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Therapeutic mechanisms of fibroblast cells in the skin conditions; trends in clinical applications- A Review

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ABSTRACT

Autologous cells in cell therapy is useful in accelerating the Curing course of lesions through reducing time required for early synthesis of new derm by the host cells to invade the lesion tissue. Collagen and hyaluronic acid matrices are also effective in accelerating the lesion curing process. They increase migration of host cells and blood vessels into the tissue and allow rapid replacement by host tissue. The cell-scaffold complex is grafted onto the defect area and covered with a polyurethane foam dressing. Practical challenges including scale up, formulation, storage, transport and delivery mechanisms are the main challenges to development and clinical translation of preclinical observations. However, recent FDA consent of fibroblast treatment applications for nasolabial folds and the expanding pipeline of other fibroblast therapies in clinical development show that cellular therapy is an expanding field.

Keywords: Therapeutic, fibroblast, derm condition, transplantation, Curing, Ulcer, Derm Rejuvenation

1. INTRODUCTION

Autologous cells in cell therapy is useful in accelerating the Curing process of lesions through reducing time required for early synthesis of new derm by the host cells to invade the lesion tissue (Tredget et al., 1997). Previous studies reported that, cell-only treatment leads to an increase in the speed of lesion curing, but does not show a beneficial effect on lesion contraction. Furthermore, cells as a tissue-engineered dermis are generally used with artificial dermis for optimizing the Curing process of lesions (Molnar et al., 2004). Moreover, cell-seeded artificial dermis graft are used for minimizing lesion contraction to treat defects in the derm and soft tissue without delaying lesion Curing. Artificial dermis are also used alone without cells for lesion coverage. However, without cells, lesions treated with artificial dermis often show delayed Curing and heal with altered patterns of scarring, resulting in scar contractures. Allogeneic cells in cell therapy has considerable value in lesion Curing (Asadbegi et al., Rosenberg et al., 2011). Initially the effect of allogeneic cells in lesion Curing was not known. Allogeneic cells are soon replaced by host cells and do not cover and attach the lesion. Due to the role of allogeneic cells in releasing growth factors, extracellular matrices and basement membrane components, these cells promote migration and proliferation of host cells from the lesion beds and edges. Eventually, allogeneic cells have key role the role in the lesion Curing process by accelerating epithelialization from the lesion edges and promote granulation formation from lesion beds. Course of cell therapy is different depending on cells. At present, keratinocytes, fibroblasts, and adipose-derived stromal vascular fraction (SVF) cells are actively used in the clinical setting (Gimble et al., 2007). Approximately, 1 cm^2 of dermal biopsy is sampled from a patient and sent to a commercial laboratory for culturing keratinocytes and fibroblasts in the clinical use. Briefly, cells are isolated using enzyme and propagated. SVF cells can be easily taken obtained from adipose tissue. SVF cells are also easily collected in large quantities without the need to cell culture. Briefly, abdominal adipose tissues are harvested from a patient by liposuction. The obtained samples are rinsed and then incubated in cell culture medium containing collagenase. The top layer of fluid is removed and then the residual liquid is centrifuged. NH_4Cl is used for treating the resulting pellet to lyse red blood cells. After washing the remaining cells, then a 100 μm nylon mesh is used for filtering them (Chow et al., 2005). The viability and density of cell is evaluated. According to the authors' experience, 6.3×104 to 2.2×105 viable SVF cells can be separated from aspirated adipose tissue (per mL) (Thirumala et al., 2005). The obtained cells are then seeded on scaffolds including artificial dermis. In order to successfully application of artificial dermis in regenerative medicine, they should have optimal physical and biochemical parameters. Hyaluronic acid sheet (HA) and collagen (Col) sponge are widely used in clinical medicine as main biomedical compounds (Lee et al., 2001). These compounds have a key role in accelerating tissue granulation. HA and Col improve migration of host cells and blood vessels into the structure, and also allow rapid replacement by host tissue. The cell-scaffold complex is grafted onto the defect area and covered with a polyurethane foam dressing (You and Han, 2014).

