Development of Full Thickness Human Skin Model Using Alvetex® Scaffold Technology

Introduction

This protocol describes the ability and advantages of using Alvetex Scaffold technology to support dermal fibroblast growth within its structure, and the co-culture of primary human keratinocytes to form a terminally differentiated, cornified human skin equivalent.

Method

1. 12-well Alvetex Scaffold inserts (AVP005) were used in 6-well plates (Figure 1). The inserts were washed twice with media. Please refer to website protocols for preparation of Alvetex Scaffold for cell culture (reinnervate.com/alvetex/alvetex-documentation/protocols/).

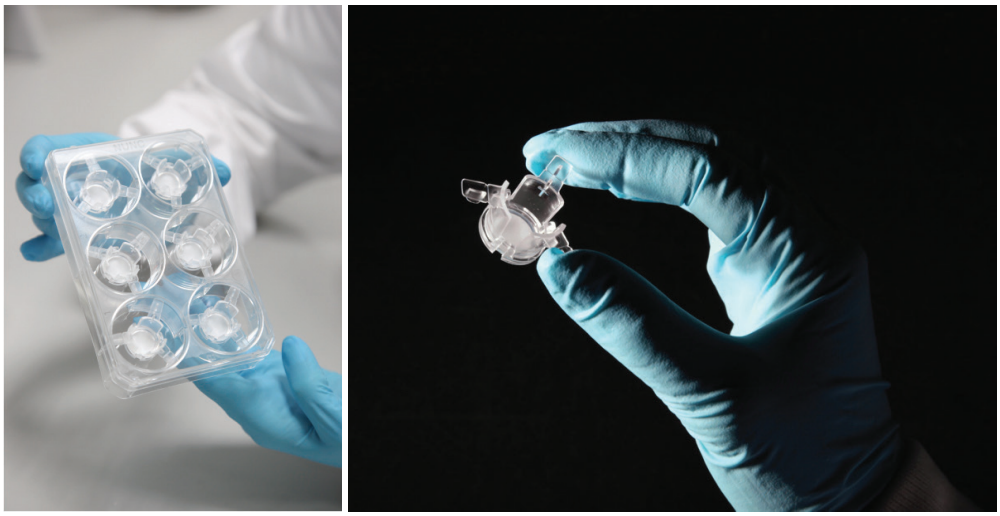

FIGURE 1. Alvetex Scaffold in 12-well insert format used in a 6-well plate.

Note: Refer to Table 1 below for all media formulations.

2. Primary fibroblasts isolated from human foreskin (P5) were incubated on the insert for 1 week. 1 million fibroblasts were seeded per insert in 100 µl of **culture medium 1** and incubated for 1 hr at 37 °C with 5 % CO_2. After 1 hr the insert was submerged in **culture medium 1** (10.5 ml) and the fibroblasts were cultured for 1 week changing **culture medium 1** every other day. (Figure 2A).

3. After 1 week of fibroblast culture, **culture medium 1** was removed and primary human keratinocytes (500,000 cells) isolated from foreskin (P4) were seeded on to the insert in 100 µl of **culture medium 2** and incubated for 1hr at 37 °C with 5% CO_2.

4. After 1 hr the inner chamber of the well insert was filled with **culture medium 2** (2 ml) and the outer well compartment (i.e. underneath the insert) was filled with 5 ml of culture medium 1. (Figure 2B).

5. After 3 days all culture medium from step 4 was removed, and the cultures were maintained

Development of Full Thickness Human Skin Model Using Alvetex® Scaffold Technology

at the air/liquid interface with culture medium 3 just touching the bottom of the well insert (4 ml from beneath the insert only, (Figure 2C)). **Culture medium 3** was changed every other day. The experiment was stopped after 1 month at the air/liquid interface and cultures were placed in the appropriate fixative for analysis (see reinnervate.com/alvetex/alvetex-documentation/protocols/).

6. Cultures were then processed for histological analysis (see reinnervate.com/alvetex/alvetex-documentation/protocols/).

