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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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Keywords: Oral keratinocytes; organotypic cell culture; oral mucosa

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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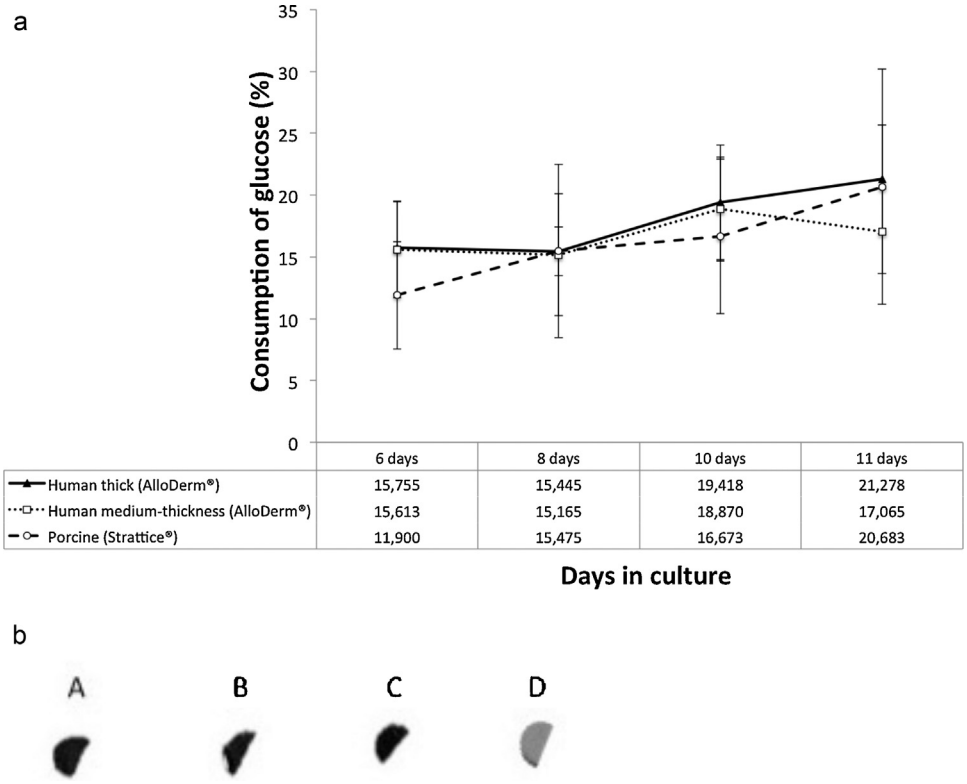

Fig. 1. (a) Glucose consumption (%) by oral keratinocytes seeded in the AlloDerm® and Strattice® (both Acelity (LifeCell Corp), Branchburg, NJ, USA) matrices ($p > 0.05$, $n = 6$). (b) MTT staining of ex vivo-produced oral mucosal equivalent (EVPOME) models: thick AlloDerm® (A), medium AlloDerm® (B), 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 μl aliquot of the culture medium with the cultured cells (GlucCell™ Glucose Monitoring System, CESCO Bioengineering, Taichung, Taiwan).[2] Data were analysed using ANOVA (α=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 μm 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).