Fibroblast cells transplantation methods: Indication can determine administration route, delivery mode, and the formulation of autologous dermal fibroblasts. Generally, the most common administration route is injection route. In direct injection route fibroblasts are injected into the dermis of the derm using a needle (27 or 30 gauge). The

injection of 1.5 million autologous fibroblasts in 0.1 milliliter per linear centimeter into dermis at 3–6 weeks intervals during three sessions is suggested for the treatment of Laviv. Direct application of fibroblasts suspended in matrix is another delivery route that is used for the lesion or ulcer sites. A mixture of cultured keratinocytes and fibroblasts that are suspended in fibrin glue is used to treat diabetic foot lesions before the area is covered with a dressing. Another method is grafting a composite bandage with cells incorporated in a matrix. Several approaches have been developed for incorporating cells in a matrix. Fibroblasts are grafted either as a dermal equivalent (matrix and fibroblast) or bi-layered dermal substitutes (dermal equivalent overlaid with autologous keratinocytes) at the lesion site. Boyce and colleagues effectively established a cultured derm replaced with autologous fibroblasts and keratinocytes that was grafted to the burn area. In this method collagen-glycosaminoglycan substrates are used as the matrix. Moreover, other matrices such as a hyaluronic acid scaffold in an autologous bioengineered dermal equivalent have been used for repairing a lesion in a nine-year-old girl with after removal of a congenital giant nevus (Kuznetsov et al., 1997).

Selecting a suitable method in lesion curing according to the lesion conditions is necessary to success in the lesion curing. Selecting an appropriate method could be effective in reducing the risk of complications, increasing the speed of lesion curing, and minimizing scar formation after the lesion has fully healed. Various strategies have been applied for lesion curing such as primary intention, secondary intention, tertiary intention, dermal grafts, and flaps. In primary intention, epithelialization process heal lesions. In the epidermis and dermis lesions without total penetration of the dermis the primary intention Curing can be used. When lesion borders come close with sutures (stitches), staples, or adhesive tape (approximated lesion), the lesion also heals by primary intention. The main purpose could be utilized for well-repaired lacerations and surgical lesions. In spite of the effect of primary intention in minimizing scarring, but its role is limited because of the size and shape of the defect. Primary intention is appropriate only for small defects with elliptical shapes. Granulation formation or fibrosis, contraction, and epithelialization are effective in secondary intention for Curing lesions. Lesion care should be carried out for preventing infection and improving the formation of granulation tissues. The secondary intention is used for unrepaired and thickness open lesions. Curing by secondary intention usually leaves significantly obvious and negative scars. Darker derm is more prone to hypertrophic scarring or keloid formation (Friedenstein et al., 1966).
In tertiary intention (delayed primary closure or secondary suture), after several days lesions are primarily left open and closed (typically 4 or 5 days) by the utilization of tissue grafts (derm grafts or flaps). During the first four to five days, the lesion is cleaned, debrided, and observed. This method can be useful for Curing of contaminated lesions. On the fourth and fifth day, phagocytosis happens on contaminated tissue and proliferative phase begins. In this step, the lesion is closed surgically. Derm grafting is one of the graft surgeries in which the derm is transferred. In derm graft without affecting blood supply relies on the growth of new blood vessels from the lesion bed. Derm grafts are mostly used in the treatment of trauma, burn, and infection lesions as well as removal of derm cancer (Ghobadi et al., 2013, Berman et al., 2008). Derm grafts uses for achieving two purposes: first, reducing the course of required treatment (time of hospitalization), and improve the function and appearance of would area which receives the derm graft. Derm grafting is generally straightforward with a relatively low risk of complications. There are two major concerns in derm grafting including poor color matching in the recipient would and complications in donor site such as pain, discomfort, and hypertrophic scarring. Most pigment mismatches occur in patients with darker derm, like Asian peoples. Different types of Flaps such as local, distant, island, and free flaps can be also applied in treating derm and soft tissue injuries. Local flap strategy is effective in treating thickness derm and soft tissue defect. Yet, in special cases, the use of a local flap is not practicable, principally due to restrictions of size and rotation arc. In these cases, an island or a free flap could be applied. However, in this method, microsurgery facilities and techniques are required. Moreover, it needs much time, and is attributed to donor site morbidity. Insomuch as the involved surgical courses, it become clear that the derm grafting or flap coverage are considered as difficult saturation for the patients, especially in ageing persons with higher chances of dermal neoplasia (Gilchrest, 1982).