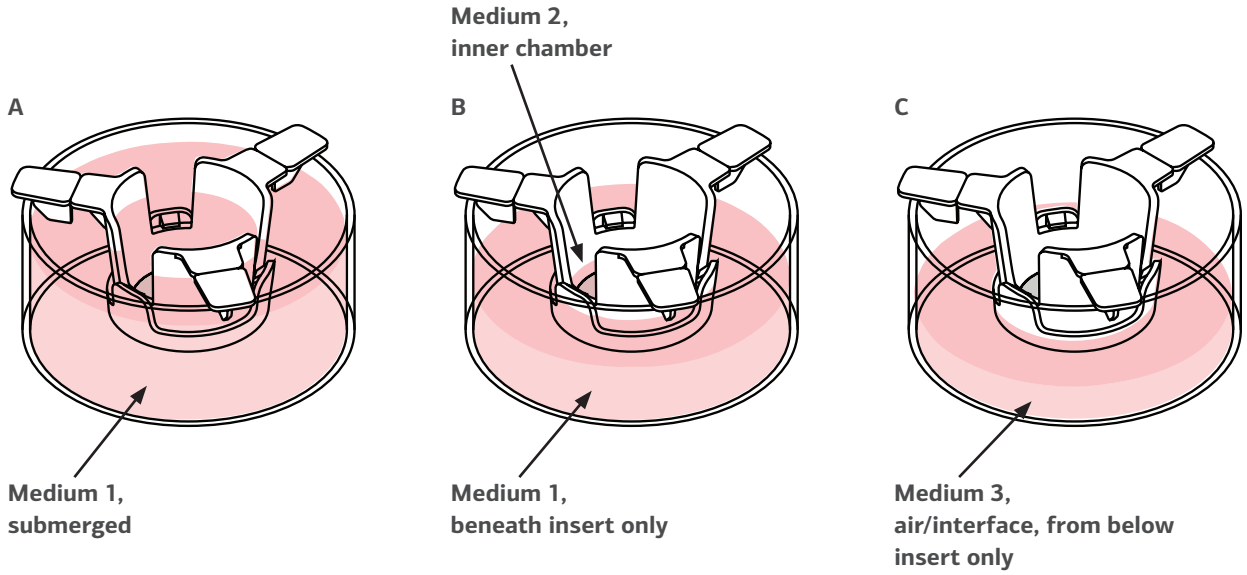

FIGURE 2: Skin culture set-up showing all media levels and types in relation to the well inserts. Note in diagram (A.) that medium 1 is common inside and outside the well insert, connected via the window in the well insert wall. In (B.), media 1 and 2 are not connected through the window. In (C.) the medium is in the lower chamber only in contact with the bottom of the Alvetex Scaffold membrane.

Development of Full Thickness Human Skin Model Using Alvetex® Scaffold Technology

	Basal Medium	Supplements
Culture Medium 1 (Fibroblast medium)	DMEM (High glucose) (PAA, E15-810)	• 10 % v/v FBS • 100 U/ml penicillin/streptomycin
Culture Medium 2 (Keratinocyte proliferation medium, submerged conditions)	Epilife (Invitrogen, M-EPI-500-CA)	• Human keratinocyte growth supplement (HKGS, Epilife, S-001-5) • 100 U/ml penicillin/streptomycin
Culture Medium 3 (Keratinocyte differentiation medium, feeding from beneath insert only)	DMEM/ Ham's F-12 (1:1) (PAA, E15-813)	• Cholera toxin, (Sigma, C8052-2mg), final concentration 10-10 M • Epidermal Growth Factor (Mouse), (Serotec, EGF-1), final concentration 10 ng/ml • Hydrocortisone, (Sigma, H4881), final concentration 0.4 µg/ml • Insulin (Sigma, I5500), final concentration 5 µg/ml • Transferrin (Sigma, T2252-500mg), final concentration 5 µg/ml • 3,3′,5-Triiodo-Lthyronine sodium salt, (Sigma T6397-100mg), final concentration 2 x 10-11 M • 10 % v/v FBS • 100 U/ml penicillin/streptomycin

TABLE 1: Basal media and supplements

Sample data

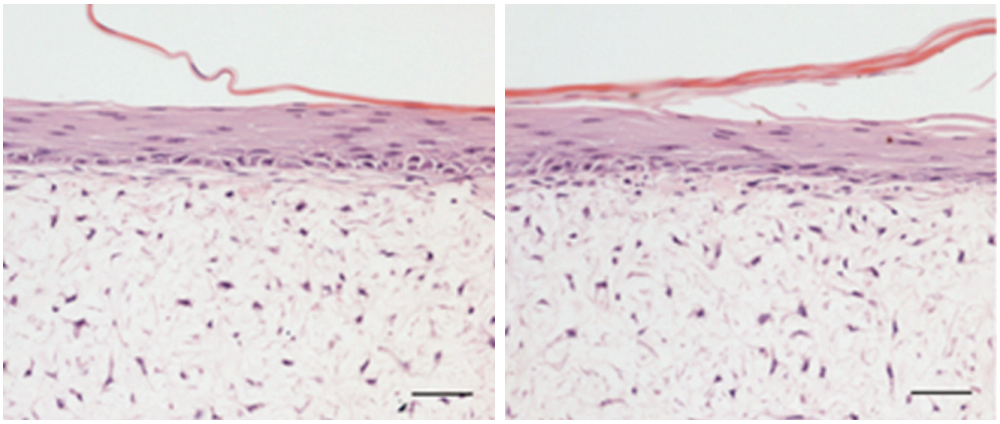

FIGURE 3. Histological analysis of skin equivalents developed using Alvetex Scaffold in 12-well insert format (AVP005) presented in a 6-well plate. (Scale bars: 50 µm).

Development of Full Thickness Human Skin Model Using Alvetex® Scaffold Technology

After one month culture at the air interface, an organised skin construct formed which showed several cornified layers lifting off the top surface of the epidermis-like zone. Good dermal fibroblast growth was also observed within the Alvetex Scaffold disc. Further functional work is required to assess the expression patterns of specific keratin isoforms, as well as basement membrane formation.

Acknowledgement

Reinnervate would like to acknowledge the contribution of Dr Supatra Marsh (Queen Mary University of London, UK) in the development of this skin application for Alvetex technology.

Development of Full Thickness Human Skin Model Using Alvetex® Scaffold Technology

Technical Support

To obtain the latest technical support information, please visit www.reinnervate.com.
You can have 24-hour access to the following information:

- Access to telephone and fax information for order support
- Search through the Reinnervate protocol video gallery
- Search product spec sheets, protocols, applications notes and product support docs
- Obtain information on customer training and workshops
- Submit a question directly to Technical Support at tech.support@reinnervate.com

Ordering Information

For the latest products and services available through Reinnervate, please visit www.reinnervate.com.

Reinnervate

NETPark Incubator, Thomas Wright Way,
Sedgefield, Co. Durham, TS21 3FD
UNITED KINGDOM

T: +44 (0) 1740 625 266

F: +44 (0) 1740 625 251

E: info@reinnervate.com

Trademarks