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**Management of Fournier's gangrene non-healing wounds by autologous skin micrograft biotechnology: A new technique**

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## MANAGEMENT OF FOURNIER'S GANGRENE NON-HEALING WOUNDS BY AUTOLOGOUS SKIN MICRO-GRAFTS BIOTECHNOLOGY: A NEW RAISING TECHNIQUE --Manuscript Draft--

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WOUNDS BY AUTOLOGOUS SKIN MICRO-GRAFTS  
BIOTECHNOLOGY: A NEW RAISING TECHNIQUE**

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# MANAGEMENT OF FOURNIER'S GANGRENE NON-HEALING WOUNDS BY AUTOLOGOUS SKIN MICRO-GRAFTS BIOTECHNOLOGY: A NEW RAISING TECHNIQUE

## ABSTRACT

Fournier's gangrene is an acute bacterial infection producing necrosis of the perineum and external genitalia which generally affects elderly men. Although skin grafts and flaps are the standard procedure for reconstruction, sometimes wounds can cronicize. Rigenera Protocol is a new raising technique based on autologous skin micro-grafts that reactivates and supports wound healing.

A 40-year-old male patient with Fournier's gangrene, due to a rectum micro-perforation following diarrhea, was treated with surgical debridement, negative pressure wound therapy and subsequently coverage with dermo-epidermal skin grafts. He developed non-healing wounds treated by Rigenera Protocol after two months of advanced wound dressings. This technique is based on skin micro-grafts obtained by mechanical dermal disgregation to provide mesenchymal stem cells and extracellular matrix to wound. The suspension injected into the wound gets reactivation of healing without significant residual scarring on both donor site and treated area. Non-healing wounds were reduced by 15% at the seventh day and by 50% after thirty days. Wounds completely healed after seventy days. The regenerated tissue appeared closer to skin graft than to scar tissue. This report shows how the use of skin micro-grafts through Rigenera Protocol can be a useful method to reactivate wound healing resulting from Fournier's gangrene, with no discomfort for patient in a practical, safe and easy way.

## INTRODUCTION

Fournier's gangrene (FG) is a soft tissue acute necrotizing bacterial infection of perineal and male genitalia, rapidly progressive, with a mortality rate between 10-83%.<sup>(1)</sup> The disease mainly affects older men (60-70 years) and can extend to anorectal region, thighs and abdominal wall.<sup>(2)</sup> It has an incidence of 1.6 cases per 100,000 people-year and represents less than 0.02% of hospitalization.

Anorectal disease is the most common cause (40%) although many comorbidities may facilitate onset of disease. The clinical manifestations are due to a mixed polymicrobial spread through a skin/mucosa laceration which leads to obliterans endarteritis of subcutaneous vessels and subsequent suppurative necrosis of tissues.<sup>(3)</sup>

A multidisciplinary prompt approach is essential to stabilize the systemic balance, remove necrotic areas, treat the infection and reconstruct the injured area.<sup>(4)</sup> The main reconstruction techniques use full or partial-thickness skin-grafts, cutaneous, fasciocutaneous or myocutaneous flaps.<sup>(5)</sup> However, due to severity of necrosis in addition with nutritional and metabolic imbalance, sometimes non-healing wounds can remain and represent a surgeon challenge. Although advanced dressings can get a quick recovery, it's necessary to produce and improve medical devices that can reduce both healing time and health care costs.

In last few years adult mesenchymal stem cells (MSCs) have attracted considerable interest due to their regenerative abilities.<sup>(6)(7)(8)</sup> There are so much tissue engineering techniques and many studies on MSCs, but actually there is not a gold standard technique to better exploit the potentiality of MSCs.<sup>(9)</sup>

The Rigenera Protocol is a new raising technique of regenerative medicine based on autologous skin micro-grafts that reactivate and support wound healing.

A case of Fournier's Gangrene healed by necrotic tissue and later reconstructed by autologous partial-thickness skin grafts followed by Rigenera Protocol application to solve residual non-healing wounds is described in this case report.

## CASE REPORT

A forty year caucasian man, without comorbidities, came to E.R. reporting a penis and scrotal swelling, pubis ecchimosys, subcutaneous emphysema, perineal and external genitalia soreness. The symptoms appeared few days before following an episode of acute diarrhea. He presented saddle perineal ischemia with genitalia skin necrosis, glans excluded, lower abdominal hardness and high fever with shivering. The clinical condition was critical and compatible with Fournier's gangrene extended to the hypogastric region.

