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Review

Fibroblast heterogeneity and its implications for engineering organotypic skin models *in vitro*

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ABSTRACT

Advances in cell culture methods, multidisciplinary research, clinical need to replace lost skin tissues and regulatory need to replace animal models with alternative test methods has led to development of three dimensional models of human skin. In general, these *in vitro* models of skin consist of keratinocytes cultured over fibroblast-populated dermal matrices. Accumulating evidences indicate that mesenchyme-derived signals are essential for epidermal morphogenesis, homeostasis and differentiation. Various studies show that fibroblasts isolated from different tissues in the body are dynamic in nature and are morphologically and functionally heterogeneous subpopulations. Further, these differences seem to be dictated by the local biological and physical microenvironment the fibroblasts reside resulting in "positional identity or memory". Furthermore, the heterogeneity among the fibroblasts play a critical role in scarless wound healing and complete restoration of native tissue architecture in fetus and oral mucosa; and excessive scar formation in diseased states like keloids and hypertrophic scars. In this review, we summarize current concepts about the heterogeneity among fibroblasts and their role in various wound healing environments. Further, we contemplate how the insights on fibroblast heterogeneity could be applied for the development of next generation organotypic skin models.

Contents

Abbreviations: 3D, three-dimensional; AdSCs, adipose-derived stem cells; AP-1, activated protein-1; Blimp1, B lymphocyte-induced maturation protein-1; BP, bullous pemphigoid antigen; CD, cluster of differentiation; Dlk1, delta-like 1 homolog; DOPr, Delta-opioid receptor; DS, dermal sheath; ECM, extracellular matrix; En1, Engrailed-1; FDP, follicular dermal papilla; FGF, Fibroblast growth factor; FSP-1, fibroblast-specific protein-1; GM-CSF, granulocyte-macrophage colony stimulating factor; HA, hyaluronic acid; HAS, hyaluronan synthase; hESCs, human embryonic stem cells; HGF, hepatocyte growth factor; IL, interleukin; iPSCs, induced pluripotent stem cells; KGF, keratinocyte growth factor; KOPr, kappa-opioid receptor; Lrig1, leucine-rich repeats and immunoglobulin-like domains 1; mAChR, mucarinergic acetyl choline receptors; MF, mitotically active fibroblasts; MMP, matrix metalloproteinases; MOPr, Mu-opioid receptor; Myb, myeloblastosis viral oncogene; nAChR, nicotinergic acetyl choline receptors; PDGFR, platelet-derived growth factor receptors; PMF, post-mitotic fibrocytes; PPAR, peroxisome proliferative-related receptors; RAR, retinoic acid receptors; RXR, retinoid X receptors; TGFβ, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor; α-SMA, α-smooth muscle actin.

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1. Introduction

Skin is the largest organ in the human body that consists of tightly organized layers of keratinocytes and the underlying dermis. The skin is highly specialized in terms of providing barrier function by preventing water loss, resisting mechanical trauma, protection against physical, chemical and/or biological agents such as temperature, light, metals, chemicals, toxins, micro-organisms (fungi, bacteria, viruses) (Simpson et al., 2011). In addition to the barrier function, the skin plays a role in sensation, thermoregulation, excretion, absorption, pigmentation and innate/acquired immunity. In addition, the skin has very complex appendages, such as hair follicles, sebaceous glands, sweat glands and nails. Recapitulating the skin with such complex functionalities *in-vitro* is a daunting task that requires the need for a complex three-dimensional (3D) microenvironment that cannot be provided by conventional monolayer cultures. Advances in cell culture methods, multidisciplinary research, clinical need to replace lost skin tissues and regulatory need to replace animal models with alternative test methods has led to development of 3D organotypic models of human skin consisting primarily of keratinocytes over fibroblasts-populated dermal matrices. The need for these organotypic models of skin are immense and have profound implications not only for basic scientists and tissue engineers, but also for clinicians, manufacturers, regulatory authorities and animal welfare organizations. Thus it is imperative that we understand how normal skin develops, is regulated, and heals during recovery from wounding. What we now know is that fibroblasts are an integral part of mesenchyme-derived signals that are essential for epidermal morphogenesis, homeostasis and differentiation.

