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MicroRNAs in skin tissue engineering ☆

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ABSTRACT

35.2 million annual cases in the U.S. require clinical intervention for major skin loss. To meet this demand, the field of skin tissue engineering has grown rapidly over the past 40 years. Traditionally, skin tissue engineering relies on the "cell-scaffold-signal" approach, whereby isolated cells are formulated into a three-dimensional substrate matrix, or scaffold, and exposed to the proper molecular, physical, and/or electrical signals to encourage growth and differentiation. However, clinically available bioengineered skin equivalents (BSEs) suffer from a number of drawbacks, including time required to generate autologous BSEs, poor allogeneic BSE survival, and physical limitations such as mass transfer issues. Additionally, different types of skin wounds require different BSE designs. MicroRNA has recently emerged as a new and exciting field of RNA interference that can overcome the barriers of BSE design. MicroRNA can regulate cellular behavior, change the bioactive milieu of the skin, and be delivered to skin tissue in a number of ways. While it is still in its infancy, the use of microRNAs in skin tissue engineering offers the opportunity to both enhance and expand a field for which there is still a vast unmet clinical need. Here we give a review of skin tissue engineering, focusing on the important cellular processes, bioactive mediators, and scaffolds. We further discuss potential microRNA targets for each individual component, and we conclude with possible future applications.

Contents

Abbreviations: APC, antigen producing cell; Ago, argonaute protein; αSMA, alpha smooth muscle actin; BSE, bioengineered skin equivalent; CEA, cultured epithelial autograft; cKO, conditional knockout; CREB1, p-cAMP response element-binding protein 1; DMD, Duchenne muscular dystrophy; E#, embryonic day #; ECM, extracellular matrix; EGF, epidermal growth factor; EMP-1, epithelial membrane protein 1; EMT, epithelial–mesenchymal transition; FGF, fibroblast growth factor; FGFR2, fibroblast growth receptor 2; G-CSF, granulocyte colony stimulating factor; hMSC, human mesenchymal stem cells; HSFB, hypertrophic scar derived fibroblast; hTERT, human telomerase reverse transcriptase; IGF1, insulin-like growth factor-1; MET, mesenchymal–epithelial transition; mRNA, messenger RNA; miRNA, microRNA; MITF, microphthalmia-associated transcription factor; PI3KR2, phosphoinositol-3 kinase regulatory subunit 2; PCL, poly (ε-caprolactone); PEG, polyethylene-glycol; PLGA, poly(lactic-co-glycolic acid); PLL, poly-(L-lysine); RNA, ribonucleic acid; siRNA, short interference RNA; TGF-β1, transforming growth factor beta 1; SHIP2, SH2 containing phosphoinositide-5′-phosphatase 2; TRE, tetracycline regulatory element; PDGF, platelet derived growth factor; VCAM-1, vascular cell adhesion molecule 1; Yap1, yes associated protein; ZEB1, zinc finger enhancing binding protein.

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1. Introduction

Skin is the largest organ in the human body. The socioeconomic burden imposed by limited availability of replacement skin is enormous. In the U.S. alone, significant skin loss requiring major clinical intervention occurs in 35.2 million cases [1]. This includes 6.5 million people who suffer from chronic wounds, for which annual treatment costs exceed $25 billion [2]. Annual estimates for burn injuries in the US are 1 million cases, with treatment estimated at $4 billion [3]. Of those treated, 32–72% of severe burn patients will experience hypertrophic scarring [4]. Current standard of care for burn wounds involve harvesting a skin graft from a donor site on the patient and placing it over the burn wound [5]. This process creates a new wound on the patient and requires enough remaining uninjured skin to cover the burn.

Tissue engineering represents a promising approach to generate replacement skin. Research into bioengineered skin over the past 30 years has yielded a number of commercial BSEs [6]. However, none of these products meet the criteria for fully-functional skin [7]. A key obstacle in the development of engineered skin is controlling cellular behavior. miRNAs are small, noncoding segments of RNA (~19–24 nucleotides) which affect gene expression by both enhancing messenger RNA (mRNA) degradation and preventing mRNA translation [8–10]. With its small size and long half-life, miRNA allows for a sustained, sophisticated, and highly customizable level of regulated cell behavior [11]. The marriage of miRNA and skin tissue engineering offers the promise of creating safer, more effective BSEs for critically important clinical conditions.

