Tissue-Engineered Skin Regenerative Units for Epidermal Keratinocytes Expansion and Wound Healing

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Financial support: This work was financially supported by the National Natural Science Foundation of China (general project No. 81272120) and the Natural Science Foundation of Zhejiang Province (LQ20H180011)

Conflict of interest: None declared

Chronic wounds have become an increasing medical and economic problem of aging societies because they are difficult to manage. Tissue engineering provides new perspectives for the clinically applicable skin substitutes. Epidermal keratinocytes play an important role in wound epithelization and construction of tissue-engineered skin substitutes. How to obtain a large number of autologous epidermal keratinocytes in a short time is the main problem that limits the application of tissue-engineered skin and epidermal cell membranes. Developing an appropriate method for reproducing the biological potential of cell-cell interactions and simulating the three-dimensional structure between cells has great significance for epidermal keratinocytes expansion and full-thickness skin regeneration. In this article, we propose the concept of tissue-engineered skin regeneration units (TESRUs) as the smallest unit with complete full-thickness skin regeneration ability. First, autologous dermal fibroblasts are cultured in biodegradable macroporous microcarriers to provide the mesenchyme support. Second, autologous epidermal keratinocytes and autologous melanocytes are incubated with the fibroblasts-loaded microcarriers and expand in vitro. Incorporating the above co-culture method into the macroporous microcarriers is reasonable for maintaining cell-cell interactions in spatial and temporal context and providing a suitable growth niche for epidermal keratinocytes. Moreover, TESRUs are composed of fibroblasts, keratinocytes, and melanocytes and have complete full-thickness skin regeneration ability. We suggest that TESRUs could be a promising strategy to repair full-thickness skin defects for clinical applications if the hypothesis proves to be practical.

Keywords: Dermis • Regenerative Medicine • Tissue Engineering • Wound Healing

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/932978

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HYPOTHESIS

Background

Full-thickness skin defects caused by trauma, burns, and non-healing ulcers are very common in the clinical setting, and represent a major burden on world health care systems. There are several strategies for wound healing, including split thickness grafts, tissue-engineered scaffolds, and cultured epidermal keratinocyte autografts [1,2]. Because skin grafting has limits, multiple approaches for engineering skin tissue products have attracted attention. However, skin substitutes with scaffolds have encountered a problem with vascularization after transplantation. At the same time, tissue-engineered skin products need a large number of epidermal keratinocytes, which are difficult to culture. Using an allogeneic epidermal keratinocyte suspension seems to be an alternative strategy but has problems caused by the lack of scaffolds and immunogenicity. Therefore, researchers have turned to directly administering cell suspensions into wounds [3,4]. Clinical trials to directly apply skin epidermal keratinocytes suspended in concentrated thrombocytes to wounds have demonstrated a significant improvement in the healing process. However, this method leads to the appearance of micro-cracks in the tissues underlying the epidermis and dermis that are populated by single skin cells, which require surgical re-intervention [4].

Wound healing is a complex physiological process that involves numerous cell types. Its mechanism is strictly organized via cell interactions. Recently, studies have focused on developing tissue-engineered skin substitutes that use synthetic biomaterials to provide structural support and incorporate human skin cell types to regenerate the damaged skin [5]. Developing an appropriate method for reproducing the biological potential of cell-cell interactions, simulating the three-dimensional structure between cells, has great significance for epidermal keratinocyte expansion and full-thickness skin regeneration.

Hypothesis

A hypothesis is proposed for a method to reproduce fibroblast–epidermal keratinocyte–melanocyte interactions and provides a temporary three-dimensional structure for living cells to construct skin substitutes with full-thickness regeneration ability. In this method, degradable macroporous microcarriers are introduced as a temporary three-dimensional structure between cells, having great significance for epidermal keratinocyte expansion in vivo and full-thickness skin regeneration. Suitable growth niche that mimics cell–cell interactions in vivo for epidermal keratinocytes expansion, and potentially form a mature skin microstructure simultaneously before final grafting. Based on these sequential steps, the concept of tissue-engineered skin regeneration units (TESRUs) as the smallest unit with complete full-thickness skin regeneration ability is proposed (Figure 1).