An endovenous antibiotic therapy with teicoplanin and imipenem was immediately administered. The patient was then sedated and underwent to three massive surgical debridement with scrotal bipartition, removal of a massive testis appendix, protective left colostomy, debridement of prevesical space and exploratory rectoscopy that found a covered rectal microperforation likely due to previous diarrhea. The intraoperative bacteriological swab showed E. Coli, Streptococcus Pyogenes and Prevotella Loeschei infection, so was carried out antibiotic therapy shift to daptomycin, metronidazole and piperacillin/tazobactam. The clinical conditions of patient gradually improved and after a month of hospitalization was suspended antibiotic therapy and applied scrotal and hypogastric two-way negative pressure wound therapy (NPWT) at -80mmHg as wound bed preparation. The NPWT therapy was removed after 60 days.

A reconstructive surgery of testes, penis and perineum by partial thickness skin-grafts was planned for this patient. Using an electrical dermatome we took partial thickness skin-grafts from anterior region of thighs to cover penis, hypogastric region, scrotum and perineum. The graft was then meshed and immobilized by moulage dressing for five days. We achieved an 80% of engraftment. The donor site was dressed intraoperatively with calcium alginate and followed by non-adherent bismuth gauze dressings every 3-5 days till complete re-epithelialization. After three weeks non-healing wounds remain in hypogastric, inguino-perineal and pubic region that were treated with advanced dressings as hydrogel, collagen, hydrofiber and polyurethane foam for three months without any improvement (healing less than 10%).

Non-healing wounds vary in surface, size and position. One was on left side of hypogastric region with a granulating bed of 12x3cm. Another one on right side was similar for type and position with a size of 7x0,5cm (Figure 1). Other were over base of the penis, with a convex surface, and a couple were on inguino-scrotal region with concave surface. This areas were subject to maceration and micro-traumatism due to deambulation. It's easy to realize the difficult to dress and/or reconstruct this areas, so we used a new raising technique: the Rigenera Protocol.

Rigenera Protocol requires an outpatient surgery in which a skin paddle of 10x20 mm was harvest from groin region as a full-thickness skin grat: the surgical incision was designed to minimize the donor site morbidity. This skin paddle was then cut into five samples of 2x2 mm followed by de-epithelialization and defatting procedure (Figure 2). Every sample was then mixed with 1,2cc of saline solution and processed for ninety seconds by Rigeneracons up to obtain skin micro-grafts in a suspension containing MSCs and extracellular matrix (ECM) derived from a mechanical disruption of skin dermis. The infiltration of suspension into the wound edges helps the reactivation of healing.

Furthermore the wound was dressed with a collagen scaffold soaked of such suspension and then covered with hydrofiber and polyurethane foam (Figure 3).

The dressing was removed after five days and then changed every 72 hours with collagen scaffold, hydrofiber and polyurethane foam. Non-healing wounds were reduced by 15% at the seventh day and by 50% after thirty days (Figure 4), with complete healing after seventy days.

After six months follow up wounds were completely healed and the patient presented regenerated tissue all over treated area. The appearance of regenerated tissue at eighteenth month follow up is closer to skin graft than scar tissue (Figure 5). The regenerated tissue had a better quality than scar tissue, with more elasticity, pliability and softness, even in sites where it was difficult to apply a dressing. It was also noticed a subjective better quality of regenerated tissue edges than to skin graft ones.

The patient reported an improvement on personal autonomy and a good functionality of penis with ability to physiological erection.

## DISCUSSION

In 1843 Fournier described five cases of rapidly progressive scrotum and penis necrosis with high mortality rate later renamed Fournier's gangrene.<sup>(1)(2)(3)</sup>

The therapy must be well-timed and based on a multidisciplinary approach in which a broad-spectrum antibiotic therapy and metabolic support is mandatory in addition to a massive surgical debridement and reconstructive surgery.<sup>(10)</sup>

Surgery can choose between many valid options to reconstruct non-healthy areas.<sup>(11)</sup> Sometimes surgery can not achieve complete healing and wounds might cronicize producing great discomfort for both patient and health care system. The advanced dressings offer a first treatment option but must be discarded when they don't get a quickly improvement of healing with significant cutback in health care costs.

In recent years regenerative medicine has shown to be able to fill the gap in treatment of non-healing wounds. Recent studies suggest that MSCs represent a valid option to help healing.<sup>(6)(7)</sup> Rigenera Protocol is a new raising technique using the ability of adult MSCs and ECM to reactivate healing without significant residual scarring on both donor site and treated area.<sup>(12)</sup> The Rigenera Protocol operates a mechanical disruption of small pieces of autologous de-epithelialized skin to obtain a suspension of adult MSCs and ECM in saline solution which is infiltrated as micro-grafts through bumps on non-healing wound edges.

