

Tissue regeneration: an overview from stem cells to micrografts

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Abstract

Regenerative medicine represents a major challenge for the scientific community. The choice of the biological sources used, such as stem cells and grafts, is crucial. Stem cell therapy is mainly related to the use of mesenchymal stem cells; however, clinical trials are still needed to investigate their safety. The micrografting technique was conceived by Cicero Parker Meek in 1958. It is based on the principle that by increasing the superficial area of skin grafts and reducing the size of its particles, it is possible to cover an area larger than the original donor site. Stem cells are pluripotent cells that have the capacity to differentiate into all cell types and are self-renewing, whereas micrografts derive from a small fragment of an autologous tissue and exhibit limited differentiative potential compared with stem cells. Therefore, stem cells and micrografts cannot be considered equivalent, although in some cases they exhibit similar regenerative potential, which is the focus of this review. Last, stem cell therapies remain limited because of complex and costly processes, making them not very feasible in clinical practice, whereas obtaining micrografts is generally a one-step procedure that does not require any advanced tissue manipulation.

Keywords

Mesenchymal stem cell, micrografts, tissue regeneration, autologous, wound management, ulcer

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Introduction

The correct histological structure of a given tissue plays a pivotal role in allowing appropriate function and optimizing the activities to which that tissue is predisposed. Unfortunately, many factors can alter the

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physiology of a tissue. Dermal tissue is the first line of defense of the human body and it is exposed to many risks; therefore, acute or chronic lesions of the skin greatly affect the quality of life of the patient. Tissue repair and regeneration represent a major challenge for the scientific community, which is continually seeking new strategies able to promote this process, especially for tissues in which the regenerative potential is very low, such as heart, nerve, and cartilage.¹⁻³

The percentage success for regenerative medicine is closely related to the biological sources used, such as stem cells, scaffolds, growth factors, and grafts. The main role of regenerative medicine is to replace damaged tissue while maintaining its original function or, alternatively, to stimulate regeneration of the tissue itself, respecting the original histological hierarchy.

The aim of stem cell therapy is to replace a damaged or aged tissue, restoring healthy, functioning cells. In practice, stem cell therapies are based mainly on the use of mesenchymal stem cells (MSCs), which are multipotent cells with unique biological properties.^{4,5} Several *in vitro* and preclinical studies have reported that MSCs might be promising in cell therapy because of their ability to differentiate into several cell types and secrete bioactive molecules capable of stimulating recovery of injured cells through a paracrine effect of inflammation inhibition. MSCs also show a lack of immunogenicity and can exert immunomodulatory functions.⁶ The use of MSCs has been evaluated under different conditions, such as ischemic cardiovascular diseases, critical limb ischemia, bone and cartilage regeneration, and neural diseases.⁷

Tissue engineering is an emerging interdisciplinary field based on the combined use of scaffolds and biologic mediators such as stem cells and growth factors, providing a new tool for regenerative medicine. An example of tissue engineering is represented

by bioengineered dermal substitutes, which not only repair wounds, but also supply growth factors, antibiotics, and anti-inflammatory drugs, which help hasten the wound healing process.⁸

Another approach is skin grafting, the success of which depends on the engraftment rate into the recipient site. Skin grafting is the most ancient healing technique used worldwide by surgeons to close wounds and, although less ideal than flap closures, can produce a good aesthetic result.⁹ Although this approach offers a rapid and temporary solution, it can be extremely expensive, especially when the defects are wide, because the availability of donor tissue is always limited.

A valid alternative to the classical skin graft is the micrografting technique, which allows a large defect to be covered using a minimal amount of donor skin by expanding it. However, although micrografting is an appealing strategy for wound coverage, additional studies are needed to identify its true potential and pitfalls.¹⁰

Stem cells and micrografts cannot be considered equivalent entities; however, in some cases, they exhibit similar regenerative potential even though some differences exist. Stem cells are self-renewing pluripotent cells with the capacity to differentiate into all cell types; in contrast, micrografts derive from a small fragment of an autologous tissue and exhibit limited differentiative potential compared with stem cells. Recent scientific evidence has shown that micrografts derived by mechanical tissue disaggregation with a cut-off of 80 μm (largest aggregates) are enriched in progenitor cells expressing MSC-like markers and have strong regenerative potential.¹¹ A progenitor cell, like a stem cell, can differentiate into a specific cell type but its differentiation is more specific and committed to its "target" cell. The most important difference between stem cells and progenitor cells is that stem cells can replicate

indefinitely, whereas progenitor cells can divide only a limited number of times.¹²

In the following sections, we will elucidate the main applications, benefits, and limitations of stem cells and micrografts.

