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Fabrication of a pumpless, microfluidic skin chip from different collagen sources

Q1 Hyun Jeong Song[a], Ho Young Lim[a], Wook Chun[b], Kyung Chan Choi[c], Jong Hwan Sung[d,*], Gun Yong Sung[a,*]

[a] Cooperative Course of Nano-Medical Device Engineering, Department of Materials Science and Engineering, and Integrative Materials Research Institute, Hallym University, Chuncheon, 24252, Republic of Korea
[b] Department of Burn Surgery, Burn Center, Hangang Sacred Heart Hospital, Hallym University Medical Center, Seoul, 150-715, Republic of Korea
[c] Department of Pathology, College of Medicine, Hallym University, Chuncheon, 24252, Republic of Korea
[d] Department of Chemical Engineering, Hongik University, Seoul, 04066, Republic of Korea

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ABSTRACT

Current skin-equivalents do not recapitulate the full functionalities of human skin, and recent efforts have been towards reproduction of 3D architecture of skin, as well as realization of vasculature using microfluidics. These microfluidic skin-on-a-chips use extracellular matrix proteins as a scaffold material for cell culture. Choice of optimal scaffold material is essential for properly recapitulating the tissue microenvironment. Here, we tested collagens from different sources, rat tail, porcine skin, and duck feet, comparing their abilities to support cell growth and differentiation. The viability was compared, and immunohistochemistry was used to evaluate differentiation of the skin constructs using different scaffold materials. The collagens from different sources had distinct mechanical properties, as well as the degree of contraction upon fibroblast culture. The morphology of skin tissue and the microstructure of the construct were also different, depending on the collagen sources and culture conditions. Our study provides valuable information about the choice of scaffold materials for constructing a 3D skin model.

Introduction

Limitations of conventional cell-based in vitro models led to a development of various novel model systems. In particular, to overcome the lack of physiological relevance observed in cell-based models, organ-on-a-chip technology attempts to recapitulate various tissue microenvironment, such as fluidic shear, 3D tissue, and cell–cell interactions [1–3] Among numerous environmental factors, 3D tissue environment plays a significant role in promoting growth and differentiation of cells, by manipulating the cell-matrix communication and transport of essential molecules [4–8]. It has been proved from numerous studies that culturing cells in 3D configuration enhances the physiology of the cells [4–8].

The skin tissue is composed of several different layers, including the dermal and epidermal layers. Artificial skin models have attempted to recapitulate the 3D architecture of these layers by using various ECM scaffolds including collagen [5,6]. Although various natural and synthetic materials can be used, collagen is the most abundant ECM material in the body and type I collagen has been the most widely used material as a cell culture scaffold for artificial skin models [9,10]. For example, Ghalbzouri et al. used a rat tail collagen to construct a long-term culture skin equivalent [11], and Kim et al. used porcine skin collagen to make a skin equivalent [12]. Kim et al. used duck feet collagen to study skin Q3 regeneration [13]. Although they all used the same type of collagen, collagens from different sources possess different mechanical and chemical properties [14], how skin-related cells respond to different collagens have not been studied in detail.

Previously, we reported the development of a microfluidic skin chip that is able to support 3D co-culture of fibroblasts, keratinocytes, and vascular endothelial cells [15]. Several different culture conditions were tested using this microfluidic skin chip. In this study, we compared the effect of collagens from different sources, including the rat tail collagen, porcine skin collagen, and duck feet collagen. Both static culture condition in a conventional transwell format, and dynamic culture condition in a chip were tested, and formation of the epidermal layer and expression of

Q2 * *Corresponding authors.
E-mail addresses: jhsung22@hongik.ac.kr (J.H. Sung), gysung@hallym.ac.kr (G.Y. Sung).

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differentiation marker proteins were examined. Our results suggest that different scaffold materials induce different responses from the cultured cells.

Materials and methods

Skin on a chip

Skin on a chip was fabricated using PDMS (base: agent = 10:1) to create an environment similar to the in vivo 3D culture. The culture solution was designed to be supplied to a 3D cell culture chamber having collagen as a support through a microfluidic channel and a membrane in a culture solution storage chamber in the chip. The culture chamber was designed to have a cylindrical shape with a diameter of 8 mm at the center of the chip, and the culture storage chamber was made to have a structure in which three cylinders having a diameter of 8 mm were connected to both sides of the chip. The culture medium of the storage chamber was designed to be supplied to the chamber through a patterned lower chip with a microfluidic channel having a width of 200 μm and a height of 150 μm. Bottom PDMS with a fluid channel was bonded to a glass substrate, and then the culture chamber was punctured using Biopsy Punch. Then, a transwell-cut membrane was placed on the culture chamber, and the PDMS upper chip was bonded to be fixed in the chip. (Fig. 1)

