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Improved Dermal Regeneration Using a Combination of Dermal Substitutes and Dermal Fibroblast Optimization: A Hypothesis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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In human adults, the repair of cutaneous wounds usually leads to scar formation rather than regeneration. Dermal substitutes have been used as a regenerative template for reducing scar formation and improving the extent of dermal regeneration. However, achievement of complete regeneration is still a long way off. Dermal substitutes are characterized by unusual regenerative activity, appearing to function by acting as temporary configurational guides for cell infiltration and synthesis of new stroma. Fibroblasts are important cells with many vital functions in wound-healing processes. They are heterogeneous with distinct characteristics according to their source location, such as subcutaneous tissue, superficial-layer dermis, and deep-layer dermis. Many studies have shown that superficial dermal fibroblasts possess the potential to form dermis-like tissue. Fibroblasts in deep-layer dermis and subcutaneous tissue may play a critical role in the formation of hypertrophic scars. Fibroblast phenotype affects the newly formed dermal architecture and influences the dermal regeneration effect induced by dermal substitutes. It is hypothesized that better regeneration of the dermis can be achieved using dermal substitutes along with dermal fibroblast optimization.

MeSH Keywords: Dermis • Fibroblasts • Regenerative Medicine

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Background

The repair of cutaneous wounds is a complex biological process that occurs throughout human life. Early in gestation, cutaneous wounds can be completely repaired via regeneration. However, in human adults, the wound-repair process mostly results in a non-functioning mass of fibrous tissue. Hypertrophic scar (HTS) tissue results from the overgrowth of fibrous tissue. Considerable research has been conducted on scars and treatments, and significant progress has been made in this field [1–3]. However, pathological scars remain an enormous medical problem, without a comprehensive solution.

Since their development by Yannas and Burke in 1980 [4], dermal substitutes have been used as regeneration templates and have become an effective method for reducing scar formation and improving the quality of wound healing [5,6]. The thickness of these scaffolds is usually between 1 and 2 mm [7, 8], allowing the composite graft to act as a full-thickness graft, improving thickness and robustness. Thick dermal substitutes, such as Integra®, need 2–3 weeks to become vascularized before skin grafting can be performed; therefore, dermal substitutes are usually applied using a two-stage procedure [9]. The 2–3-week delay is necessary for scaffold angiogenesis so that sufficient oxygen and nutrients are available for secondary skin grafting. Thinner dermal substitutes with thicknesses ≤1 mm can be applied using a one-step procedure in which the dermal substitute is immediately covered by an ultra-thin autograft [10–12].

A dermal substitute is a macromolecular network synthesized as a highly porous analogue of the extracellular matrix (ECM), with a highly specific structure that degrades *in vivo* at a controlled rate. It is characterized by unusual regenerative activity, appearing to function by blocking contraction and acting as a temporary configurational guide for the synthesis of new stroma [5,6,13].

Short-term clinical and histological studies of up to 2 years show that newly formed collagen within the scaffold is histologically indistinguishable from normal dermal collagen [9]. Although an extensive body of literature exists on the experimental and clinical use of dermal substitutes to reduce scar formation and improve the quality of wound healing [6], achieving complete dermal regeneration is still a long way off.

Fibroblasts are an important part of the vital functions involved in the wound healing process, such as proliferation, migration, matrix synthesis, and tissue remodelling. Fibroblasts are a heterogeneous population of cells with distinct characteristics defined according to their source location, such as subcutaneous tissue, superficial-layer dermis, and deep-layer dermis [14,15]. The origin and phenotype of fibroblasts are important factors influencing the outcome of dermal regeneration.

In clinical cases, the wound bed of a full-thickness skin defect will usually be surgically treated by exposing the subcutaneous tissue. After application of a thin split-thickness skin autograft and dermal substitute, fibroblasts infiltrate into the dermal substitute. These fibroblasts generally originate from subcutaneous tissue, and their phenotype is relatively unfavorable to dermal regeneration [16]. In fact, this type of fibroblast is likely to result in scar formation [17]. Dermal regeneration may be optimized by controlling the fibroblast phenotype or source.

