Biomaterials 116 (2017) 48–56

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Skin integrated with perfusable vascular channels on a chip

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ARTICLE INFO

Article history:
Received 6 October 2016
Received in revised form
15 November 2016
Accepted 23 November 2016
Available online 27 November 2016

Keywords:
Skin-equivalent
Skin model
Vascular channel
Perfusion
Blood vessel
Percutaneous absorption

ABSTRACT

This paper describes a method for fabricating perfusable vascular channels coated with endothelial cells within a cultured skin-equivalent by fixing it to a culture device connected to an external pump and tubes. A histological analysis showed that vascular channels were constructed in the skin-equivalent, which showed a conventional dermal/epidermal morphology, and the endothelial cells formed tight junctions on the vascular channel wall. The barrier function of the skin-equivalent was also confirmed. Cell distribution analysis indicated that the vascular channels supplied nutrition to the skin-equivalent. Moreover, the feasibility of a skin-equivalent containing vascular channels as a model for studying vascular absorption was demonstrated by measuring test molecule permeation from the epidermal layer into the vascular channels. The results suggested that this skin-equivalent can be used for skin-on-a-chip applications including drug development, cosmetics testing, and studying skin biology.

1. Introduction

The skin is the largest human organ and functions as a barrier between the internal and external environments. The functions of skin include temperature regulation, tactile sensing, and the prevention of water loss in association with its vascular network, nervous system, and appendages (e.g. sweat glands, sebaceous glands, and hair follicles) [1–3].

To assist studies on the medical treatment of the skin, a skin model consisting of dermal and epidermal layers, known as a skin-equivalent, was developed in the 1980s [4]. The skin-equivalent model has been used not only as a tool in clinical dermatology and for wound coverage, but also as an alternative to animal experiments in the development of drugs and cosmetics [5]. In addition, studies have integrated skin-equivalents with various cells, structures, and appendages to mimic human skin [3,6–10]. In particular, the construction of vascular channels within a skin-equivalent has attracted considerable attention because a skin-equivalent with vascular channels could be used in applications including angiogenesis studies [11], graft survival improvement [12], angiostatic drug evaluation [13], and cancer research [14]. Moreover, the construction of vascular channels would be an important step towards implementing hair, sweat glands, nervous system, and immune system components because vascular channels can deliver nutrition to these features.

To construct vascular channels, skin-equivalents containing capillary networks have been developed by seeding stem cells or endothelial cells into the dermal layer and inducing vascularization. Such models have been used in studies of angiogenesis and skin grafting, and for drug testing [13,15–17]. However, the vascular channels formed in those studies, which were composed of capillary networks, could not be perfused using an external pump because they were inaccessible owing to their spontaneous and random formation in the dermal layer. Consequently, supplying nutrients or sampling media via the vascular channels remains difficult, limiting the applicability of those models.

Here, we fabricated perfusable vascular channels in a skin-equivalent model (Fig. 1a). The vascular channels were coated with endothelial cells and supplied nutrition to the skin-equivalent. Both edges of the vascular channels were fixed to the connectors of a culture device attached to a perfusion system composed of a peristaltic pump and silicone tubes. We cultured the skin-equivalent under perfusion conditions and confirmed that it had

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http://dx.doi.org/10.1016/j.biomaterials.2016.11.031
0142-9612/

