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Human melanocytes mitigate keratinocyte-dependent contraction in an in vitro collagen contraction assay

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ABSTRACT

Scarring is an extensive problem in burn care, and treatment can be especially complicated in cases of hypertrophic scarring. Contraction is an important factor in scarring but the contribution of different cell types remains unclear. We have investigated the contractile behavior of keratinocytes, melanocytes and fibroblasts by using an in vitro collagen gel assay aimed at identifying a modulating role of melanocytes in keratinocyte-mediated contraction. Cells were seeded on a collagen type I gel substrate and the change in gel dimensions were measured over time. Hematoxylin & Eosin-staining and immunohistochemistry against pan-cytokeratin and microphthalmia-associated transcription factor showed that melanocytes integrated between keratinocytes and remained there throughout the experiments. Keratinocyte- and fibroblast-seeded gels contracted significantly over time, whereas melanocyte-seeded gels did not. Co-culture assays showed that melanocytes mitigate the keratinocyte-dependent contraction (significantly slower and 18–32% less). Fibroblasts augmented the contraction in most assays (approximately 6% more). Non-contact co-cultures showed some influence on the keratinocyte-dependent contraction. Results show that mechanisms attributable to melanocytes, but not fibroblasts, can mitigate keratinocyte contractile behavior. Contact-dependent mechanisms are stronger modulators than non-contact dependent mechanisms, but both modes carry significance to the contraction modulation of keratinocytes. Further investigations are required to determine the mechanisms involved and to determine the utility of melanocytes beyond hypopigmentation in improved clinical regimes of burn wounds and wound healing.

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1. Introduction

Wounding of the skin is a potentially life-threatening condition due to the unhindered exposure to environmental pathogens. Wounds primarily heal by re-epithelialization and scar formation after partial contraction, providing a rapid biological response to restore the skin barrier. In normal wound healing in humans contraction has a limited impact on the process but is important for maintaining a functional barrier. Pathological contractures such as keloids and hypertrophic scars present serious clinical challenges, also evident within burn care [1]. Improving our understanding of skin wound contraction paves the way for improved clinical regimes and useful scientific models. Wound contraction has been studied with cell-embedded hydrogels since the model was first described by Elsdale and Bard [2]. This assay allows investigators to analyze the motility, adhesion and growth of cells as well as their effect on extracellular-matrix (ECM) contraction and remodeling in a three-dimensional manner that imitates the wound environment of the granulation tissue [3]. The manipulation of tissue-engineered constructs allows for control of independent variables necessary for scientific experimentation, and the development of such tissue constructs leads to minimized use of animals in preclinical research.

Dermal fibroblasts have received the most attention as mediators of wound contraction. They are able to differentiate into myofibroblasts, a phenotype characterized by expression of α-smooth muscle actin (α-SMA) [4]. This cell type has been shown to be induced in anchored collagen gels that mimic the stressed granulation tissue of a wound [5] and their numbers are known to increase due to mechanical tension [6], explaining the higher incidence of hypertrophic scars in areas of high tension and movement of the skin (e.g. hands, neck and knees) [7]. Besides myofibroblasts, the keratinocytes are also important sources of wound contraction [8–11], being responsible for an early wound closure of up to 25% before fibroblasts or myofibroblasts are even able to migrate into the wound bed [12]. The interest in the contractile properties of keratinocytes has recently increased due to the popularity of employing in vitro engineered skin models [13]. The role of keratinocytes in the etiology of keloids or hypertrophic scarring remains elusive [14]. Recent research has shown that keratinocytes of the basal layer are also able to switch to a myo-phenotype similar to myofibroblasts by expressing α-SMA [15], perhaps contributing more to the etiology of pathological scarring than has been previously appreciated.

Melanocytes reside along the dermo-epidermal junction, interspersed in the basal layer of the epidermis where they form the epidermal melanin unit (EMU). Each melanocyte connects to approximately 36 keratinocytes in its surroundings to synthesize and distribute the pigment melanin via dendrites to protect cells against UV radiation [16]. Scarring and follicular disorders are more common with darker skin, suggesting a link with pigmentation [17,18]. Clinical and experimental observations suggest that melanocytes influence the rigidity of the scarring under certain conditions [19], motivating further research on melanocytes during wound-healing. A recently published report by Gao et al. [20] has demonstrated the contribution of melanocytes to pathological scar formation through interaction with fibroblasts. By activating TGF-beta signaling pathways in fibroblasts the melanocytes may significantly contribute to keloid formations [20]. Clearly, melanocytes play important roles in wound healing and subsequent scar formation. Our aim was to determine the influence of melanocytes on the contractile behavior of keratinocytes by using a collagen type I gel contraction assay, with fibroblasts for comparison.

2. Methods

2.1. Cell cultures

Primary human cells were isolated from skin obtained from routine breast reduction surgeries of Caucasian women. Tissue samples were stored in DMEM with antibiotics at 4 °C, and processed within 12 h. Tissue was obtained in accordance with the Declaration of Helsinki ensured by ethical regulations at the University of Linköping and the County Council of Östergötland, Sweden. Keratinocytes were isolated by overnight treatment with dispase (#17105, Life Technologies) to separate the epidermis from the tissue sample, followed by mincing of the epidermis and seeding into T-75 flasks in complete Keratinocyte Serum Free Medium (KSFM, #17005-034, Life Technologies) with antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin, #15140122, Life Technologies). Complete KSFM was supplemented with 0.033 μl/ml epidermal growth factor (EGF, #13028-014, Life Technologies), 2.5 μl/ml bovine pituitary extract (BPE, #13028-14, Life Technologies) as specified by manufacturer.

Melanocytes were isolated by mincing the epidermal layer, followed by incubation in Trypsin–EDTA (1:1) for 40 min at 37 °C while continuously stirring the suspension. The tissue was allowed to sediment and the supernatant was collected and transferred into T-75 flasks. Melanocyte Growth Medium (MGM) contained PC-1 base medium (#344018, Lonza) supplemented with 2% PC-1 sterile supplement (#344022, Lonza), 1% L-glutamine (200 mM, #G7513, Sigma–Aldrich), 5 ng/ml Fibroblast Growth Factor-Basic (#F0291, Sigma–Aldrich), 246 μg/ml N6,2′ O-dibutyryladenosine 3′,5′-cyclic monophosphate sodium salt (#D0627, Sigma–Aldrich) and antibiotics.

Fibroblasts were isolated by mincing the dermal compartment and incubating the pieces in a solution containing 25 U/ml dispase and 165 U/ml collagenase (#17101-015, Life Technologies) overnight at 37 °C, followed by seeding in T-75 flasks with Fibroblast Medium (FM). FM contained 10% FCS (HyClone FetalClone II, #SH30066.03, Thermo Scientific) and antibiotics in DMEM (#31885-023, Life Technologies).

All media were changed three times per week, and kept at 37 °C (5% CO_2, 95% humidity). Cells were expanded at a ratio of 1:3 at 80% confluency. Melanocytes and keratinocytes were used for experiments between passages 2–5 and fibroblasts at passages 3–6. Cell populations were verified microscopically during selective expansion culture, and confirmed by histology and immunohistochemistry after the assays.

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