Dermal substitutes can improve dermal regeneration. During wound healing, fibroblasts from surrounding sites, including residual dermis and subcutaneous tissue, infiltrate into the dermal substitutes, and are guided by the porous scaffold to assemble an ordered ECM. These fibroblasts are heterogeneous with distinct characteristics that affect the quality of the newly formed dermal architecture.

The Hypothesis

Improved dermal regeneration can be achieved using dermal substitutes and by optimizing the phenotype of infiltrated dermal fibroblasts, specifically, by increasing the number of superficial dermal fibroblasts.

Evaluation of the Hypothesis

Fibroblasts are a heterogeneous population of cells defined according to their origin. During wound healing, the majority of fibroblasts originate from subcutaneous tissue underlying the wound edge (hypodermal fibroblasts) rather than from the dermis adjacent to the wound edge (dermal fibroblast) [18]. Unlike dermal fibroblasts, hypodermal fibroblasts lack the potential to regenerate the normal dermal architecture [19]. Fibroblasts of dermal origin have a different phenotype from those derived from subcutaneous fat or eschar tissue. Fibroblasts originating from subcutaneous fat and eschar tissue exhibit a relatively more contractile phenotype *in vitro*, with elevated myofibroblast numbers and greater contraction of collagenous matrices. Dermal fibroblasts have better characteristics for use in dermal regeneration in terms of phenotype and cell proliferation [16].

Dermal fibroblasts are heterogeneous and consist of 3 subpopulations that exhibit distinct characteristics [14]. Two of these subpopulations reside in the papillary and reticular dermis, and the third subpopulation is associated with hair follicles. The papillary and reticular fibroblasts are also known as superficial and deep dermal fibroblasts, respectively. They have distinct physical and biochemical characteristics, including size, packing density, rate of proliferation, growth kinetics,

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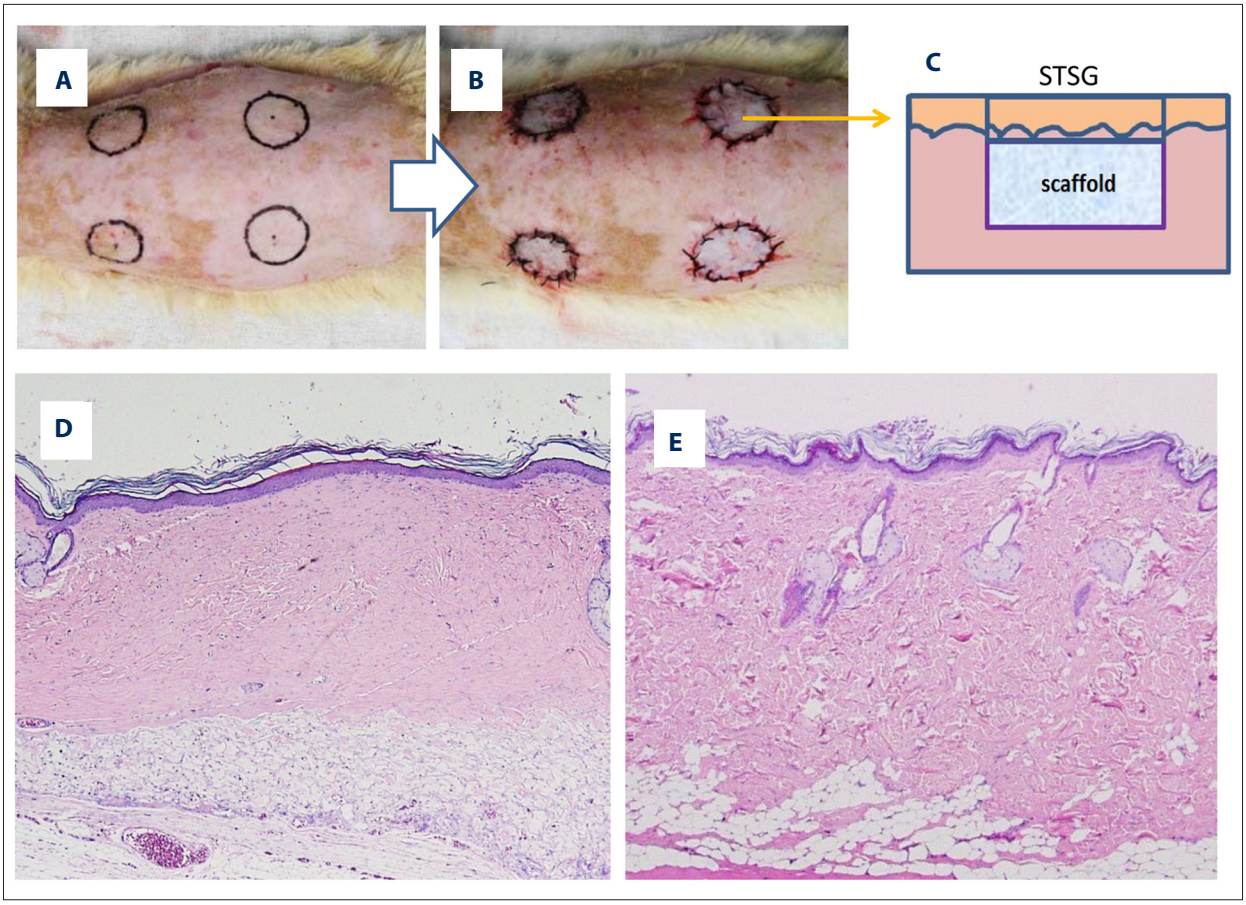

