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CASE REPORT

Acellular Fish Skin for Deep Dermal Traumatic Wounds Management; Introducing a Novel Dressing

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Abstract: The optimal therapy for deep wounds is based on the early debridement of necrotic tissue followed by wound coverage to avoid a systemic inflammatory response and optimize scar-free healing. The outcomes are affected by available resources and underlying patient factors, which cause challenges in wound care and suboptimal outcomes. Here we report a patient with deep dermal injury wounds, who was treated with platelet-rich fibrin (PRF) gel, plasma rich in growth factor (PRGF) gel, and acellular fish skin. Patient's outcomes regarding healing and scar quality were collected objectively and subjectively for one year after the injury. Wounds treated with acellular fish skin demonstrated accelerated wound healing, a significantly higher water-storage capacity, and better pain relief. Furthermore, improved functional and cosmetic outcomes, such as elasticity, skin thickness, and pigmentation, were demonstrated. It seems that, the PRGF gel and PRF in combination with acellular fish skin grafts resulted in the faster healing of wounds and better functional and aesthetic outcomes than split-thickness skin grafts treatment.

Keywords: Wound healing; platelet-rich fibrin; Transplantation, heterologous

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1. Introduction

Over the past 50 years, advances in wound care have led to a massive improvement in clinical care and a subsequent decrease in patient mortality (1). The systemic inflammatory response to wounds reduces muscle mass and impairs wound healing (2, 3). In the wound environment, an adverse reaction leads to either the rapid degradation or reabsorption of the matrix or the encapsulation of the matrix by the host, with both outcomes resulting in little contribution to healthy tissue generation. Thus, the ideal response to an advanced therapy is one that does not elicit an adverse reaction but rather allows for repopulation of the tissue matrix with cells and vasculature, leading to the generation of new tissue over time (4, 5). The studies have shown that matrices, as compatible biological products, constitute an advanced wound therapy for wound healing (6, 7). Matrices contain the 3-dimensional architecture and non-denatured biochemical components of the collagen fiber matrix found in fetal dermis.

This acellular matrix provides the structure and function of the native extracellular matrix, it replaces and provides the ideal extracellular environment for tissue generation. It has been demonstrated that the implanted collagen matrix goes through a biological progression initiated by a transient in-

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filtrate of inflammatory cells followed by progenitor cell recruitment and vascular development (8).

In these cases, autologous skin grafts are limited in availability; allogenic and xenogeneic skin substitutes have gained traction and are commonly used for temporary coverage until donor sites can be re-harvested for grafting or healing through secondary intention (9-11).

Skin substitutes from pigskin or human cadaver skin carry the risk of autoimmune responses and infections, and additional challenges include cultural and religious barriers, as well as the disruption of the skin's architecture (12). A novel skin substitute from acellular fish skin graft (6) has gained attention for the treatment of burn patients. The gentle processing of Grass Carp skin allows it to maintain its nutrients, providing potential anti-inflammatory and antimicrobial benefits. Fish skin has not been linked to autoimmune reactions (13) and does not have any cultural or religious barriers to use. Its porous microstructure supports dermal cell migration, proliferation, and ingrowth (6, 13).

Here we report a patient with deep dermal injury wounds, who was treated with platelet-rich fibrin (PRF) gel, plasma rich in growth factor (PRGF) gel, and acellular fish skin. Patient's outcomes regarding healing and scar quality were collected objectively and subjectively for one year after the injury.

Figure 1: Skin defect of distal anteromedial of tibia.

2. Case presentation and procedure

An Iranian 8-year-old girl with traumatic wounds receiving treatment in Taleghani Hospital, Tehran, Iran, was evaluated. She was damaged by car accident with direct trauma and had deep-thickness wounds on anteromedial surface of distal tibia (figure 1). Vital signs of patient were stable and her erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were 70 mm/hour and 50 mg/dL, respectively. Hygrine level of the case was reported as sufficient. Size of wound was measured as 6 × 4 cm. The wound was debrided and the patient received the tetanus prophylaxis. The patient had no comorbidities, such as diabetes, malnutrition, vascular disease, or immunodeficiency.