Stem cells: definitions, therapy, and limitations

The concept of stem cells was introduced at the end of the last century to account for the regenerative capabilities showed by some tissues. “Stemness” is a defining characteristic of cells able to differentiate into more specialized cells because of pluripotency and self-renewal properties. Pluripotency is the ability to differentiate into all three germ layers: endoderm, mesoderm, and ectoderm,¹³ whereas self-renewal is the capability of unlimited cell replications while maintaining the same differentiative stages.¹⁴ Therefore, stem cells can duplicate practically limitlessly and, to different extents, differentiate into various lineages, making their use appealing when the aim is regenerating damaged tissue in complex injuries.^{5,15–17} The clinical significance of these populations of stem cells led to 30 years of research in which the greatest challenge was identifying human stem cells and determining how to isolate them. Today, stem cells are classified as embryonic stem cells, adult stem cells, MSCs, and, finally, induced pluripotent stem cells.¹⁸ A significant milestone was the pioneering work by Owen and Friedenstein, who first described a population of clonogenic, non-hematopoietic stem cells that were able to differentiate into several lineages, including cartilage, bone, and adipocytes.¹⁹ Because of their multipotency, they were named mesenchymal stem cells.²⁰ Contradictory findings about their origins, development, and biological function have prompted the scientific community to better investigate the role of MSCs; however, despite

the inadequate understanding of MSCs, ongoing efforts are being made to use these cells in a clinical setting.²¹

MSCs are applied in different fields of medicine, including orthopedics, wound healing, neurology, oncology, and many others. MSCs are the most investigated stem cell population because of their biological and immunomodulatory properties in addition to ease with which they can be collected from many tissues. A recent review summarized MSC-based clinical trials, either complete or ongoing in the database of the United States National Institutes of Health, including trials for hematological disease, graft-versus-host disease (GvHD), organ transplantation, diabetes, and other diseases involving liver, kidney, lung, cardiovascular system, bone, cartilage, neurological, and autoimmune. The authors of these studies concluded that, in most cases, MSC therapy was efficient and promising in terms of safety, indicating that MSC infusion and administration are well tolerated.⁷ However, the potential risk of MSC transplantation should be considered based on long-term observations. Other studies focused on MSC paracrine properties, including the release of extracellular vesicles containing mRNAs and regulatory micro-RNAs and secretion of a large number of regulatory substances.^{22,23} However, if the paracrine effect of MSCs represents a risk, it can also offer an opportunity to develop new therapeutic approaches combining nanomedicine and stem cell therapy to create a novel class of next-generation drug delivery systems. By far, the most widely used source of MSCs is adult bone marrow followed by adipose tissue and, in rare cases, discarded tissues, such as umbilical cord and placental cells.²⁴ In this regard, human adult adipose tissue may be a suitable alternative source of MSCs because adipose stem cells can be largely extracted from subcutaneous adipose tissue and used

in surgical practice for tissue remodeling.^{25,26} In fact, adipose-derived stem cells can undergo multi-lineage differentiation and secrete growth factors that enhance wound-healing processes by promoting angiogenesis, and hence increase local blood supply.²⁷

In recent years, the first stem cell-based therapy—named Holoclar—received market authorization by the European Medicines Agency (EMA).²⁸ This therapy can efficiently replace damaged corneal epithelial cells and is based on the use of ocular limbal cultured stem cells. The most important characteristic of a new therapy for wound healing or epithelial graft is the maintenance of an adequate number of stem cells or progenitors to ensure engraftment of the injured area and increase the success rate of treatment.²⁹

Despite these efforts, very few of the 900 clinical trials officially registered have reached clinical significance (<https://clinicaltrials.gov/>); among 19 reported industry-sponsored phase III clinical trials, only 10 were completed and even fewer had publicly available data. Even though those clinical studies using “mesenchymal stem cells” or “medicinal signaling cells” are indicated as safe, results for the majority of listed trials are unavailable.