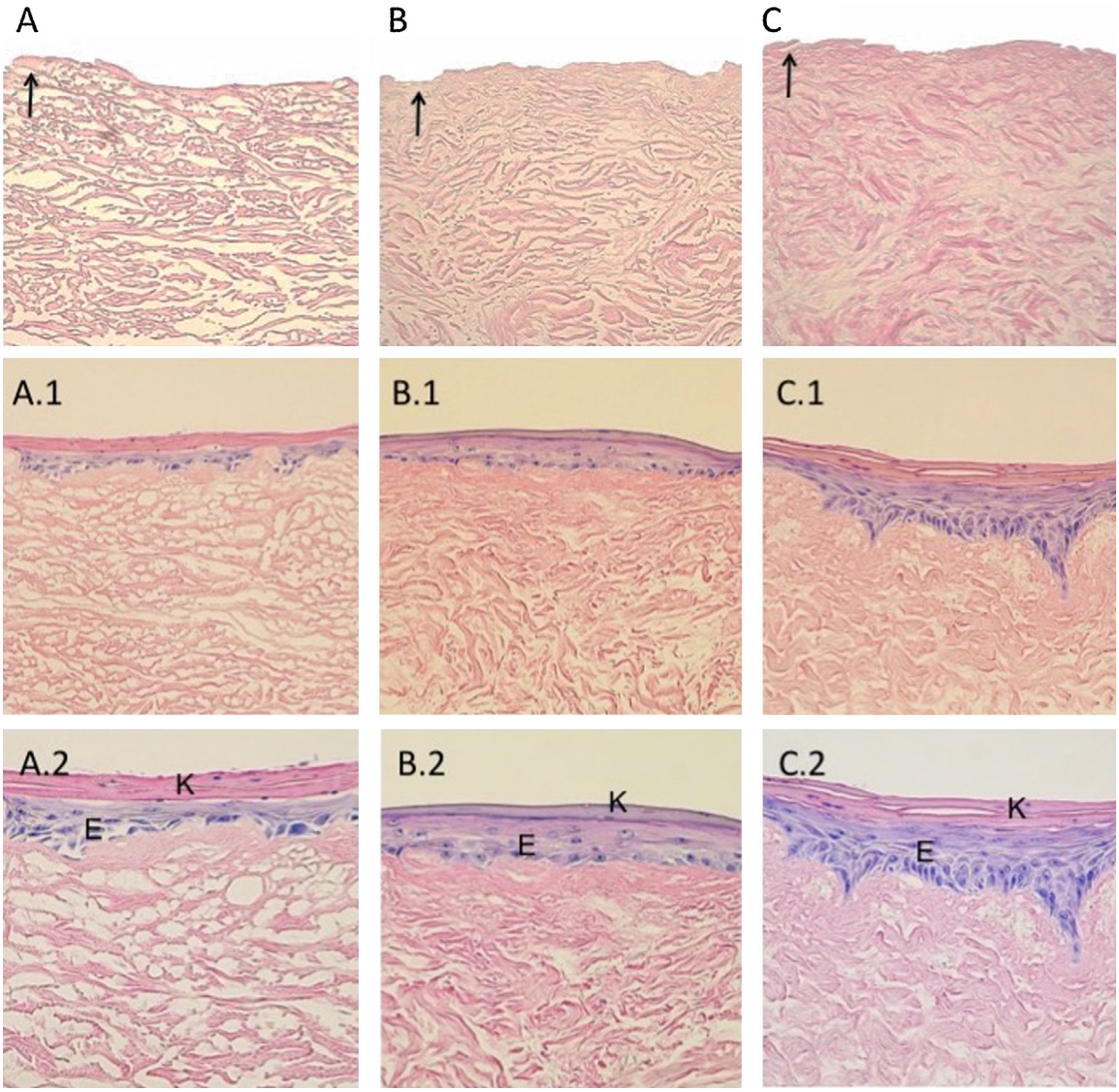

Fig. 2. Photomicrographs of AlloDerm® and Strattice® (both Acelity (LifeCell Corp), Branchburg, NJ, USA) matrices before seeding with oral keratinocytes, showing the intact basement membrane (arrows) ((A) thick AlloDerm®; (B) medium AlloDerm®; and (C) Strattice®) and EVPOME after 11 days of culture. Oral keratinocytes adhered to the surfaces of the matrices by recognition of the basement membrane, and proliferated. EVPOME shows stratification of epithelial cells (E) and differentiation, shown by the synthesis of keratin (K). All matrices produced 3-dimensional oral mucosal cells, but stratification was greater in the medium (B1 and B2) than in the thick AlloDerm® (A1 and A2). The porcine matrix (C1 and C2) provided an equally suitable environment for the attachment and proliferation of epithelial cells, as there was even thicker epithelial tissue and greater synthesis of parakeratin than in the AlloDerm® matrices (haematoxylin and eosin, original magnification upper row x 100; lower rows x 400).

Discussion

Histological analysis showed good adherence, stratification, and differentiation of epithelial cells (Fig. 2). The thickness of the matrix seems to have interfered with the proliferation and differentiation of oral keratinocytes, as the medium human matrix produced a thicker and more differentiated EVPOME.[5] The epithelial tissue produced on the surface of the thinner human matrix had more layers of cells and greater synthesis of keratin, probably because of better nutrition by the culture medium during the air-liquid interphase.[6] However, despite these differences, oral keratinocytes remained viable and presented good metabolism in all matrices.

The porcine matrix produced a differentiated oral mucosal equivalent, which would provide a 3-dimensional oral mucosa with similar patterns to the human matrix. It could be a reliable, non-human, and cheaper option for the evaluation of several oral conditions and the biological behaviour of various treatments.

Conflict of interest

We have no conflicts of interest.

Ethics statement/confirmation of patients' permission

Ethics approval was obtained. Patients' permission was not applicable.

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