Construction of a 3D skin tissue model

Using the primary cells obtained from humans, two cells of fibroblast (2.0×10^6) and keratinocyte (6.0×10^6), which are dermal cells and keratinocytes. The fibroblast culture medium was DMEM (Gibco, 10% v/v FBS 1% penicillin/streptomycin) and keratinocyte culture medium was replaced with KGM (Lonza) every 24 h. Fibroblasts were seeded on collagen gel, cultured for 5–7 days, seeded with keratinocyte, and stabilized for 3–5 days. (EGF-1 10 ng/ml, Hydrocortisone 0.4 μg/ml, Insulin 5 μg/ml, Transferrin 5 μg/ml, and DMEM/Ham's F12) The cells were cultured using 3,3,5-triiodo-L-thionine sodium salt 2×10^{-11} M, Cholera toxin 10^{-10} M, 10% v/v FBS 1% penicillin/streptomycin and replaced every 24 h. The above process is shown briefly in Fig. 2.

Immunohistochemistry and tissue staining

The samples were fixed in 4% paraformaldehyde and processed for paraffin embedment. After rehydration, tissue sections (5 mm) were stained with hematoxylin and eosin (H & E) for histological examination or processed for immunohistochemistry (IHC). Fibronectin (ab2413, abcam), Cytokeratin 10 (ab76318, abcam), CD34 (ab81289, abcam), and Collagen IV (ab6586 abcam) were used as primary antibodies. Rabbit specific HRP/DAB (ABC) Detection IHC Kit (ab 64261, abcam) was used as secondary antibody. For Special staining, MT (Masson Trichrome Stain Kit Procedure, K7228, IMEB INC) and Sirius red/Fast green staining were used. The microscopic slides were visualized and recorded with a microscope (OLYMPUS IX7).

Results and discussion

Contraction of collagen matrix

During cell culture, collagen goes through a considerable amount of contraction due to cell growth and differentiation. The contraction of collagen matrices from different sources was tracked for 21 days (Fig. 3). Rat tail collagen (0.85 wt%), porcine skin (3 wt %), and duck feet collagen (3 wt%) were used for this experiment. The choice for the concentrations of collagen from different sources were determined based on the rigidity and the mechanical properties of the collagens, since collagens from different sources possess different mechanical properties even at the same concentrations. Briefly, we chose concentrations that show similar degree of rigidity for each collagen source.

The degree of contraction was evaluated by measuring the thickness of collagen matrices after determined amount of culture time. The porcine skin and rat tail collagen showed higher degree of contraction, compared to duck feet collagen. The rat tail collagen started contracting early, within the first day, and showed approximately 30% contraction after three weeks. In case of the porcine skin, contraction started after two days of fibroblast seeding, and during the initial 10 days, approximately 30% contraction was observed. In case of duck feet collagen, contraction started after two days, and the final contraction ratio was approximately 25%, which was smaller than the other two collagens. This result implies that the duck feet collagen maintained the original structure better than the other collagens.

When media was switched from DMEM to DMEM + KGM at day 6, the porcine skin collagen contracted 10%, and duck feet collagen contracted 7.5%. After 4 days of culture in DMEM + KGM media, porcine skin collagen contracted 15% and duck feet collagen

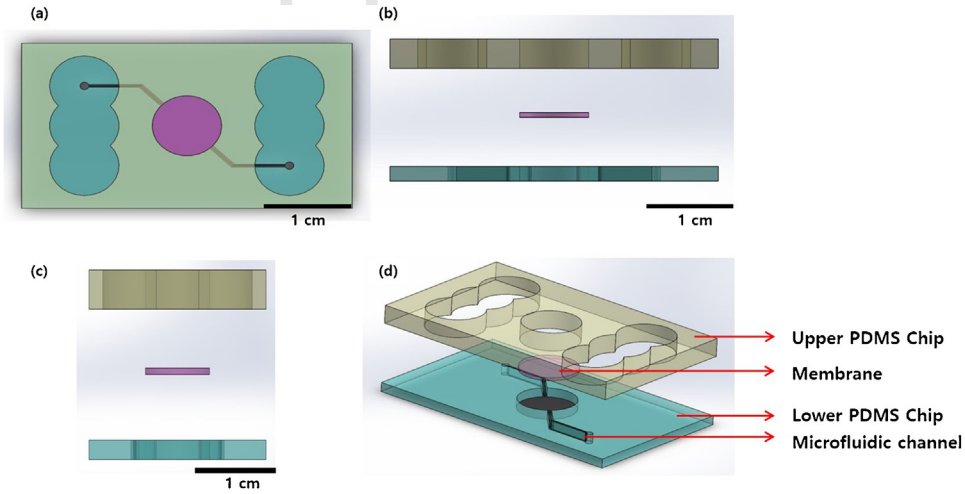

Fig. 1. A schematic diagram of a skin on a chip. (a) Top view, (b) a side view, (c) a side view, and (d) perspective view.

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