The patient was treated with fish skin graft (0.2 mm, meshed 1:1.5) and PRGF.

Acellular Fish Skin (AFS) as a scaffold was designed in the previous study (6).

AFS was subjected to in vitro (6) and in vivo evaluations and finally (13) received the permission for clinical evaluation from the Ethics Committee of Shahid Beheshti University of Medical Sciences (Ethics code: IR.SBMU.REC.1398.059 and Clinical Trials code: IRCT20190826044620N1). In brief, Fresh Grass Carp skin (Mazandaran Fisheries Department, Tonekabon, Iran) was prepared, cleaned, and then plunged in hypertonic solution for a significant time. The solution contained 0.5M sodium chloride and 25–50mM tris (hydroxyl methyl) aminomethane and 10mM ethylene di-amine tetra-acetic acid (EDTA). The skin was exposed to Triton X100 (0.5% w/v) for 24hours, then washed with PBS and cold distilled water after exposure to hypertonic saline, and was finally freeze-dried. The resulting acellular fish skin was about 0.25mm thick. The samples were sterilized by gamma radiation.

Blood samples (60 ml) were collected using syringes equipped with 18G needles and, were added to DNase, Proteas, and endotoxin-free tubes with an anticoagulant, acid citrate dextrose (200μL in each tube) (ACD-A1; Terumo, Tokyo, Japan). The tubes were centrifuged at 580g for 8minutes. The fraction above the interface of the red thrombus fraction was collected as PRGF in this study and subjected to the following experiments.

After obtaining PRGF, calcium gluconate (300 microliters for every 10 cc) was added and incubated at 37°C for 90 minutes. Two phases were created in the tube, one was liquid and the other was gel-like. The gel-like part (PRGF gel) was placed in the center and deep part of the wound using sterile tweezers, and its soluble PRGF, was injected at the edge of the wound with a 14 needle. The wound was debrided before surgery.

As shown in figure 2, obvious improvement of wound oc-

Figure 2: Wound healing process of patient during a one-year period.

curred. Sizes of wound at 0, 30, and 60 days were reported as 6 x 4, 3 x 2, and 0, respectively. The wound surface area measured at different times after the initial injury revealed accelerated wound healing in the fish skin-treated wounds based on multiple-column t-tests ($p < 0.001$). All the relative measurements were performed with the patient's healthy skin as a control for the graft-treated areas.

3. Discussion

The presence of a rich source of growth factors and cytokines in platelets can accelerate wound healing (14). In the process of re-epithelialization, growth factors such as EGF, IGF-1, FGF-2, and TGF, as key elements, play an important role in processes such as angiogenesis, cell migration, cell proliferation, and differentiation to ultimately enhance extracellular matrix production (15). Growth factor-rich plasma (PRGF) is considered as rich source of proteins and circulating growth factors for tissue regeneration (15, 16). Simple extraction, availability, cost-effectiveness, and safety are some of the advantages of PRGF in wound healing (17). Our previous study on rat model showed that wound healing using PRGF/PRF with AFS is faster than the others, which may be due to the protection of growth factors against enzymes, as well as control of their release in the damaged area by scaffolds (13). Several studies have shown that such factors may inhibit hyper-inflammation, and affect macrophages to improve wound healing (18, 19). Studies have shown that type I collagen in fish skin stimulates Fibroblast Growth Factors (FGF), as well as Keratinocyte Growth Factor (KGF), as two important cytokines for wound healing. Moreover, the use of skin dressings keeps tissue moisture, which is a key factor in speeding up the epithelialization process (20, 21).

A report for the phase II randomized controlled trial of Nile Tilapia skin graft was published in January 2020. The efficacy of Nile Tilapia skin was tested in 62 participants in terms of healing time, number of dressing changes during treatment, and pain intensity, assessed via the Visual Analogue Scale, as well as the Clinical Global Impression Scale-Improvement. Results of the study showed that less dressing changes were required for patients using Nile Tilapia skin compared to patients under other treatments when there was no significant change in their re-epithelialization time. Also, significant pain reduction was reported by in-patients treated with Nile Tilapia skin (22). Fish Skin is an outstanding candidate among skin substitutes in treating partial thickness burns due to its efficacy and reduced price; however, larger scale studies are needed before it is officially used in burn cares.