Common conditions now treated with fibroblast cells: Cell therapy may be effective in treatment of various types of derm defects including trauma, burn lesions, scar excision, leg ulcer, donor sites of split-thickness derm, and excision of derm tumor. Cell therapy can be suitable especially in elderly patients in whom flap coverage or derm grafting is a burden for them or patients who do not agree to undergo major surgery. In addition, this method could be applied as a suitable and appropriate for the coverage of defects involving the face and upper extremities. In this method the safest and least invasive strategy should be selected with a goal of achieving optimal functional and cosmetic outcomes. Cell therapy may be also useful for treating chronic lesions such as the lesions caused by diabetes mellitus. Due to the involvement of multiple factors lesion Curing cannot often be effective for chronic lesions. One of the main contributing factors for the non- or delayed Curing lesion is diminished role of the key cells for the lesion curing together with fibroblasts and keratinocytes. In patients with chronic lesion these cells have markedly declined mitotic activity compared to the normal healthy controls. Besides, there is an deficient degree of synthesis of extracellular matrices and growth factors that are necessary for lesion Curing. In contrast, secretion of matrix

metalloproteinase becomes activated and is effective in destroying tissue proteins. Cell migration degree is also decreased. So, the lesion remains open for long periods of time without Curing, which could cause infections eventually. Therefore, it is mandatory to activate the cells within treatment. By transplanting cells with an excellent profile of lesion curing ability to the lesion bed (the term may sound grand, but this method actually denotes the addition or spraying the cells to the lesion bed), attempts are made to convert the lesion bed into the environment where maximum lesion curing can be achieved (Wynn, 2007).

Lesion Curing: Lesion Curing involves four phases: hemostasis, inflammation, proliferation and remodeling. Fibroblasts have key role during the proliferative phase of lesion Curing, which begins a few days after lesioning, when fibroblasts begin to proliferate and migrate into the lesion bed to produce extracellular matrix (ECM) proteins. ECM proteins act as a scaffold for inflammatory cell migration and granulation tissue generation. The temporary substrate upon is provided by granulation tissue which re-epithelialization takes place by keratinocytes. During proliferation, fibroblasts also have potential to further discriminate into myofibroblasts that produce lesion contracture. Then, during the remodeling phase of lesion Curing, myofibroblasts undergo apoptosis cause a decrease in cellular density. While the remaining dermal fibroblasts begin to produce type I collagen. The plasticity feature of the fibroblast makes it as an appropriate candidate for cellular based therapies in lesion Curing. Based on this feature it can transition from producing ECM to improve lesion contracture to the synthesis of Type 1 collagen. This future of fibroblasts during lesion Curing indicates that the regulation of fibroblast phenotype is complex. For example, the differentiation of fibroblasts into myofibroblasts is regulated by members of the TGF-β family that are released early in lesioning by platelets. Platelet derived growth factor-CC (PDGF-CC) stimulates myofibroblast differentiation, which it produced by M2 macrophages and by Interleukin-22 (IL-22) produced by adaptive and innate lymphoid cells. , fibroblasts release a variety of growth factors, ECM and proteases that improve or regulate lesion Curing. For example, soluble factors improve lesion Curing such as fibroblast growth factor 2 (FGF2, also known as basic FGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (Meyer-Lueckel and Paris, 2008). FGF2 promotes lesion Curing and adjusts ECM production and degradation, while VEGF improves angiogenesis. Dermal fibroblasts in a fibrin matrix exposed to thrombospondin-1 generate increased amounts of VEGF that stimulates endothelial cell tubulogenesis. Hepatocyte growth factor (HGF) has various effects on lesion Curing, such as stimulation of angiogenesis, regulation of matrix deposition and degradation, and stimulation of keratinocyte migration and proliferation. The production of HGF includes intracellular signaling through molecules such as c-JUN and integrin-linked kinase (ILK), demonstrating that HGF production is secondary to growth factor stimulation and cell-matrix interactions. This complex web of interactions in the lesion bed relies heavily on the role of fibroblasts as mediators of lesion Curing. Although, pathologic states are not uncommon, lesion Curing is a well synchronized event generally. For example, fibroblasts from hypertrophic scars release increased amounts of TGF-β1. By contrast, fetal fibroblasts in relation to scarless Curing produce TGF-β3. The differential expression of TGF-β1 vs. TGF-β3 in hypertrophic vs. regular scars represents another target for cellular based fibroblast therapies. Along the lines of differential expression of proteins by fibroblasts, specific proteases produced by fibroblasts, such as matrix metalloproteinase 2 (MMP-2), are involved during the remodeling phase of lesion curing. Fibroblast expression of MMP-9 and MMP-13 in the late remodeling phase is believed to promote scar-free Curing. This various expression of favorable proteases represents another advantage of fibroblast therapies (Pappone et al., 2000).