Among the completed studies, three have been disclosed allowing for public consideration: allogenic marrow MSCs for GvHD, autologous marrow MSCs for heart diseases, and allogenic adipose MSCs for Crohn’s fistula diseases.²⁷ Osiris Therapeutics (Columbia, MD, USA) received Health Canada approval via a Notice of Compliance with Conditions for Prochymal for the treatment of children with refractory GvHD. Prochymal is a cryobanked product containing marrow-derived MSCs sourced from healthy volunteers. Subset analysis showed that children with GvHD were responsive to MSCs.³⁰

MSCs were the first approved allogeneic regenerative medicine product in Japan. In 2003, JCR Pharmaceuticals (Hyogo, Japan), using the licensed technology of Osiris Therapeutics, developed TEMCELL for acute GvHD, which was approved in 2015 by the Japanese Ministry of Labour. The first unambiguously successful use of MSCs resulted from the TiGenix-sponsored trial, NCT01541579. It demonstrated that allogeneic stem cells were significantly and substantially superior to placebo in treating perianal fistulas associated with Crohn’s disease. In that trial, MSCs were retrieved from cryo storage and cultured for a few days before being administered to patients.³¹

The reason for major concern is not the contradictory research on stem cells, that has led to very few market approvals, but the increasing number of unregistered treatments largely in private clinics around the world that lack approval from regulatory bodies. There is an urgent need to educate patients, doctors, and researchers about MSCs and what the therapeutic opportunities truly are.³²

From a regulatory perspective, any treatment or procedure that involves manipulation of stem cells is classified as an advanced therapy medicinal product, which is subject to strict criteria from the regulatory agencies.³³ In the last 20 years, the scientific community has experienced exponential growth in clinical trials involving such cells, but unfortunately the scientific rationale and clinical efficacy are unclear, highlighting the need for a different approach to hasten their translational application. Cells should be described not only based on their origin tissue but primarily on their differentiation capacity, as established by rigorous assays.

In summary, despite advances in stem cell therapy, very few clinical results have been achieved until recently. Large-scale clinical trials are necessary to investigate

the safety of stem cells, especially immediately and years after transplantation.

Micrografting: theory and applications

The micrografting technique was conceived by Cicero Parker Meek at the University of South Carolina Aiken in 1958.³⁴ Micrografting is based on the concept that by increasing the superficial area of a skin graft by cutting the graft into smaller “micrografts,” it is possible to cover a wound larger than the original donor site. Micrografting was first applied to the treatment of burns because of a lack of available donor sites for skin grafting. The original Meek technique was complex because the micrografts were placed with the dermal side down to achieve optimal survival. Because this requirement of dermal orientation was impracticable, especially with very small graft fragments, the technique did not gain widespread clinical application, and it was eclipsed in 1964 after the introduction of mesh skin grafts by Tanner et al.³⁵ Subsequently, several modifications were made to overcome the limitations; in 1993, Kreis et al. modified Meek’s technique by using a dermatome running on compressed air to obtain widely expanded postage stamp autografts. This modification, combined with the use of cultured grafts or allografts, improved the treatment of severe burns, reaching a percentage of wound coverage of 75%.³⁶ A recent literature review of Meek and mesh techniques indicated that micrografting can be used in burns of >30% total body surface area (TBSA), even with inadequate donor sites and in patients with comorbidities, such as in patients with diabetes with very low vascularity and low metabolic demand.³⁷ To date, the Meek micrografting technique is often used for extensive burn management,

and the micrografting concept is strongly related to this context.^{38,39}

Micrografting has subsequently been applied in other clinical settings, such as hair transplantation, hair restoration of the scalp, and regeneration of the face after burn injuries, particularly eyebrow reconstruction.^{40,41} The micrografting approach can be also used for treatment of vitiligo by melanocyte transplant or autologous punch grafting, resulting in rapid and satisfactory pigmentation.^{42,43}

In recent years, a new clinical approach has been developed related to the micrografting concept, called “Rigenera micrografting technology” (Human Brain Wave LLC, Turin, Italy). The objective of this technology is to mechanically disaggregate autologous tissue, with a calibrated size of 80 μm , collecting autologous micrografts enriched in progenitor cells, growth factors, and particles of extracellular matrix derived from the patient’s own tissue. This technology was developed after several years of experimental and clinical research on stem cell isolation from dental pulp⁴⁴ to obtain highly viable calibrated micrografts that express MSC markers. In vitro studies have shown that micrografts obtained mechanically by selecting particles with a cut-off of 80 μm are positive for MSC markers such as CD73, CD90, CD115, and CD146 and negative for hematopoietic markers such as CD34 and CD45^{14,45–47} only. Additionally, these studies have reported cell viability ranging from 70% to 90%. These data support the regenerative potential of micrografts, and several studies have reported the ability of micrografts to differentiate into chondrocytes, osteocytes, and adipocytes.^{47,48}