In 2019, Alam et al., at the Burn centre of the Queen Elizabeth Hospital Birmingham, used fish skin as wound dressing on ten burn casualties in both their donor sites and burn sites. The fish skin was soaked in saline and then applied to both the donor sites and burn sites with dry gauze as a secondary dressing. Seven days after split thickness skins were harvested, dressing was changed for the first time and every three days afterward, until healing. In the donor site study, no signs or symptoms of infection and adverse reactions was shown. All the patients reached 100% re-epithelialization within 16 days and low pain scores (1–4 out of 10) were reported by the patients. In the burn site study, patients including a burn in the thigh caused by cooking-oil and a flame injury on a hand were treated with fish skin. Feedbacks from the patients showed that no discomfort by the fish skin except for the "fish smell" of the product and there was an immediate analgesic effect after dressing. All wounds completed epithelialization within two weeks. In comparison with other wound dressing products, reduced healing time was shown when Kerecis was applied in the study (12). Since Alam's study was a pilot case series, larger scale experiments must be conducted to further investigate the effectiveness of fish skin in burn injuries. In the present study, the wound located below the knee was treated successfully using platelet-rich fibrin (PRF) gel and acellular fish skin. The scaffold with gel were applied directly to the open wound repeatedly every week for a period of one month. The wound started healing immediately, and 11 days later it was almost closed. With healing process completed seven days later. The wound surface area measured over different times after initial injury revealed accelerated wound healing in the fish skin-treated wounds. Efforts to improve the healing process of decubitus or mechanical wounds is a permanent duty of specialists in hospitals and relevant institutions trying to reduce suffering of patients and also of those responsible for the economic cost of its treatment (6, 13). Due to its distinct properties compared to other biological skin substitutes, the acellular fish skin can act well in healing the decubitus wounds. It could be mentioned that the activity of scaffold is also antimicrobial, as described in previous studies (23). This study is an important step toward standardizing and optimizing wound care after damage. The results demonstrate a significant reduction in the healing time for fish skin-treated wounds compared to normal healing process. The hydration was significantly high in the fish skin-treated case. Fish skin graft treatment tended to increase the skin elasticity and sebum content. In this study, fish skin graft with PRGF gel from blood taken from the patient herself was applied on full-thickness wound.

4. Conclusion

In conclusion, a combined treatment consisting of growth factor with acellular fish skin graft, was found to be effective in healing deep traumatic wounds. Along with a reduction in wound-healing time, the advantages of fish skin graft treatment were better outcomes in the objective and subjective evaluation of scar quality. Therefore, a potential improvement in the quality of life of damaged patients can be envisioned through the use of fish skin graft. Nevertheless, larger randomized controlled trials are needed to confirm these results.

5. Declarations

5.1. Acknowledgments

This project was supported by Shahid Beheshti University of Medical Sciences.

5.2. Competing interests

None

5.3. Funding source

Proteomics Research Center, Shahid Beheshti University of Medical Sciences

5.4. Authors' contribution

Esmaeil Biazar, Saeed Haidari-Keshel, and Mostafa Rezaei Tavirani: Project design, material preparation, data interpretation, manuscript writing and final approval of proof. Reza Zandi, Majid Rezaei Tavirani, Reza M Robati, and Reza Vafaee: Clinical administration, data interpretation, manuscript writing and final approval of proof. All authors read and approved the final version of manuscript.

5.5. Patient consent statement

Parents of the patient participated voluntarily and consented to the publication of clinical information related to their case. The study follows the principles outlined in the Declaration of Helsinki and is in accordance with the Ethical Code: IR.SBMU.REC.1398.059 and Clinical Trials code: IRCT20190826044620N1

5.6. Funding

None

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