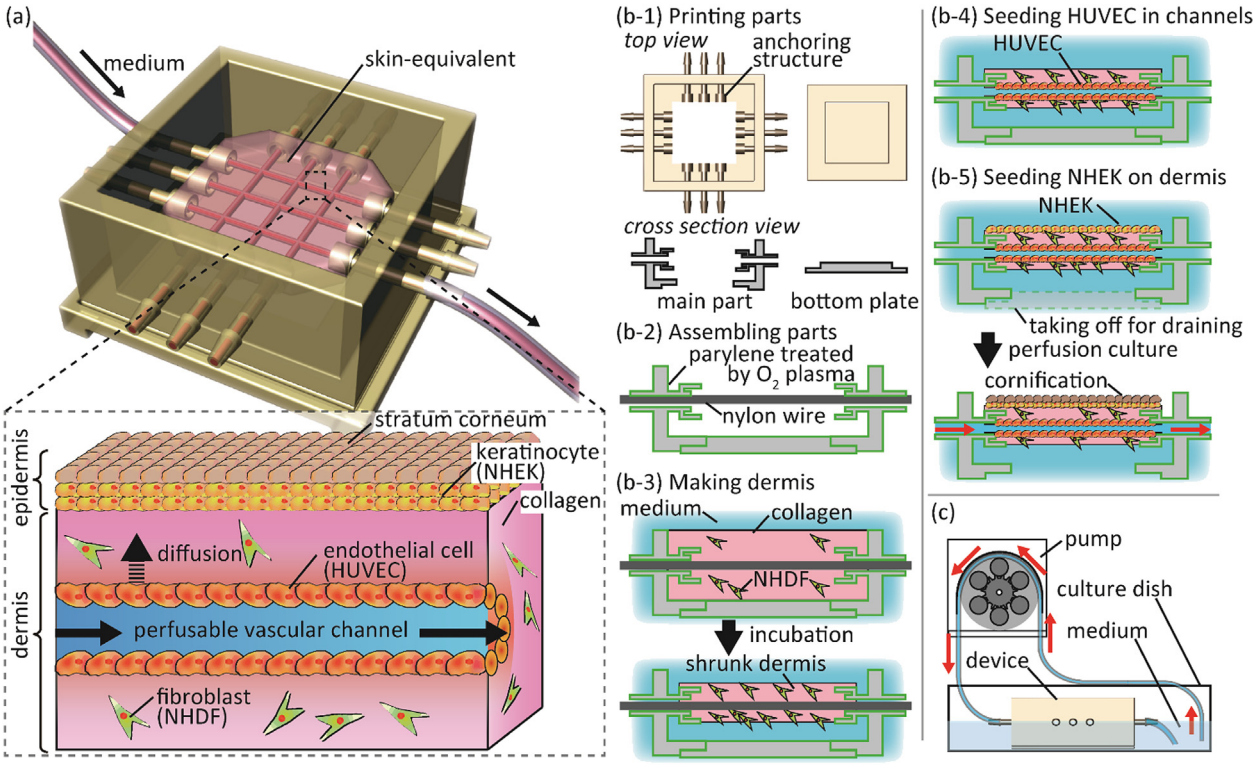

Fig. 1. Schematic of skin-equivalent integrated with perfusable vascular channels and the fabrication process. (a) Conceptual illustration of the skin-equivalent and culture device. (b) Fabrication of the culture device and skin-equivalent. (c) Perfusion system composed of a peristaltic pump, silicone tubes, and a culture device.

dermal/epidermal layers and a barrier function. Moreover, we investigated the influence of medium perfusion on the skin-equivalent by analyzing the cell distribution following perfusion and non-perfusion. Finally, to demonstrate the potential applications of the skin-equivalent in dermatological studies and for drug testing, we applied test drugs to the epidermis and measured the amounts absorbed into the vascular channels.

2. Materials and methods

2.1. Reagents

Fibroblast growth medium (FGM)-2, endothelial growth medium (EGM)-2, and keratinocyte growth medium-Gold™ were purchased from Lonza, Ltd. (Basel, Switzerland). Dulbecco's modified Eagle's medium (DMEM), Ham's nutrient mixture F-12 (Ham's F-12), penicillin-streptomycin, hydrocortisone, adenine, caffeine, isosorbide dinitrate (ISDN), rhodamine B, fluorescein isothiocyanate-dextran (FITC-dextran, 20 kDa), and rhodamine B isothiocyanate-dextran (RITC-dextran, 70 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum was purchased from Biosera (Kansas City, MO, USA). Phosphate-buffered saline without Mg^{2+} and Ca^{2+} (PBS^{-}) was purchased from Cell Science & Technology Institute (Sendai, Japan). Type I collagen solution (IAC-50) was purchased from Koken Co. (Tokyo, Japan). L-ascorbic acid phosphate magnesium salt *n*-hydrate (ascorbic acid), insulin, vascular endothelial growth factor (VEGF), and acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). Four percent paraformaldehyde solution (PFA) was purchased from Muto Pure Chemicals Co., Ltd. (Tokyo, Japan). Dimethyl sulfoxide was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). The topical skin adhesive DERMABOND ADVANCED® was purchased from Ethicon LLC (Somerville, NJ, USA). Type IV collagen antibody, cytokeratin 10 (CK10) antibody, and cytokeratin 15 (CK15) antibody were purchased from Abcam PLC (Cambridge, UK). CD31 antibody was purchased from BD Biosciences (San Jose, CA, USA). ZO-1 antibody was purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Cell culture

Normal human dermal fibroblasts (NHDFs) and normal human epidermal keratinocytes (NHEKs) were purchased from Lonza. Human umbilical vein endothelial cells (HUVECs) were purchased from PromoCell GmbH (Heidelberg, Germany). NHDFs, NHEKs, and HUVECs were maintained in FGM-2, keratinocyte growth medium-Gold™, and EGM-2, respectively, at 37 °C under a 5% CO_2 atmosphere.

2.3. Fabrication of the culture device and skin-equivalent

The culture device was composed of a main part and a bottom plate fabricated using a 3D printer (AGILISTA-3100, KEYENCE Corp., Osaka, Japan) (Fig. 1b). Anchoring structures on the connectors kept the skin-equivalent fixed to the device. The main part and the bottom plate were assembled and coated with parylene C (2–4 μm thick) to increase biocompatibility and seal the gap (Figs. 1b–2). Then, nylon wires (0.52 mm diameter) were strung across the connectors of the device. The device and nylon wires were sterilized using 70% ethanol and ultraviolet irradiation. Next, the parylene layer of the device was treated with O_2 plasma using a plasma etcher (FA-1, Samco, Inc., Kyoto, Japan) to increase cell and collagen gel adhesion onto the device. Within 1 h after O_2 plasma treatment, the device was filled with neutralized collagen solution composed of IAC-50, 10 × PBS^{-}, and FGM-2 containing 8 × 10^5 cells/mL of NHDFs and incubated at 37 °C for 30–60 min until the collagen gel