In this review, we summarize work in the field of skin tissue engineering and provide a review of the cellular events of skin development and wound healing. Particular attention is given to the role of miRNA in these processes, and we conclude by discussing future avenues for the use of miRNA in skin tissue engineering.

2. Skin anatomy, physiology, and repair

The skin is divided into three main layers: the epidermis, the dermis, and the hypodermis (Fig. 1). The hypodermis is a subcutaneous layer of connective tissue, fat, and large blood vessels, which serves primarily in cushioning and thermoregulation. Thus, the vast majority of efforts in skin tissue engineering have focused on de novo generation of epidermis and dermis. The epidermis is relatively thin (10–100 μm) and comprised mainly of keratinocytes, which serve a barrier function [12]. Wounds extending only through the epithelium heal primarily by keratinocyte migration from the wound edge. Basal stem cells contribute primarily to the homeostatic pool of keratinocytes, but may also assist in repair [13,14]. The epidermis possesses dermal projections called *appendages*. One such appendage, the hair follicle, supplies a major pool of new keratinocytes from stem cells located within its bulge; these epithelial stem cells migrate up from the level of the dermis in response to injury [15–17]. Sebaceous glands also possess a pool of epithelial stem cells which are thought to be populated by bulge stem cells [18]. The role of sebaceous gland stem cells in tissue regeneration is still under investigation [18]. In addition to keratinocytes, the epidermis also contains pigment-producing melanocytes, which serve to color the skin and protect it from ultraviolet radiation.

The dermis is thicker than the epidermis (400–2000 μm) and is comprised mainly of fibroblasts, which provide strength to the skin in the form of their secretory extracellular matrix (ECM) products [19]. The dermis also contains blood vessels, which provide transport for nutrients, wastes, bioactive mediators, and immune cells within the skin. When designing a BSE, the extent of damage and the condition of the wound must be considered (Fig. 2). Injuries extending partially into the dermis (partial thickness) heal primarily by keratinocyte contribution from the hair follicle bulge and migration of cells from the wound edge. Skin's protective and retentive functions are dependent upon proper epithelialization. Re-epithelialization is impaired in chronic wounds. Re-epithelialization also depends upon the ability of epithelial stem cells and fibroblasts to proliferate, differentiate, and migrate from their respective niches to the site of repair. Interestingly, keratinocytes surrounding chronic wounds are highly proliferative, undifferentiated, and unable to migrate; these observations suggest that chronic wound keratinocytes may be trapped in a proliferative stage [20]. If an injury extends past the deep dermal elements (full thickness), the ability of skin to regenerate may be impaired, requiring the use of a BSE and/or skin graft. In fact, full thickness wounds greater than 1 cm in diameter necessarily require such measures to prevent excessive scarring [6,21]. Scarring occurs when wound healing fails to resolve to normal levels [22]. The result is a shrunken and stiff tissue that can impair function and produce significant cosmetic consequences [23,24]. Thus, there is a need to engineer improved BSEs to tackle complex clinical problems associated with skin.

3. Morphogenesis: miRNA and skin development

The embryo develops from three primary germ layers: endoderm, mesoderm, and ectoderm. The ectoderm gives rise to the epidermis, the hair follicle, and the sebaceous gland [25]. Following gastrulation, a layer of epidermal cells forms and persists from embryonic days 9.5 (E9.5) to E12.5 [18]. As mesenchymal cells (from the mesoderm) populate the skin, they communicate signals, which direct the stratification of the epidermis, the formation of an ECM-rich basement membrane, and the positioning of downgrowths which become hair follicles. The mesenchymal cells eventually go on to develop into the dermis. During early epidermal stratification (E12.5–E15.5), cell division occurs only rarely suprabasally, and stratification completes around E17.5. Once stratified, the epithelium consists of an inner layer of basal cells with high proliferative potential and subsequent layers of terminally-differentiating suprabasal cells.

Skin embryogenesis and development were recently found to be dependent upon miRNA expression [26,27]. miRNAs possess unique sequences, but they share common synthetic pathways in which their precursor transcripts fold back into a hairpin structure (Fig. 3) [8,28]. This hairpin is released from the primary transcript by the nuclear Drosha–DGCR8 complex [29–33]. Following exportation to the cytoplasm by the RanGTP-dependent Exportin-5, the hairpin is processed into a dsRNA duplex by the RNase III enzyme Dicer [34,35]. One strand

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