Evaluation of Hypothesis

Epidermal keratinocytes, which comprise ~95% of the cells within the epidermis, have been recognized as a powerful tool in skin regeneration. Epidermal keratinocytes play an important role in wound epithelization and would healing. However, it is still difficult to obtain adequate numbers of epidermal keratinocytes that retain their differentiation potential. How to obtain a large number of autologous epidermal keratinocytes in a short time is the main problem that limits the application of tissue-engineered skin and epidermal cell membrane. It is known that culture confluence can induce commitment to terminal differentiation by expression of suprabasal keratin 1 (K1) and 10 (K10) genes [6]. The keratinocytes were always expanded and isolated by enzymatic detachment from culture flasks and directly engrafted onto patients. However, the use of enzymes and lack of three-dimensional structure limited its application. It is known that epidermal keratinocytes are regulated by their niches to maintain tissue homeostasis and repair. Other studies that cultured human keratinocytes on a fibrin indicated that it facilitated the formation of dermo-epidermal junction and favored the regeneration of normal epidermis [7]. Co-culture methods establish and mimic the synthetic interactions between cell populations in vivo. A theory of obtaining a large volume of autologous epidermal keratinocytes in suspension through multiple cell interactions seems to be reasonable. Rheinwald et al developed a method for the long-term expansion of primary human epidermal keratinocytes by co-culture with 3T3 mouse embryonic fibroblasts. Fibroblasts provide a supportive environment for the expansion of human stratified epidermal keratinocyte cells [8]. This shows that the co-culture of fibroblasts and epidermal keratinocytes promotes the proliferation of epidermal keratinocytes, maintains their stemness, and delays their differentiation [9]. In addition, fibroblasts can facilitate re-epithelialization in wounded human skin equivalents [10]. Moreover, evidence shows that cultured epidermal keratinocytes on collagen implanted with fibroblasts for a period of time can form an artificial dermis and epidermis. This has already been used for venous ulcer and diabetic foot treatment, with Apligraf as a well-known example [11].

Melanocytes are another cell type that can be included to mimic the cell–cell interactions in vivo and improve the regeneration ability of a tissue engineering product. Melanocytes synthesize...
the melanin that produces the skin’s color, and thus fill a cosmetic need. Liu et al [12] developed a tissue engineering skin substitute composed of human fibroblasts, melanocytes, and keratinocytes in a type I collagen gel. The results showed proper integration and morphology and successful repair of skin defects in athymic mice, and black skins were observed by 6 weeks after grafting. In addition, melanocytes protect epidermal keratinocytes from ultraviolet radiation-induced changes in their DNA structure [13,14]. Melanocytes in autologous engineered skin substitutes can restore photoprotection after grafting to full-thickness skin wound, regardless of whether light or dark pigmentation phototype melanocytes were used [15].

A three-dimensional structure is required to deliver adequate numbers of epidermal keratinocytes to a wound, which is necessary for epithelialization and wound healing. Biodegradable microcarriers provide a temporary three-dimensional structure for cell adhesion and proliferation and help to form a three-dimensional skin substitute after delivery to a poorly vascularized wound. A biodegradable microcarrier-based suspension culture containing various cell types has also been reported to be a good alternative for effective ex vivo expansion [16]. It was found that isolated human epidermal keratinocytes cultured on Cultispher-G microcarriers multiplied by 44.9-fold in a microcarrier-bioreactor culture in 17 days, whereas two-dimensional cultures reached confluence in 9 days and only expanded by 7.4-fold. Moreover, microcarrier-expanded epidermal keratinocytes retained their capacity to form an epidermis and exhibited a morphology similar to that of native skin [17]. In addition, it is possible to promote the proliferation and maturation of epidermal keratinocytes and melanocytes by adjusting the culture medium, such as through serum, epidermal growth