Figure 1. One-stage procedure to transplant collagen-based scaffold and split-thickness skin graft (STSG) for skin defects in rats, and the results of HE staining. (**A**) Recipient sites where the full-thickness skin defects will be created. (**B**) Skin defects were covered by the combined transplantation of collagen-based scaffold and STSG. (**C**) Cross-sectioned structure after one-stage procedure. (**D**) Image of HE staining 1 week after one-stage transplantation. The thin layer of dermis contained by STSG is an important source of dermal fibroblasts, contributing to the induced dermal regeneration from the upper side of the collagen-based scaffold. There is little tissue formation in the lower side of the scaffold. (**E**) Histological structure of normal skin.

and production of collagenase and type I and III procollagen [14,15,20,21]. Deep dermal fibroblasts resemble fibroblasts from HTS tissue [22]. Compared to superficial dermal fibroblasts, they produce more transforming growth factor-β1 (TGF-β1) [22], a pro-fibrotic cytokine that plays a critical role in scar formation and is over-expressed in HTS fibroblasts [23,24]. Recently, Varkey et al. indicated that the use of a specific subpopulation of dermal fibroblasts such as superficial fibroblasts, rather than heterogeneous fibroblasts, may improve wound healing and minimize post-burn HTS tissue [25]. These findings suggest that superficial dermal fibroblasts may possess dermal regeneration potential similar to that of normal dermal fibroblasts, while deep dermal fibroblasts may play a key role in the formation of HTS tissue.

Dermal substitutes are effective in reducing scar formation and improving the quality of wound healing. Histological studies of dermal reconstruction sites with two-stage grafting of Integra® dermal regeneration templates have shown a collagen pattern similar to that of normal skin after 1 year [26] and a normal organized pattern of mature collagen after 2 years [9,27,28]. Typically, parallel patterns of collagen fibres are seen in the normal dermis, a more random pattern with little organization is observed in immature scars, while a nodular arrangement is usually observed in hypertrophic scars [29,30]. A study of Integra® reconstructions after 35 months suggested that collagen arrangement is not always normal; the majority of the specimens had a random and/or parallel pattern of collagen fibres, with 7 specimens (30.4%) showing a nodular pattern [31]. The nodular pattern was located in the middle and/or lower layer of the reticular dermis, not in the outer reticular dermis, which may be because the more superficial dermal layers originated from the host autografts above the dermal substitutes [31]. In the two-stage grafting procedure, dermal substitutes are grafted to the wounds first, and need 2–3 weeks to become vascularized before autografts can be performed. During this time, hypodermal

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fibroblasts (from subcutaneous tissue) infiltrate upwards into the dermal substitutes and become the main source of fibroblasts in the scaffold, particularly in the middle and lower layers of the scaffold. After the thin autografts have been transplanted onto the dermal substitutes, dermal fibroblasts from the autografts may start migrating downwards into the dermal substitutes, mainly within the upper layer of the scaffold. The different sources and distributions of fibroblasts may explain the asymmetric collagen arrangement pattern observed throughout dermal reconstruction sites.

