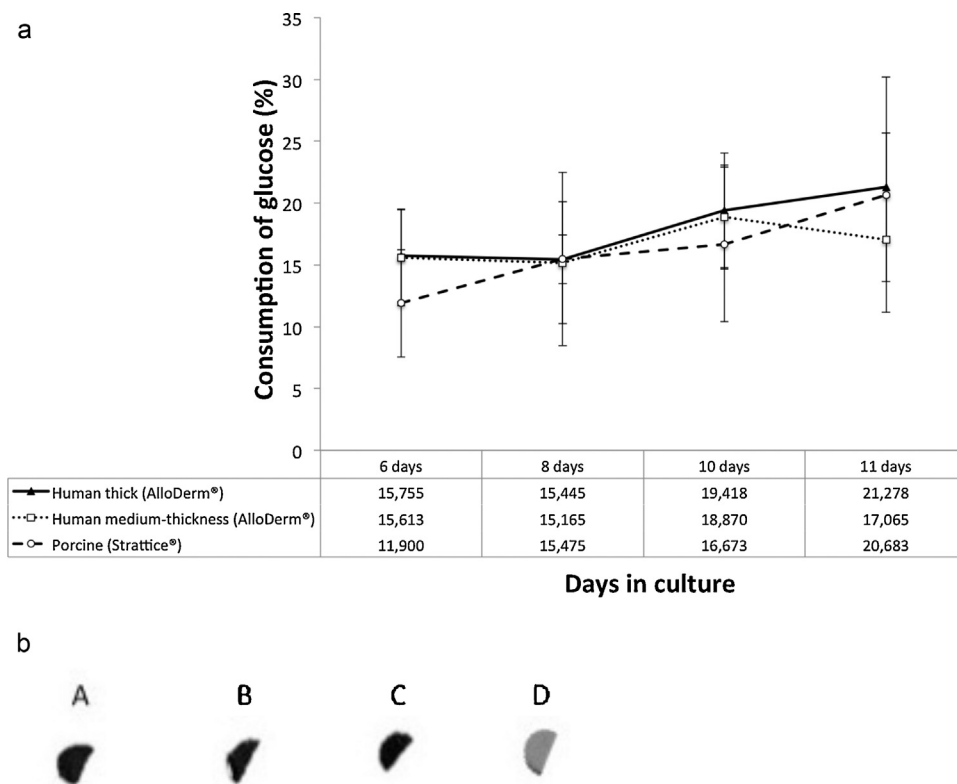


Fig. 1. (a) Glucose consumption (%) by oral keratinocytes seeded in the AlloDerm® and Strattice® (both Acclity (LifeCell Corp), Branchburg, NJ, USA) matrices ($p > 0.05$, $n = 6$). (b) MTT staining of ex vivo-produced oral mucosal equivalent (EVPOME) models: thick AlloDerm® (A), medium AlloDerm® (B), Strattice® (C), and medium AlloDerm® stained with MTT without cell seeding (D, control). The intensity of the purple staining indicates the viability of the oral keratinocytes seeded on the epidermal surface of the matrices.

(PBS) (Gibco™), and coated with human collagen type IV (0.05 mg/ml) (Sigma-Aldrich).³

The matrices were placed at the bottom of 48-well plates and EpiLife® (Gibco™) 1 ml containing calcium 1.2 mmol (Sigma-Aldrich) was added. Oral keratinocytes were placed on to the matrices (2×10^5 cells) and were submerged for four days. Matrices were then transferred to an air-liquid interphase in Transwell® inserts (Corning Inc, Corning, NY, USA) for seven days.²

Glucose consumption

Glucose consumption was measured on days 6, 8, 10, and 11 using a 10 μ l aliquot of the culture medium with the cultured cells (GlucCell™ Glucose Monitoring System, CESCO Bio-engineering, Taichung, Taiwan).² Data were analysed using ANOVA ($\alpha = 0.05$).

Cell viability

We analysed cell viability qualitatively by staining with MTT.⁴ The matrices were incubated in a solution of MTT (1/10 EpiLife®) at 37 °C for 4 hours. Purple staining indicated viable adherent cells.

Histological analysis

Matrices were fixed in 10% formalin for 24 hours and then transferred to flasks containing 70% ethanol until analysis. Samples were washed in deionised water, deparaffinised with xylene, rehydrated with decreasing concentrations of alcohol, and embedded in paraffin. Sections 5 μ m thick were obtained, stained with haematoxylin and eosin, and analysed in a photomicroscope (Leica DMI4000B, Leica Microsystems, Wetzlar, Germany).

Results

Cells seeded on the AlloDerm® matrices maintained the same glucose consumption for all periods, while glucose consumption was increased for porcine matrices (Fig. 1a). However, there was no significant difference among periods or matrices (ANOVA, $p > 0.05$).

All matrices stained strongly for MTT, which showed that the cells were adherent and viable on the EVPOME model (Fig. 1b).

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