Burn Ulcer: In cutaneous burns there are following goals: restoring barrier function, preventing infection, minimizing scarring, and preventing disfigurement. Lesion dressings and synthetic substitutes provide a temporary barrier to minimize infection. However, the tissues of lesion cannot fully replaced with the normal derm barrier, and burns often cause scarring leading to psychological distress. Derm transplants from split- or full-thickness grafts are well-established techniques for restoring lost derm. However, donor derm availability and the size of the area involved have led to the development of techniques to expand cells in vitro for generation of engineered full thickness tissue substitutes. Caruso et al. reported the restriction of epithelial autografts in the treatment of burn lesions and developed a fibroblast-keratinocyte composite from a burn victim's normal derm. This composite could be used to cover third degree burns. In another study, a cultured autologous fibroblast and keratinocytes suspension were used for a patient with 19 years-old suffering from a third degree burn involving 76% of the body surface area was treated after stabilizing the burn area with a commercial dermis equivalent. It was shown that in the patient lesion closure improved with the development of functional derm over a one year follow up. Boyce et al. developed the bi-layered derm substitute through autologous fibroblasts and keratinocytes, which lead to faster lesion closure and decreased the requirements for donor derm harvesting for additional grafting in full-thickness burns. Another study on the use of autologous bioengineered derm in two patients with extensive burns showed that it can cause the restoration of bi-layered derm that was maintained after two years of follow up. The probability of using synthetic derm from autologous fibroblasts and keratinocytes for an indication other than burns was demonstrated by Llames et al. They treated patients with big lesions from the removal of giant nevi with bio-engineered derm. In these patients, the bio-engineered derm resulted in permanent re-epithelialization in all cases without blistering or derm retractions. These

mentioned studies indicate that autologous fibroblast therapies alone or as part of bio-engineered derm equivalents can be useful in burns and other large epithelial defects (Curling, 1842).

Scars treatment: Here are many filler agents for augmentation of static wrinkles and atrophic scars. These products are synthetic, biosynthetic or derived from the cadaver, animal and human sources. However, many side effects were reported for the application of fillers is linked to. Collagen, a major constituent of extra cellular matrix, derived from bovine source has been widely used as filler. It is reported that 6% of patients suffer hypersensitivity responses to bovine collagen, which can be clear as granulomatous inflammation, necrosis, or abscess formation. Rare systemic complications have also been reported. The ideal filler would be an autologous, injectable material that provides long-term results, involves minimal surgery and tissue removal for initial tissue harvest, and has unlimited yield without the need for additional tissue harvest. The possibility of allergy and short duration of their effect are mentioned as some disadvantages of synthetic or biosynthetic fillers. Therefore, recent studies have focused on trying to use autologous approaches like injecting the autologous collagen and autologous cultured fibroblasts transplantation. The latter course satisfies the criteria for suitable filler. Therefore, in the current study, 20 patients were presented whose facial wrinkles and lines were cured through injecting of autologous cultured fibroblasts with 6 month follow ups. Results show out of 20 individuals participating in this study, 17 patients (85%) were female and 3 patients (15%) were male with the minimum and maximum age of 40 and 62, respectively. All the 20 individuals received autologous fibroblast in the nasolabial fold. The mean improvement for autologous fibroblast transplantation at 6 month follow up was 41 ± 13% with minimum of 20% and maximum of 60%. We cannot find a study on the side effects or allergic reaction, except small reddening around the treatment area, which disappeared within 24 h of cell transplantation. It is stated that autologous transplantation of derm fibroblast may be a safe product for sustained improvements in contour defects without surgery and virtually zero risk of hypersensitivity reactions. Histological analysis in previous studies have demonstrated that fibroblast injections increase the formation of collagens In line with increasing thickness and density of dermal collagen without induction of an inflammatory response. The efficacy of fibroblast injection for treatment of nasolabial fold was evaluated. The obtained results indicate that fibroblast injection is useful for improvement of nasolabial fold and possibly for scars and wrinkles. Although the degree of improvement is variable between individuals, no significant side effect was observed in the present patients. To our best knowledge, it is the first study on the application of autologous fibroblast in Asia. In Weiss et al. study, the efficiency and side-effects of autologous living fibroblast injections compared to placebo in a randomized phase III trial, were investigated in the treatment of various facial contour defects. The results showed that living fibroblasts are more effective than placebo in dermal deformities and acne scars. At 9- and 12-month follow-ups, live fibroblast treated patients continued to show obtained advantages from treatment with response rates of 75.0% and 81.6%, respectively. Similar to the present study no serious treatment-related adverse events were reported. In another study, the efficiency of intradermal injections of autologous fibroblasts for the treating facial rhytids and dermal depressions was studied on ten adults aged between24 to 69 years, each of who demonstrated a prominent rhytid or depressed facial scar. Nine of 10 patients noted a 60% to 100% improvement with the treatment; clinicians made similar observations. Size reduction of 10% up to 85% of the study site was demonstrated by optical profilometry for every patient. According to the results of microscopic examination, there was evidence of increased thickness and density of dermal-layer collagen. The different improvement rate between patients can be due to the age variation, proper injection of cell in the right derm level, differences in the degree of nasolabial fold, number of injection and duration of assessment. Hyaluronic acid belongs to a family of macromolecules known as glycosaminoglycans. There are these molecules, consist of chains of repeating disaccharide units, in the native extracellular matrix of connective tissues. The hydrophilic part of glycosaminoglycans adsorbs water into the extracellular matrix conferring a degree of turgor to the tissue. After transplantation, the hyaluronic acid derivatives undergo local degradation. The metabolic products are then further catabolized by the liver into carbon dioxide and water. Commercially available injectable are gels of hyaluronic acid derivatives that relates closely to prolong their degradation in vivo. Two large groups of patients have confirmed the duration of the augmentation achieved with hyaluronic acid derivatives to be nine months. However, recent studies in relation to fibroblast injection, have shown objectively and subjectively measured improvements in facial contour defects lasting at least 12 to 48 months. Despite the injection of hyaluronic acid derivatives is cheaper than first glance, but the need of repeated injection for hyaluronic acid derivatives makes the cost of both treatment methods same. In contrast, there is still a need to perform a small punch biopsy for obtaining tissue needed to perform culture. The results of immnocytochemical staining and flow cytometery showed that the recovered cells have fibroblast identity and over 91.84% of cells were antivimentin positive. The trypan blue staining indicates that over 95% of cells were observable before transplantation (Chermol, 1985).