In vitro studies have shown that micrografts exhibit a fibroblast-like morphology when cultured and have confirmed the expression of MSC markers. When combined with collagen sponges, micrografts can form a viable and proliferative

bio-complex, enhancing their regenerative potential.⁴⁹

Clinically, these micrografts have been applied in different fields of medicine such as dentistry, dermatology, orthopedics, and especially wound care. The first clinical application of autologous micrografts was reported in the dentistry field, showing that micrografts derived from human dental pulp or periosteum promote periodontal regeneration⁵⁰ and bone regeneration in the atrophic maxilla⁵¹ and can preserve the alveolar socket after tooth extraction by reducing bone resorption and increasing new bone formation.⁵² Furthermore, a recent study reported the ability of autologous micrografts to promote sinus lift augmentation.⁵³

In contrast, the first application of micrografts in wound care was reported 5 years ago, in which the authors described two clinical cases of complex postoperative wounds. In the first case, after abdominoplastic surgery, a woman developed necrosis at the end of the wound flaps and was initially treated with vacuum-assisted closure (VAC) therapy without any improvement of wound margins with respect to skin surface. The application of autologous skin micrografts showed a gradual improvement of the wound's margins with leveling to the skin surface.⁵⁴ In the second case, a man underwent numerous different surgical interventions due to the presence of adhesions on the colon, ascites, an entero-cutaneous fistula, and other complications. He was first treated with VAC therapy and then with autologous micrografts, resulting in progressive improvement of wound surface and size reduction.⁵⁴

One advantage of a micrograft suspension obtained by mechanical disaggregation is that the suspension can be used alone or in combination with different scaffolds or biomaterials to form a bio-complex. Micrografts have been combined with a collagen sponge to treat a post-traumatic

lesion previously treated with two radical debridements and negative pressure therapy; the authors reported improvements of both the re-epithelialization process and softness of the lesion.⁴⁶

Micrografts obtained by mechanical disaggregation of autologous tissue have also been widely used in the management of post-surgical dehiscence in a set of patients. One case report described an oncological and immunocompromised patient who underwent decompressive spinal laminectomy and vertebral fixation, after which a dehiscence occurred. The patient was treated with advanced dressings without any benefit and started a cycle of chemotherapy. Subsequently, the patient was treated with negative wound pressure therapy for 2 months, which resulted in a reduction in diameter and depth of the wound dehiscence but not complete re-epithelialization. After application of micrografts, complete re-epithelialization was achieved within 70 days despite the patient's comorbidities.⁵⁵ A similar result on wound dehiscence was achieved in another case that showed good remission of the wound within 1 month, on average, of micrograft application and a complete re-epithelialization after 1 year.⁵⁶

Chronic wounds represent a major challenge for wound care specialists in terms of management and time, as well as representing an economic burden for the healthcare system.⁵⁷ Among chronic wounds, non-healing ulcers, especially venous ulcers in the lower extremity, occur very frequently; these wounds reduce the quality of life of affected patients and represent a social issue due to reduced mobility and social isolation. The treatment of chronic venous ulcer includes several approaches, such as compression therapy and debridement when necessary, even if these ulcers do not completely heal in some cases, even after several months.⁵⁸

The role of micrografts in the treatment of ulcers has been demonstrated in several

studies in which micrografts promoted healing of chronic leg ulcers of different etiologies, including venous, diabetic, and post-traumatic ulcers. In the study of Trovato et al., the authors reported the appearance of granulation tissue 3 weeks after micrograft application and the formation of new tissue covering the area around the lesion after 4 weeks. The authors also reported no signs of inflammation in the skin around the wound and a reduction of pain after micrograft application.⁵⁹ Similar results were observed in another independent study in which the authors evaluated the ability of dermal micrografts to improve the healing of venous, diabetic, pressure, and post-traumatic ulcers. Reductions in wound size, increased granulation, and reduced exudation were reported.⁴⁹ Another study reported that 15 patients showed wound reduction of $37.33 \pm 19.35\%$ after 2 weeks, 9 patients were healed after 8 weeks, and 13 patients were

healed 16 weeks following micrografting. The quality of scars was good, and they did not deteriorate at the 6-month follow-up.⁶⁰ In a recent study, the Rigenera micrografting technique was used in 70 cases of traumatic wounds of the lower and upper limbs; in 69 cases, complete healing of the wounds was achieved in a period between 35 and 84 days. This clinical trial was registered at <https://clinicaltrials.gov/ct2/show/NCT04030832>. On day 0, the mean surface area of the wounds was 14 cm^2 ; on day 14, the mean surface area was $9.3 \pm 1.6 \text{ cm}^2$, representing a reduction of $33.6\% \pm 11.4\%$. In the same clinical trial, patients using a visual analog scale to self-assess pain, used the Vancouver Scar Scale to evaluate the aesthetic characteristics of the lesions, and the Wound Bed Score to track to progress of the wound; all scored significant results with optimum outcomes.⁶¹

