Acta Biomaterialia 9 (2013) 9474–9484

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Micropatterned dermal–epidermal regeneration matrices create functional niches that enhance epidermal morphogenesis

Amanda L. Clement, Thomas J. Moutinho Jr., George D. Pins *

Biomedical Engineering Department, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609, USA
Bioengineering Institute, Worcester Polytechnic Institute, Worcester, MA 01609, USA

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form 1 August 2013
Accepted 9 August 2013
Available online 17 August 2013

Keywords:
3-D organ model
Keratinocyte function
Microtopography
Dermal–epidermal junction

ABSTRACT

Although tissue engineered skin substitutes have demonstrated some clinical success for the treatment of chronic wounds such as diabetic and venous ulcers, persistent graft take and stability remain concerns. Current bilayered skin substitutes lack the characteristic microtopography of the dermal–epidermal junction that gives skin enhanced mechanical stability and creates cellular microniches that differentially promote keratinocyte function to form skin appendages and enhance wound healing. We developed a novel micropatterned dermal–epidermal regeneration matrix (μDERM) which incorporates this complex topography and substantially enhances epidermal morphology. Here, we describe the use of this three-dimensional (3-D) in vitro culture model to systematically evaluate different topographical geometries and to determine their relationship to keratinocyte function. We identified three distinct keratinocyte functional niches: the proliferative niche (narrow geometries), the basement membrane protein synthesis niche (wide geometries) and the putative keratinocyte stem cell niche (narrow geometries and corners). Specifically, epidermal thickness and keratinocyte proliferation is significantly ($p < 0.05$) increased in 50 and 100 μm channels while laminin-332 deposition is significantly ($p < 0.05$) increased in 400 μm channels compared to flat controls. Additionally, $\beta_1^{bri}p63^+$ keratinocytes, putative keratinocyte stem cells, preferentially cluster in channel geometries (similar to clustering observed in native skin) compared to a random distribution on flats. This study identifies specific target geometries to enhance skin regeneration and graft performance. Furthermore, these results suggest the importance of μDERM microtopography in designing the next generation of skin substitutes. Finally, we anticipate that 3-D organotypic cultures on μDERMS will provide a novel tissue engineered skin substitute for in vitro investigations of skin morphogenesis, wound healing and pathology.

1. Introduction

Annually, three to four million diabetic and venous ulcers require clinical intervention in the United States [1]. In addition to profoundly impacting patient quality of life, the cost of treatment for venous and pressure ulcers exceeds $8 billion USD per year [1,2]. With the average age of the population and the incidence of diabetes on the rise, these numbers are expected to increase, leading to a demand for wound closure products such as skin substitutes [1–3]. Tissue engineered skin substitutes represent a promising therapy option and have had limited clinical success; however, there remains a significant clinical need for a robust tissue engineered skin substitute that reduces healing time while eliminating mechanically induced graft failure [1,2,4–7].

Skin, a complex multilayered tissue, is primarily responsible for maintaining a highly regulated semi-permeable barrier between the body and the environment [8]. Following traumatic disruption of the skin, rapid restoration of the barrier function, which is the first line of defense against infection and dehydration, is critical for a ensuring a positive clinical outcome [1]. To better under the cellular mechanisms which direct these tissue responses, several groups have developed in vitro models of native skin [9–11]. While these models elucidate mechanisms that guide epidermal regeneration, they are limited in their capacity to precisely analyze the key topographic and biochemical roles of keratinocyte microniches at the dermal–epidermal junction (DEJ) in directing critical wound healing processes.

In native skin, the topography of keratinocyte microniches at the DEJ plays a critical role in maintaining the structure and mechanical properties of the tissue, as well as in directing its regenerative potential [12–14]. The DEJ is characterized by finger-like dermal papillae (DP) and epidermal rete ridges that conform to the dermal topography. These features create microniches with

* Corresponding author at: Biomedical Engineering Department, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609, USA. Tel.: +1 508 831 6742; fax: +1 508 831 5541.
E-mail address: gpins@wpi.edu (G.D. Pins).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.08.017

dimensions ranging from 50 to 400 μm in width and from 50 to 200 μm in depth [15,16]. This interdigitated topography increases the surface area between the dermis and the epidermis, enhancing both the mechanical shear resistance of the skin and the paracrine diffusion between layers. Additionally, microtopographic niches create distinct cellular microenvironments that differentially direct keratinocyte phenotype and cellular function. For example, numerous laboratories have demonstrated that keratinocyte stem cells preferentially locate to the tips of the DP or the bases of the rete ridges, depending on their anatomical location [13,14,17–19]. Interestingly, changes in DEJ topography are noted in skin diseases such as psoriasis (lengthening of rete ridges) and in aged skin (flattening of the DEJ), further suggesting an important role for topography in epidermal function [20,21]. To further investigate the roles of three-dimensional (3-D) microniches on keratinocyte functions, several groups developed in vitro models to characterize the effects of cell geometries and surface chemistries on keratinocyte function [22–25]. However, a greater understanding of how 3-D topographic, biochemical and cellular cues direct keratinocyte proliferation and cellular function will further our understanding of epidermal morphogenesis and skin wound healing, and will allow us to harness these cues to develop the next generation skin substitutes.