This new device is based on two components: a Rigenera Machine and a Rigeneracons (HBW - Human Brain Wave, Italy), a biological disruptor able to disaggregate small pieces of human tissues and select a specific viable cell population including progenitor cells that can differentiate in several cell types and regenerate damaged tissue. The Rigeneracons is a grid of one hundred hexagonal holes in which each hole has six microsurgical blade. Below grid there is a cellular filter that allows the pass of cells below 50 microns of diameter. Cells with this size represent the population side of adult MSCs.<sup>(13)</sup>

In vitro studies have shown that the characteristic of Rigenera is the high cell viability (92%) despite the mechanical disruption, and the high regenerative potential indicated by positivity to adult MSCs markers such as CD34, CD73, CD90, CD105.<sup>(14)</sup> This cell population is negative to hematopoietic markers as CD45 and CD14. The suspension also carry pericytes and endothelial cells promoting angiogenesis and stimulating engraftment of micro-grafts.<sup>(15)</sup>



Rigenera Protocol seems to possess an action very similar to platelet-rich plasma (PRP)<sup>(16)</sup> although it departs from it for the outpatient procedure, rapid preparation of tissue, mini-invasiveness and more lasting duration of cell viability (90% after a week). This method promotes immediately re-epithelialization creating multiple islands of proliferating epithelium which gradually provide to wound healing. Skin micro-grafts can regenerate and restore pliability, softness and strength of the skin also in presence of folds and grooves. The patient can pinch regenerated tissue almost as physiological skin due to MSCs therapy.<sup>(17)</sup> The procedure is valid, simple, practical and repeatable several times even on the same area and the same patient.

The results that we obtained well accord with other recent studies reporting an improvement of the healing of complex wounds through the use of Rigenera Protocol.<sup>(18)(19)</sup>

In our opinion skin-grafts and flaps are the best solution to cover wounds due to Fournier's gangrene although our experience say that Rigenera Protocol can be a valid and safe option to solve cases of non infected non-healing wounds compared to the use of numerous and too much expensive advanced dressings. This new raising technology allows to reactivate the wound re-epithelialization with quick and complete healing of patient with a significant reduction of patient discomfort. It can also be a valid option where other treatments are contraindicated.

## **CONCLUSION**

The use of skin micro-grafts as Rigenera Protocol can be a useful method to reactivate wound healing not only resulting from Fournier's Gangrene, but it's potentially applicable on any non-healing wound. It's evident that further studies should be conducted to better define the areas of application and the therapeutic management. However the method we used allow to heal chronic wound in a practical, safe and minimally invasive way with a great improvement of patient's life quality and a potential reduction of health care costs.

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## **FIGURE LEGENDS**

Figure 1. Hypogastric region with three chronic wounds (left side, right side and base of penis) after reconstructive surgery by skin graft

Figure 2. Rigenera Protocol - Step One: harvesting of skin samples

Figure 3. Rigenera Protocol - Step Two: processing of skin samples and application of micro-grafts suspension

Figure 4. Follow up after thirty days

Figure 5. Follow up after eighteen months

Figure 1

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Figure 2

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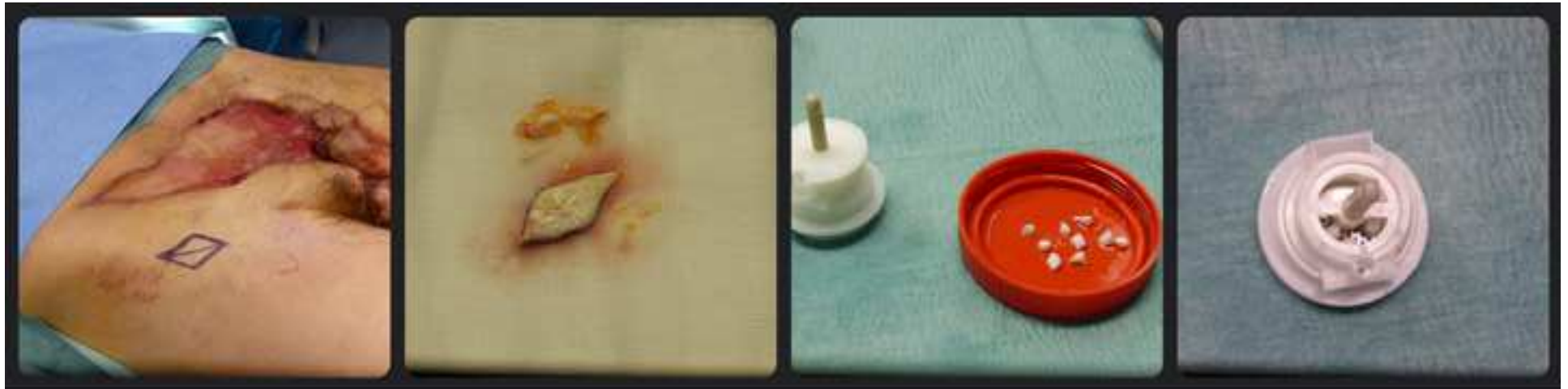


Figure 3

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Figure 4

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Figure 5

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