Micrografts have also been used for other non-healing wounds, such as those

Micrografting technology

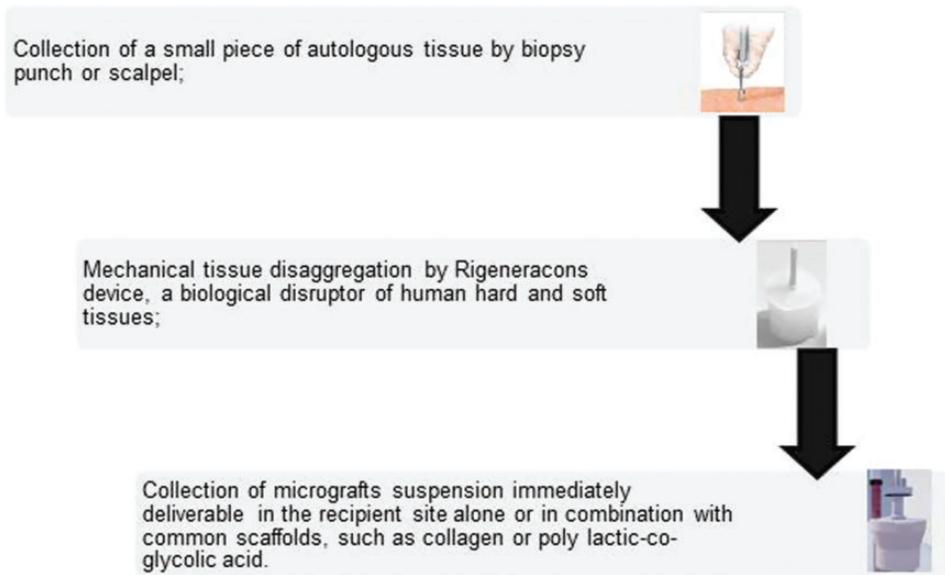


Figure 1. Representative diagram of micrografting technology.

developing following Fournier's gangrene or chronic osteomyelitis, showing a reduction in wounds after approximately 1 month of treatment.^{62,63} The efficacy of micrografts has also been reported in repairing cartilage defects, for cartilage regeneration in patients affected by external nasal valve collapse,⁶⁴ and the treatment of function-limiting and painful knee chondral injuries.⁶⁵ An *in vitro* study showed that autologous micrografts influenced chondrocyte differentiation, increasing deposition of glycosaminoglycans and the presence of collagen II in primary human cells cultured in the presence of micrografts, which supported the formation of chondrogenic micro-masses and acted as a scaffold for chondrocytes.⁶⁶

Finally, a recent clinical trial reported good outcomes in terms of safety and feasibility using autologous micrografts derived by atrial appendages during on-pump coronary artery bypass graft surgery for epicardial transplantation.⁶⁷ Figure 1 shows the different steps to obtain autologous micrografts by mechanical disaggregation. From a biological point of view, a recent *in vivo* and *in vitro* study showed that micrografts improve skin re-epithelialization by accelerating the migration of fibroblasts and keratinocytes by activation of the extracellular signal-regulated kinase (ERK) signaling pathway. Specifically, micrograft-treated wounds showed an improvement in granulation tissue formation, organized collagen content, and newly formed blood vessels.⁶⁸

In summary, good clinical evidence supports the role of autologous micrografts in different medical settings of tissue repair and regeneration, such as bone and cartilage regeneration, cardiac applications, and wound care management.

Conclusions

Despite recent progress in stem cell therapy, few clinically significant results have been

reported. The clinical applications of stem cell therapy are reduced by the complexity of the technique and the enormous expense, making this approach time consuming and not very feasible. There is a growing need for more practical and reasonable approaches, especially for diseases that are not life threatening. In this context, micrografts represent an innovative tool for clinicians because of their ability to stimulate tissue regeneration. Additionally, from a regulatory perspective, micrografts are considered a normal graft and do not require extensive manipulation before application, resulting in a greater safety profile.

Declaration of conflicting interest

All authors belong to the Scientific Division of Human Brain Wave SRL (Turin, Italy), the owner company of the Rigenera technology cited in this review and developed to obtain autologous micrografts.

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