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Reconstructive

Regenerative Wound Healing by Open Grafting of Autologous Fat and PRP-Gel – A New Concept and Potential Alternative to Flaps

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Summary: A new regenerative technique is described that enabled in situ formation of soft tissue bridge for difficult wounds, which otherwise needed flaps, resulting in complete healing in 3/3 cases. The wounds were debrided till healthy bleeding and covered with a triple-layer matrix of platelet-rich fibrin gel, condensed liposuctioned autologous fat, and porous collagen dressing. Negative pressure wound therapy was applied after 48 hours for 4–5 days in 2 cases and after 11 days for 3 weeks in the third case. Rapid vascularization was noted, with regeneration of mature tissue bridge and subsequent epithelization even over the bone. One patient underwent elective replacement of healed epithelium by split skin graft for better aesthetics and durability. This technique, if further validated, may herald a new concept of in situ regeneration, namely transformation of autologous tissue (grafted in gel form) to viable soft tissue. *(Plast Reconstr Surg Glob Open 2021;9:e3349; doi: 10.1097/GOX.0000000000003349; Published online 25 January 2021.)*

INTRODUCTION

Flap surgery is advocated for deep wounds and for those with exposed bone with the primary aim of providing a vascularized cover over the bone and secondary aims of contour fill and skin cover. The ability to do this without harming other tissues is the holy grail of tissue regeneration. Scientific progress in the laboratory with regenerative stem cells, growth factors, and biomimetic scaffolds is translating into clinical applications.[1] Peri-wound injection of fat has been used successfully to hasten healing in difficult wounds with or without negative pressure wound therapy (NPWT).[2] Tissue regeneration templates can cover but small areas of bone exposure; cost, availability, lack of insurance coverage, and difficulties with vascularization are significant downsides.[3]

The purpose of this report was to describe a new, simple regenerative method combining platelet-rich fibrin matrix (PRFM), autologous fat, and a collagen dressing as a triple layer matrix to bridge across wounds, leading to rapid in situ regeneration of vascularized tissue cover even over the bone. It has the potential to provide customized soft tissue cover at point of care.

PATIENTS AND METHODS

Case 1

An 11-year-old boy presented with a painful infected wound on the anterior aspect of right leg 1 month following radiofrequency needle ablation done for an osteoid osteoma of the posterior tibial cortex. Surgical debridement and application of NPWT had failed. Examination revealed an infected wound of 80 × 50 mm, covered with necrotic tissue over the subcutaneous tibial bone. An inside-out fourth degree burn due to leakage of RF current was suspected and confirmed by photon deficiency

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on a bone scan. A second debridement exposed 45 × 16 mm of bare bone; cortical drill holes did not bleed and the marrow looked dead (Fig. 1). NPWT and antibiotics for 3 weeks cleaned the wound but did not initiate any healing. A flap cover was essential but disapproved by his parents for fear of further scars and deformities. Continuing NPWT for several months was risky, with an uncertain outcome.

Forced to innovate, the author proposed covering the wound with a regenerative matrix of PRFM and autologous fat, hoping to harness their wound healing powers; the author was aware of studies about the benefits of peri-wound fat injections.[3] Written consent was obtained after stressing the novelty of the procedure and potential for failure.

Surgical Technique

Wound edges were refreshed; clinically necrotic bone and marrow were debrided. Autologous PRFM gel was prepared from 30 ml of blood that was centrifuged for 10 min without anticoagulant and then allowed to clot. It was placed at the depth as the first layer to enable bio-stimulation of the bone marrow.[4] Syringe-aspirated fat from the abdomen was injected into the wound edges (See **Supplemental Digital Content 1**, deep wound after final debridement with exposed bone, peri-wound fat injection in progress prior to open grafting. http://links.lww.com/PRSGO/B533). More fat was also condensed into "dry" fat by the Telfa rolling technique. The PRFM-gel-covered wound was filled completely with condensed fat, hoping to create a bridge for cellular migration or in situ transformation. This was covered with a sheet of porous collagen (Biopad equine collagen) soaked in leached serum (Fig. 2). This was meant to act as space definer and potentially provide bio-induction. NPWT was applied 48 hours later (intermittent protocol 5/2 at −75 mm Hg vacuum) for 4 days. The wound was inspected on the 6th day, and NPWT reapplied for 3 days followed by conventional dressings.

Fig. 1. Appearance of the wound 2 weeks after the second debridement and NPWT showing the extent of bone exposure, absence of bleeding from cortical drill holes, and dead marrow seen through the cortical window.