Keratinocyte stem cells remain a controversial area of research, with ongoing debate surrounding epidermal stem cell models as well as specific locations and appropriate markers for epidermal stem cells. Some of this confusion originates from the heterogeneous nature of the epidermal stem cell population. The epidermis contains several distinct stem cell populations, including interfollicular (IF) stem cells, hair follicle stem cells and sebaceous gland stem cells, which support epidermal maintenance and tissue regeneration [26]. These physical microniches contain several distinct keratinocyte sub-populations located throughout the epidermis, including both keratinocyte stem cells and terminally committed keratinocytes. In particular, evidence suggests that basal keratinocytes, once considered a homogeneous population, represent a heterogeneous cell reservoir. Studies of native skin demonstrated clustering of phenotypically similar keratinocytes. Lavker and Sun [13,14] suggested the existence of two spatially segregated keratinocyte subpopulation: nonserrated, slow-cycling keratinocytes located at the base of deep rete ridges and serrated keratinocytes located in shallow rete ridges. Later, Watt et al. [12,17–19] examined stem cell patterning in the IF epidermis by correlating the regional variation in integrin expression levels in human skin with the colony forming efficiency of keratinocytes. Additionally, a recent study of keratinocytes seeded on a microfabricated dermal papilla template showed differential gene expression on micropatterned membranes compared to flat controls [27]. Given the continued lack of understanding of the role of microtopography in directing cellular phenotype, function and fate, we suggest that the topography of the dermal–epidermal junction is largely responsible for driving the clustering of these keratinocyte sub-populations and hypothesize that topographical cues can be used to direct keratinocyte phenotype and create stem cell reservoirs. Further, we propose that micropatterned dermal–epidermal regeneration matrices (μDERMs) provide a novel platform for systematically investigating the roles of microtopography on cellular function as well as diverse keratinocyte stem cell niches.

In this manuscript, we investigated the influence of cellular microniche topography on keratinocyte function. We recently described a method to create μDERMs that facilitate the investigation of cellular responses to microtopographies [28]. In this study, we integrated fibroblast co-culture into our model system and specifically probed the formation of distinct proliferative and synthetic niches within micropatterned keratinocyte microniches. We demonstrated that keratinocytes in narrower channels exhibit a more proliferative phenotype, while keratinocytes in wider channels exhibit enhanced synthesis of the basement membrane (BM) protein laminin-332. Additionally, keratinocytes exhibiting the putative stem cell markers β_1 integrin and p63 preferentially localized to the base of narrow channels and the corners of wider channels. This novel approach to creating 3-D model systems with topographical cues that mimic native cellular compartments at the DEJ will enable systematic investigation of the biophysical and biochemical cues that direct cutaneous tissue morphogenesis, and epidermal pathologies.

2. Materials and methods

2.1. Cell culture and media formulations

Neonatal primary human foreskin keratinocytes (NHKs) and primary human foreskin fibroblasts (NHFs) were isolated from foreskins, obtained as non-identifiable discard tissue from the University of Massachusetts Memorial Medical Center (Worcester, MA) and approved with exempt status from the New England Institutional Review Board, using previously described methods. Prior to seeding on μDERMs, keratinocytes were cultured on a feeder layer of mitomycin C treated J2s (generously donated by Dr. Stelios Andreadis, State University of New York at Buffalo, Buffalo, NY). The NHF medium consisted of Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 1% penicillin–streptomycin. The NHK medium was composed of a 3:1 blend of high glucose DMEM and Ham's F12 with 10% fetal bovine serum and 1% penicillin–streptomycin supplemented with adenine (1.8×10^{-14} M), cholera toxin (10^{-10} M), hydrocortisone (0.4 μg ml^{-1}), insulin (5 μg ml^{-1}), transferrin (5 μg ml^{-1}) and triiodo-L-thyronine (2×10^{-9} M). After initial NHK plating, medium was replaced with NHK medium containing 10 ng ml^{-1} epidermal growth factor (EGF). The seeding medium for μDERM culture consisted of a 3:1 blend of high glucose DMEM and Ham's F12 with 1% fetal bovine serum and 1% penicillin–streptomycin supplemented with cholera toxin (10^{-10} M), hydrocortisone (0.2 μg ml^{-1}), insulin (5 μg ml^{-1}) and ascorbic acid (50 μg ml^{-1}). The priming medium consisted of seeding medium supplemented with 24 μM BSA, 25 μM oleic acid, 15 μM linoleic acid, 7 μM arachidonic acid, 25 μM palmitic acid, 10 μM L-carnitine and 10 mM L-serine. The air–liquid interface (A/L) medium was serum-free priming medium supplemented with 1 ng ml^{-1} EGF.

2.2. In vitro μDERM culture model

2.2.1. μDERM specifications

The microtopographic features of our μDERMs were designed specifically to enable concurrent histological evaluation of multiple geometries on a single construct to minimize experimental variations and analysis time. Specifically, 1.25 cm × 2.0 cm patterns were designed with three series of parallel channels 200 μm deep, 2500 μm long and with variable widths (50, 100, 200 or 400 μm). For histological analysis, μDERMs were sectioned perpendicular to the channels to provide multichannel analysis on each section.

2.2.2. μDERM fabrication

μDERM fabrication was modified from a previous method [28] by incorporating fibroblasts into the dermal sponge and decreasing the thickness of the collagen gel matrix (Fig. 1). Specifically, a pattern was designed with a series of 200 μm deep channels with variable widths (50, 100, 200 and 400 μm) [28,29]. Type I acid-soluble collagen (10 mg ml^{-1} in 5 mM HCl) was purified from rat tail tendons [30,31] and 0.3 ml of this collagen solution was self-assembled on the micropatterned molds using 5× DMEM (Invitrogen) with 0.22 M $NaHCO_3$ and 0.1 M NaOH (Sigma) for 18 h at 37 °C