**Figure 1.** Schematic diagram of construction the TESRUs: (A) First, autologous fibroblasts are loaded on a biodegradable macroporous microcarrier. (B) Second, autologous epidermal keratinocytes and melanocytes are incubated with the fibroblast-loaded microcarriers. (C) Autologous epidermal keratinocytes and autologous melanocytes are expanded in the fibroblast-loaded microcarriers, which are regarded as the smallest unit with complete full-thickness skin regeneration ability. (D) Multiple TESRUs are delivered to the wound surface to regenerate the full-thickness skin.
factor (EGF), and fibroblast growth factor-basic (bFGF) supplements [18]. In this way, the fibroblasts in the microcarriers are inhibited by competition from other cell populations and provide a supportive in vitro environment for the expansion of epidermal keratinocytes and melanocytes [10]. It is speculated that the crosstalk between the fibroblasts, epidermal keratinocytes, and melanocyte in the microcarriers could facilitate epithelialization and have potential for full-thickness skin regeneration.

It seems that fibroblasts and the biodegradable microcarriers, as well as melanocytes, might provide a suitable growth niche for epidermal keratinocytes. One possible mechanism is the crosstalk between the fibroblasts, epidermal keratinocytes, and melanocytes via secreted factors [19-21], and via extracellular matrix (ECM) deposition [22]. The feeder cell culture system is an example that demonstrated that the epidermal keratinocyte stem cell phenotype depends on the interaction with fibroblasts. The fibroblasts are inhibited by competition from the surrounding epidermal keratinocytes and melanocytes, and serve as a source of growth factors and cytokines to support the functions of these epidermal keratinocytes and melanocytes, including transforming growth factor beta (TGF-β), keratinocyte growth factor (KGF), EGF, bFGF, and tumor necrosis factor-alpha (TNF-α), which bind to receptors and modulate intracellular signaling cascades related to the epidermal keratinocyte and melanocyte proliferation and functions [19-21]. Such cascades include Wnt/β-catenin, PI3K/Akt, and the MAPK/ERK signaling pathway [19,23]. In addition, there is a stronger increased secretion of IL-6 (Interleukin-6) [24], TGF-β [25], collagenases, MMP-3 (matrix metallopeptidase 3), and TIMPs (tissue inhibitors of metalloproteinases) [26] in epidermal keratinocyte–fibroblast co-cultures in comparison to monocultures, which has been shown to stimulate epidermal keratinocyte proliferation, differentiation, and ECM deposition [22]. Another possible mechanism is the crosstalk between fibroblasts, epidermal keratinocytes, and melanocytes via extracellular vesicles (EVs) from those cells. EVs have been implicated in many mechanisms, such as cell–stroma interactions and angiogenesis [27,28]. It is reported that fibroblasts can produce EVs that can stimulate mesenchyme growth when cultured with serum or plasma [29]. On the other hand, exosomes derived from keratinocytes can not only stimulate fibroblast migration and promote wound healing [30], but also modulate pigmentation in melanocytes [31].

Conclusions

We present the novel concept of TESRUs as the smallest unit with complete full-thickness skin regeneration ability. TESRUs can potentially form and simultaneously deliver to the wound a mature skin microstructure to regenerate full-thickness skin, independent of scaffolds and vascularization. It is believed that TESRUs will provide a promising strategy in skin tissue engineering if a thorough understanding can be gained of the intimate interactions between the fibroblasts, epidermal keratinocytes, and melanocytes. However, further in vivo and clinical trials are still necessary.

Declaration of Figures’ Authenticity

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References:

1. Bhardwaj N, Chouhan D, Mandal BB. Tissue engineered skin and wound healing: Current strategies and future directions. Curr Pharm Des. 2017;23(24):3455-82
2. Ho J, Walsh C, Yue D, et al. Current advancements and strategies in tissue engineering for wound healing: A comprehensive review. Adv Wound Care (New Rochelle). 2017;6(6):191-209
3. Velander P, Theopold C, Bleiziffer O, et al. Cell suspensions of autologous keratinocytes and melanocytes of autologous keratinocyte and melanocyte proliferation and functions [19-21]. Such cascades include Wnt/β-catenin, PI3K/Akt, and the MAPK/ERK signaling pathway [19,23]. In addition, there is a stronger increased secretion of IL-6 (Interleukin-6) [24], TGF-β [25], collagenases, MMP-3 (matrix metallopeptidase 3), and TIMPs (tissue inhibitors of metalloproteinases) [26] in epidermal keratinocyte–fibroblast co-cultures in comparison to monocultures, which has been shown to stimulate epidermal keratinocyte proliferation, differentiation, and ECM deposition [22]. Another possible mechanism is the crosstalk between fibroblasts, epidermal keratinocytes, and melanocytes via extracellular vesicles (EVs) from those cells. EVs have been implicated in many mechanisms, such as cell–stroma interactions and angiogenesis [27,28]. It is reported that fibroblasts can produce EVs that can stimulate mesenchyme growth when cultured with serum or plasma [29]. On the other hand, exosomes derived from keratinocytes can not only stimulate fibroblast migration and promote wound healing [30], but also modulate pigmentation in melanocytes [31].