Fig. 2. PRFM has been placed at the wound depth and the cavity filled with condensed fat graft. The petri dish holds the collagen cover sheet soaked in fat and serum.

Result

The grafted fat remained in place and was well vascularized on the 6th day, except for the central area (Fig. 3). By the second week, there was epithelialization from the edges and healthy granulation on the floor. The central non-vascularized fat was removed on the 20th day, revealing a layer of vascularized tissue covering the bone, which was again covered with PRFM sheets prepared by the Choukroun technique, resulting in marked improvement.[5] Epithelization was almost completed at 8 weeks. Elective over-grafting was done with parental consent to improve the aesthetics and durability. The neo-epithelium was removed and the tissue bridge was covered by ADM sheet (Matriderm 1 mm) and skin graft, resulting in 100% graft "take." The area has been stable for 35 months with good contour and radiological bone healing (Fig. 4).

Case 2

A 60-year-old diabetic patient presented with recurrent ulcerations over the left medial malleolus following minor trauma 5 months ago despite diligent advanced dressing care. His limb was heavily scarred due to multiple skin grafts and LD flap over an old tibia fracture. There were 2 necrotic ulcers adherent to the bone on a highly scarred

Fig. 3. Appearance 6 days after matrix grafting. NPWT foam has been removed. Note the bright red vascularization of the matrix except in the middle and the total filling of wound space.

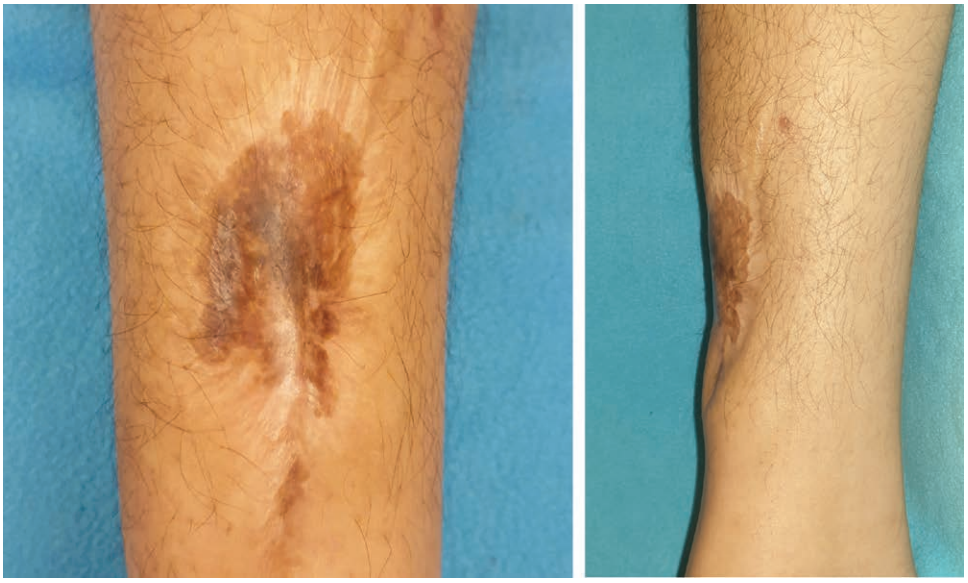

Fig. 4. Late result after 30 months showing stable skin graft and significant contour improvement.

bed in a woody limb. Local flap options were exhausted and he refused excision of ulcers with ADM-assisted skin grafting. The same triple matrix technique was applied after fresh re-excision of the ulcers; fat with PRP was also injected to the surrounding scars.

Result

The matrix got partly vascularized in 7 days and completely in 21 days proceeding to full epithelization in 7 weeks. The surrounding tissues became softer, and the area is stable for the past 24 months. (See **Supplemental Digital Content 2**; Right: larger ulcer covered with PRFM and fat, smaller ulcer already covered with collagen. Middle: vascularized matrix and epithelization. Left: result after 2 years. http://links.lww.com/PRSGO/B534.)

Case 3

A 43-year-old quadriplegic man with chronic grade III Ischial pressure sore measuring 4 × 2.5 cm, 1.5-cm deep, agreed for the above protocol because he did not have insurance or finances for a flap surgery. The ulcer edges were sharply excised, and criss-cross cuts were made in the fibrotic base. (See **Supplemental Digital Content 3**; a) Refreshed ulcer with cross-cuts at the base and peri-wound fat injection b) second episode of open grafting c) peripheral core biopsy 1 week later, fat is already vascularized d) result 2 months after healing. http://links.lww.com/PRSGO/B535.). The same triple layer matrix was applied but could not be covered by NPWT initially due to cost issues. The matrix became vascularized in a week. A portable NPWT could be applied on the 11th day, after which vascularity improved but the graft became reduced in volume; it was topped up with more fat harvested in clinic. Peripheral and central core biopsies were done 7 days later (28 days after first procedure), NPWT was removed, and simple collagen dressings were continued.