Fibroblasts are the most abundant cell type within all the body's connective tissues, and their primary role is secretion of the components of the extracellular matrix (ECM). In the case of the skin, the epidermal keratinocyte layer is underpinned and intimately connected to the dermis, which contains the dermal fibroblasts as well as immune cells, blood vessels, nerve fibers, hair follicles and secretory glands. Early attempts to model the human skin *in vitro* used monolayer cultures of keratinocytes and/or fibroblasts, and while these studies formed the foundation of our current knowledge, they were unsuitable for studying the interaction between the two cell types as they were unable to take into account the effect of spatial organization of the skin layers. In addition, they do not have a corneal layer and a differentiated epidermis with the variety of properties of the keratinocytes in different differentiation stages. Advances in culturing techniques have since led to the development of organotypic culture systems that mimic the 3D organization of keratinocytes and fibroblasts observed *in vivo* (Auxenfans et al., 2009). Keratinocytes are seeded onto fibroblast-populated dermal matrices then cultured at the air–liquid interface to drive epidermal differentiation, stratification and cornification, which results in an engineered skin tissue that closely mimics the native skin (Bell et al., 1981; Boehnke et al., 2007; Butler et al., 2008; Cario-Andre et al., 2006; Chen et al., 1995; El Ghalbzouri et al., 2002a,b, 2005; Lamb and Ambler, 2013; Liu et al., 2007; Muffler et al., 2008; Ponec et al., 1997, 2001; Pontiggia et al., 2013; Smola et al., 1994; Stark et al., 1999, 2004a,b, 2006). These engineered skin mimics have successfully been used to study various aspects of skin biology including epithelial-mesenchymal interactions (Maas-Szabowski et al., 1999; Smola et al., 1993, 1994), growth and differentiation of keratinocytes (Boukamp et al., 1990; Maas-Szabowski et al., 2000; Muffler et al., 2008; Stark et al., 2004b, 2006), development of epithelial barrier properties (Pasonen-Seppanen et al., 2001; Ponec et al., 1997, 2001; Regnier et al., 1993; Thakoersing et al., 2012), dynamics of the basement membrane (Breitkreutz et al., 1997, 2004; El Ghalbzouri et al., 2005; Fleischmajer et al., 1998; Nischt et al., 2007; Smola et al., 1998; Stark et al., 2004b), wound healing (Boyce and Warden, 2002; Geer et al., 2002; Harrison et al., 2006; Laplante et al., 2001) and dermatopathology (Barker et al., 2004; Butler et al., 2008; Chiu et al., 2005; Eves et al., 2000). They have also revealed new and intriguing properties of the dermal fibroblast population with profound implications for our understanding of how the skin works and how we might be able to engineer optimal skin substitutes for industrial and clinical use.

In this review we will discuss current knowledge and recent breakthroughs in our understanding of fibroblasts, with particular reference to the dermal fibroblast population and its effects on human skin development, homeostasis and healing. We will look at how knowledge gained from *in vivo* wound healing studies and scarring phenotypes can be combined with the latest insights from reconstituted skin models to inform the next steps in development of biological skin substitutes. Finally, we will highlight several questions in the field and consider how the next generation of *in vitro* models might be optimized to find the answers and facilitate development of clinically-appropriate skin replacements.

2. Fibroblasts in the skin

Conventionally, fibroblasts are defined by their spindle-shaped morphology, adhesive growth on tissue culture plastics, expression of mesenchymal markers that include vimentin and collagen I, and the lack of expression of markers related to other specific cell lineages. While traditionally considered a static population of spindle-shaped cells that maintain and support the skin through secretion and degradation of ECM, we now know that fibroblasts play an important role in almost every skin process throughout life: in the embryo, fibroblasts direct skin morphogenesis; in the mature organism they contribute to homeostasis of the skin; and their involvement in various physiopathological conditions including healing, fibrosis, aging, psoriasis, and skin cancer, is just beginning to be understood. The dermal fibroblast population also undertakes dynamic and reciprocal interactions with other resident cell types (epithelial cells, endothelial cells, neural cells, adipocytes, inflammatory cells, and resident stem cells) through direct cell–cell communications, cell–matrix interactions, and the secretion of soluble factors (growth factors and cytokines) (Borchers et al., 1994; Costea et al., 2003; Eming et al., 2007; Maas-Szabowski et al., 1999;

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