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References:
17. Borg DJ, Dawson RA, Leavesley DI, et al. Functional and phenotypic characterization of human keratinocytes expanded in microcarrier culture. J Biomed Mater Res A. 2009;88(1):184-94
18. Blaimauer K, Watzinger E, Erovic BM, et al. Effects of epidermal growth factor and keratinocyte growth factor on the growth of oropharyngeal keratinocytes in coculture with autologous fibroblasts in a three-dimensional matrix. Cells Tissues Organs. 2006;182(2):98-105
19. Werner S, Krieg T, Smola H. Keratinocyte-fibroblast interactions in wound healing. J Invest Dermatol. 2007;127(5):998-1008
20. Peura M, Siltanen A, Saarinen I, et al. Paracrine factors from fibroblast aggregates in a fibrin-matrix carrier enhance keratinocyte viability and migration. J Biomed Mater Res A. 2010;92(2):658-64
21. Stunova A, Vistejnova L. Dermal fibroblasts-A heterogeneous population with regulatory function in wound healing. Cytokine Growth Factor Rev. 2018;39:137-50
22. Benny P, Badowski C, Lane EB, Raghunath M. Making more matrix: Enhancing the deposition of dermal-epidermal junction components in vitro and accelerating organotypic skin culture development, using macromolecular crowding. Tissue Eng Part A. 2015;21(1-2):183-92
23. Qiang L, Yang S, Cui YH, He YY. Keratinocyte autophagy enables the activation of keratinocytes and fibroblasts and facilitates wound healing. Autophagy. 2020 [Online ahead of print]
24. Lichtman MK, Otter-Vinas M, Falanga V. Transforming growth factor beta (TGF-beta) isoforms in wound healing and fibrosis. Wound Repair Regen. 2016;24(2):215-22
25. Maas-Szabowski N, Starker A, Fusenig NE. Epidermal tissue regeneration and stromal interaction in HaCaT cells is initiated by TGF-alpha. J Cell Sci. 2005;116(Pt 14):2937-48
26. Sawicki G, Marcoux Y, Sarkhosh K, Tredget EE, Ghahary A. Interaction of keratinocytes and fibroblasts modulates the expression of matrix metalloproteinases-2 and -9 and their inhibitors. Mol Cell Biochem. 2005;269(1-2):209-16
27. Rackov G, Garcia-Romero N, Esteban-Rubio S, et al. Vesicle-mediated control of cell function: The role of extracellular matrix and microenvironment. Front Physiol. 2018;9:651
28. Carrasco E, Soto-Heredero G, Mittelbrunn M. The role of extracellular vesicles in cutaneous remodeling and hair follicle dynamics. Int J Mol Sci. 2019;20(11):2758
29. Moulin VJ, Mayrand D, Messier H, et al. Shedding of microparticles by myofibroblasts as mediator of cellular cross-talk during normal wound healing. J Cell Physiol. 2010;225(3):343-40
30. Sjoqvist S, Kasai Y, Shimura D, et al. Oral keratinocyte-derived exosomes regulate proliferation of fibroblasts and epithelial cells. Biochem Biophys Res Commun. 2019;514(3):706-12
31. Lo Cicero A, Delevoye C, Gilles-Marsens F, et al. Exosomes released by keratinocytes modulate melanocyte pigmentation. Nat Commun. 2015;6:7506