Result

Fat was seen vascularized 7 days after each session, rapid epithelization was noted, culminating in full healing by 53 days. Core biopsies showed new vessels in the midst of adipocytes even in the superficial 7-day-old fat graft. At intermediate depth, larger vessels perpendicular to the surface and a profusion of fibroblasts oriented parallel to the surface were seen. Deeper layers showed a well-organized tissue consisting of collagen bundles, fibroblasts, foamy histiocytes, and some surviving fat (See **Supplemental Digital Content 4**; Histopathology of peripheral core (H&E stain): a) Superficial part showing peripheral host tissue, new blood vessels in recently grafted fat and advancing epithelium b) intermediate part (older fat graft) showing intact adipocytes, foamy histiocytes, new blood vessels, fibroblasts, collagen and sparse lymphocytes with no evidence of necrosis. Arrows code: brown-new epithelium, light green-host tissue, red-blood vessel, blue-lymphocytes, yellow-adipocytes, orange-foamy histiocytes, black-fibroblasts, dark green-collagen. http://links.lww.com/PRSGO/B536).

DISCUSSION

The author could not find any published clinical reports of open fat grafting used as a regenerative tissue bridge in deep wounds. Peri-wound injections have been reported in many studies to accelerate healing in burns and chronic wounds of diverse etiologies.[6–10] In 1 study, the authors also deposited some fat on shallow surface wounds and observed graft survival.[10] Platelet-derived fibrin matrix seeded with keratinocytes and ADSC was able to regenerate skin across a defect in a rat study.[11]

The apparent survival and rapid revascularization of open grafted fat noted in all the 3 cases here (even without NPWT in case 3) is intriguing and needs an explanation. The following hypotheses are proposed to explain

first the rapid vascularization and then the subsequent transformation to granulation tissue.

Vascularization

Liposuctioned fat is made up of intact particles of cell nests in their natural environment of extra cellular matrix (ECM) and contain intact adipose-derived microvascular fragments (AD-MVF). These particles, along with PRFM, can form a continuous sheet of extra cellular matrix, fat tissue, and fibrin saturated with platelet-derived growth factors. Linking up of AD-MVFs in situ and their inosculation by new budding capillaries from the periphery results in rapid vascularization (similar to the "take" of a full-thickness skin graft). Stimulation by PGDF and VEGF from platelets and pressure gradients set up by NPWT accelerate the process. This hypothesis also explains the lack of vascularization in the central superficial zone in case 1, being farthest away from a source of blood vessels. There is experimental support for this theory.[12,13] Compaction of fat before grafting is perhaps important, as close spacing is necessary for rapid link up of the MVFs.[13]

Transformation

The small fat particles (1–2 mm) surviving by diffusion of nutrients suffer hypoxia due to open grafting and lowering of ambient PO2 by NPWT. Relative ischemia can kick-start proliferation and transformation of ADSCs into many cell lines needed for soft tissue regeneration (mesenchymal, neural, ectodermal, and vascular); they also exert multiple paracrine effects in synergy with growth factors from platelets.[14] The particulate fat grafts in matrix can thus transform into a sheet of well-vascularized granulation tissue, as seen in the highly cellular histology of the core biopsies in case 3. Prompt and rapid epithelialization from the periphery without wound contraction in all cases and progressive radiological bone healing in case 1 is proof that this new tissue is vital and functional.

The role of NPWT is unclear; it is however very effective in retaining and protecting all the components and almost certainly helps speed up vascular ingrowth, as was noted in case 3.

CONCLUSIONS

The described method was able to regenerate vascularized soft tissue in deep wounds in 3 challenging cases, even covering the bone in 2. It suggests a tantalizing possibility of a simple regenerative alternative to flaps in selected cases. It can provide essential tissue cover without adding new scars or deformity. It is inexpensive, easy to learn, applicable to any level of care, is possible under local anesthesia even at the point of care, and does not compromise other methods of reconstruction. If further validated, it will give a simple yet powerful tool to plastic and regenerative surgeons.

There are limitations to the study. The report is preliminary and based on a small series. The first case was the trigger where innovation was made to address a need. The other 2 cases seem to confirm the success of the first. It needs more clinical and animal studies for independent validation, elucidation of the biological mechanisms, the role of each component, and understanding its place in surgical therapeutics.

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