In a previous study, we used a single-layer collagen/chitosan porous scaffold as a dermal substitute to treat full-thickness wounds using a one-step grafting procedure in a rat model. In this model, full-thickness incisional wounds (including the panniculus carnosus) with a diameter of 2 cm were made by scalpel excision on the back of the rat. The thin split-thickness autografts (0.38±0.1 mm) were made from the excised skin by removing the panniculus carnosus and greater part of the reticular dermis with a scalpel blade. The dermal substitutes were grafted to the wounds and then covered by the autografts. After 1 week, the fibroblasts from the autografts (mainly superficial dermis) and subcutaneous tissue started to infiltrate into the dermal substitutes and produce collagen (Figure 1). In some harvesting samples, it was found that the arrangement pattern of the newly formed collagen in the upper scaffold layer, which was mainly produced by superficial dermal fibroblasts, was almost the same as the collagen pattern in the normal dermis, while the collagen arrangement in the lower scaffold layer, mainly produced by hypodermal fibroblasts, was significantly distinct from the normal arrangement (Figure 1). The different sources and distributions of fibroblasts may explain these different collagen arrangement patterns observed throughout the dermal reconstruction sites.

Various bioactive factors such as proteins or peptides that exist *in vivo* have significant biological activity involved in regulating the cell growth and development and play an important role in tissue repair and regeneration [32]. In addition to suitable biomaterial scaffolds and optimized dermal fibroblasts, the roles of some related bioactive factors should also be further explored for improved dermal regeneration.

Conclusions

In both the one-step and two-step grafting procedures, abundant fibroblasts from subcutaneous tissue infiltrate into the dermal regeneration templates. More scar formation possibly occurred in the two-step grafting procedures compared to the one-step method. This may be due to increased hypodermis fibroblast infiltration into the dermal regeneration template during the 2–3-week delay of the two-step procedure, before autograft transplantation. Dermal regeneration could potentially be improved by promoting increased superficial dermal fibroblast generation, or by preventing hypodermis fibroblasts from migrating into the dermal substitutes. Further studies are needed to determine if higher-quality dermal regeneration can be achieved with a combination of dermal substitutes and dermal fibroblast optimization.

Conflicts of interest

None.

References:

1. Gurtner GC, Werner S, Barrandon Y et al: Wound repair and regeneration. Nature, 2008; 453(7193): 314–21
2. Liu W, Wu X, Gao Z: New potential antiscarring approaches. Wound Repair Regen, 2011; 19 (Suppl.1): s22–31
3. Tziotzios C, Profyris C, Sterling J: Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics Part II. Strategies to reduce scar formation after dermatologic procedures. J Am Acad Dermatol, 2012; 66(1): 13–24; quiz 25–26
4. Yannas IV, Burke JF: Design of an artificial skin. I. Basic design principles. J Biomed Mater Res, 1980; 14(1): 65–81
5. Yannas IV, Orgill DP, Burke JF: Template for skin regeneration. Plast Reconstr Surg, 2011; 127(Suppl. 1): 60S–70S.
6. Yannas IV, Tzeranis DS, So PTC: Regeneration mechanism for skin and peripheral nerves clarified at the organ and molecular scales. Curr Opin Biomed Eng, 2018; 6: 1–7
7. van der Veen VC, van der Wal MB, van Leeuwen MC et al: Biological background of dermal substitutes. Burns, 2010; 36(3): 305–21
8. Teot L, Otman S, Trial C et al: The use of noncellularized artificial dermis in the prevention of scar contracture and hypertrophy. Wound Repair Regen, 2011; 19(Suppl. 1): s49–58
9. Moiemen NS, Vlachou E, Staiano JJ et al: Reconstructive surgery with Integra dermal regeneration template: Histologic study, clinical evaluation, and current practice. Plast Reconstr Surg, 2006; 117(7 Suppl.): 160S–74S
10. Callcut RA, Schurr MJ, Sloan M et al: Clinical experience with Alloderm: A one-staged composite dermal/epidermal replacement utilizing processed cadaver dermis and thin autografts. Burns, 2006; 32(5): 583–88
11. Ryssel H, Radu CA, Germann G et al: Single-stage Matriderm(R) and skin grafting as an alternative reconstruction in high-voltage injuries. Int Wound J, 2010; 7(5): 385–92
12. Koenen W, Felcht M, Vockenroth K et al: One-stage reconstruction of deep facial defects with a single layer dermal regeneration template. J Eur Acad Dermatol Venereol, 2011; 25(7): 788–93
13. Yannas IV: Facts and theories of induced organ regeneration. Adv Biochem Eng Biotechnol, 2005; 93: 1–38
14. Sorrell JM, Caplan AI: Fibroblast heterogeneity: More than skin deep. J Cell Sci, 2004; 117 (Pt 5): 667–75
15. Ali-Bahar M, Bauer B, Tredget EE et al: Dermal fibroblasts from different layers of human skin are heterogeneous in expression of collagenase and types I and III procollagen mRNA. Wound Repair Regen, 2004; 12(2): 175–82
16. van den Bogaerdt AJ, van Zuijlen PP, van Galen M et al: The suitability of cells from different tissues for use in tissue-engineered skin substitutes. Arch Dermatol Res, 2002; 294(3): 135–42
17. Middelkoop E: Fibroblast phenotypes and their relevance for wound healing. Int J Low Extrem Wounds, 2005; 4(1): 9–11
18. Gross J, Farinelli W, Sadow P et al: On the mechanism of skin wound "contraction": A granulation tissue "knockout" with a normal phenotype. Proc Natl Acad Sci USA, 1995; 92(13): 5982–86