Derm Rejuvenation: Facial contour deformities such as acne scars and nasolabial fold wrinkles can impair derm function and lead to psychological discomfort, which eventually can cause decreased quality of life. Dermal fillers are one of the main therapeutic approaches for soft tissue augmentation, but complications such as bruising, unwanted

swelling, derm dyspigmentation, derm infections, and subcutaneous nodules can occur. Autologous fibroblasts as natural filler is available for generating matrix proteins like collagen. West and Alster in their study reported that injecting autologous dermal fibroblasts can change intradermal injection of silicone and other fillers in the treatment of facial wrinkles. This new merthod has the potential to avoid hypersensitivity reactions associated with dermal fillers and result in a sustained therapeutic effect. Watson et al. reported that nine out of ten patients with facial rhytides had some improvement by injecting autologous fibroblasts in the target site, and increases in collagen were noted in the dermis. In previous studies on autologous fibroblast therapy, a significant improvement was observed in facial contour when 20 million/mL autologous cells were injected into the dermis in three doses spaced one month apart. Moreover, acne scars also improved without adverse events when studied. Given the initial successes, multicenter placebo control trials were expanded to develop an aesthetic indication in treating nasolabial fold wrinkles and facial contour deformities with autologous fibroblast. These research led to the confirmation in 2011 of LAVIV™, an autologous cellular therapy product manufactured by Fibrocell Science, Inc. (Exton, PA, USA) for treating nasolabial wrinkles. Autologous fibroblasts can be used to acne scars and peri-orbital derm flaccidity (Teimourian and Adham, 2000).

2. CONCLUSION

As reviewed in all investigated tissue and organ systems fibroblasts represent a functional diverse population of cells. Differences in phenotypic are characterized by various methods: extracellular matrix production and organization as well as the secretion of and response to growth factors. The functional diversity of various fibroblast subtypes have to be considered when aspects of tissue homeostasis, especially in regard to stromal or mesenchymal-epithelial interactions and crosstalk are discussed. All investigations into phenotypic characteristics of stromal fibroblasts indicated that these cells represent a dynamic and diverse population of functional cell types. A specific nomenclature should reflect this fact and greater care should be taken when defining the population of fibroblasts used in experimental studies. The terminology fibrocyte should be reserved for the postmitotic subtype of fibroblasts as the term ‘cyte’ implicates a non-proliferative cell population. Based on autologous dermal fibroblast cellular therapy has become a new topic in regeneration medicine holds enormous promise to the field of. It offers a safe, immunologically acceptable and simple alternative for tissue regeneration applications (Rodemann and Rennekampff, 2011).

Basic studies on the mechanisms of regional phenotype determination using fibroblasts can be useful for improving reprogramming options in cellular therapy applications. Practical challenges including scale up, formulation, storage, transport and delivery mechanisms are the main challenges to development and clinical translation of preclinical observations. However, recent FDA approval of fibroblast healing applications for nasolabial folds and the growing channel of other fibroblast remedies in clinical development appearance that cellular healing is a growing field.

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