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 (AMS.AVP005-34) 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 www.reinnervate.com/alvetex/workflow.

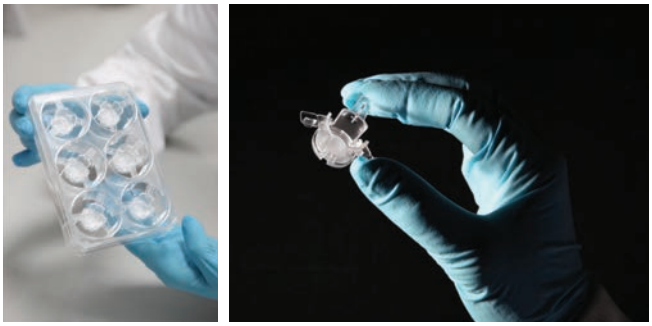

Figure 1 *Alvetex®Scaffold in 12-well insert format used in a 6-well plate.*

NB. Refer to Table 1 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 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 'choosing the right fixative to preserve 3D cultures' section of website protocols).

6) Cultures were then processed for histological analysis (see website protocols). www.reinnervate.com/alvetex/workflow

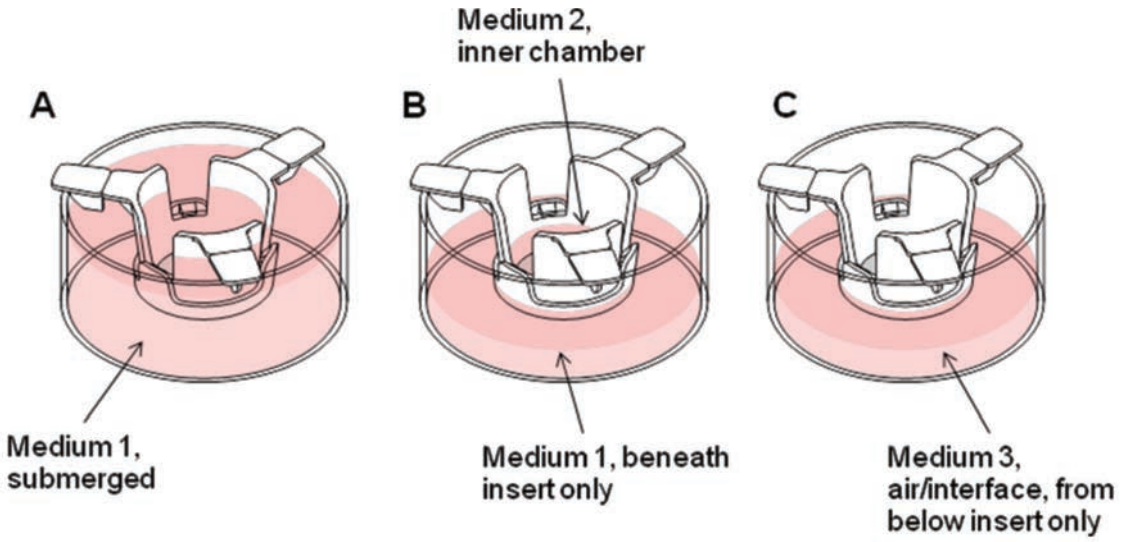

Figure 2 *Skin culture set-up showing all media levels and types in relation to the well inserts. Note in diagram A) that medium 1 is common inside and outside the well insert, connected via the window in the well insert wall. In B), media 1 and 2 are not connected through the window. In C) the medium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

	Medium 1 (Fibroblast medium)	Medium 2 (Keratinocyte proliferation medium, submerged conditions)	Medium 3 (Keratinocyte differentiation medium, feeding from beneath insert only)
Basal Medium	DMEM (High glucose, PAA, E15-810)	Epilife (Invitrogen, M-EPI-500-CA)	DMEM/ Ham's F-12 (1:1) (PAA, E15-813)
Supplements	10 % v/v FBS 100 U/ml penicillin/ streptomycin	Human keratinocyte growth supplement (HKGS, Epilife, S-001-5) 100 U/ml penicillin /streptomycin	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-L-thyronine sodium salt, (Sigma T6397-100mg), final concentration 2×10^{-11} M 10 % v/v FBS 100 U/ml penicillin/streptomycin

Table 1 Basal media and supplements

Sample data

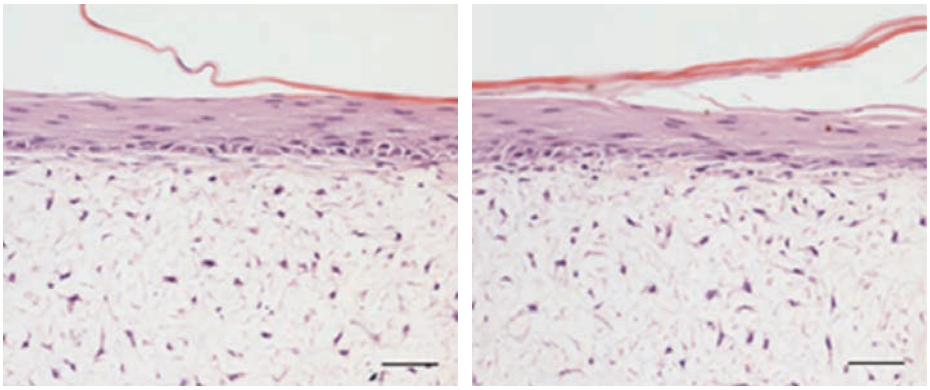

Figure 3 *Histological analysis of skin equivalents developed using Alvetex® Scaffold in 12-well insert format (AVP005) presented in a 6-well plate. (Scale bars: 50 µm).*

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.

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.

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
AMS Biotechnology (Europe) Ltd
184 Park Drive, Milton Park
Abingdon, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
amsbio LLC
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
AMS Biotechnology (Europe) Ltd
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
AMS Biotechnology (Europe) Ltd
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85