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19. Gross J: Getting to mammalian wound repair and amphibian limb regeneration: A mechanistic link in the early events. Wound Repair Regen, 1996; 4(2): 190–202
20. Schafer IA, Pandy M, Ferguson R et al: Comparative observation of fibroblasts derived from the papillary and reticular dermis of infants and adults: Growth kinetics, packing density at confluence and surface morphology. Mech Ageing Dev, 1985; 31(3): 275–93
21. Azzarone B, Macieira-Coelho A: Heterogeneity of the kinetics of proliferation within human skin fibroblastic cell populations. J Cell Sci, 1982; 57: 177–87
22. Wang J, Dodd C, Shankowsky HA et al: Deep dermal fibroblasts contribute to hypertrophic scarring. Lab Invest, 2008; 88(12): 1278–90
23. Wang R, Ghahary A, Shen Q et al: Hypertrophic scar tissues and fibroblasts produce more transforming growth factor-beta1 mRNA and protein than normal skin and cells. Wound Repair Regen, 2000; 8(2): 128–37
24. Tredget EE: Pathophysiology and treatment of fibroproliferative disorders following thermal injury. Ann NY Acad Sci, 1999; 888: 165–82
25. Varkey M, Ding J, Tredget EE: Differential collagen-glycosaminoglycan matrix remodeling by superficial and deep dermal fibroblasts: Potential therapeutic targets for hypertrophic scar. Biomaterials, 2011; 32(30): 7581–91
26. Molnar JA, DeFranzo AJ, Hadaegh A et al: Acceleration of Integra incorporation in complex tissue defects with subatmospheric pressure. Plast Reconstr Surg, 2004; 113(5): 1339–46
27. Palao R, Gomez P, Huguet P: Burned breast reconstructive surgery with Integra dermal regeneration template. Br J Plast Surg, 2003; 56(3): 252–59
28. Jeng JC, Fidler PE, Sokolich JC et al: Seven years' experience with Integra as a reconstructive tool. J Burn Care Res, 2007; 28(1): 120–26
29. Scott P, Ghahary A, Wong J et al: Molecular and cellular basis of hypertrophic scarring, In: Herndon DN. Total Burn Care, 3rd ed. Philadelphia: Saunders; 2007: chap. 47: 596–607
30. Linaes H, Kischer C, Dobrkovsky M et al: The histotypic organization of collagen of the hypertrophic scar in humans. J Invest Dermatol. 1972;59: 323–31
31. Moiemen N, Yarrow J, Hodgson E et al: Long-term clinical and histological analysis of Integra dermal regeneration template. Plast Reconstr Surg, 2011; 127(3): 1149–54
32. Zhou H, You C, Wang X et al: The progress and challenges for dermal regeneration in tissue engineering. J Biomed Mater Res A, 2017; 105(4): 1208–18

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