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Concise Review

Adipose-Derived Stromal Cells for Chronic Wounds: Scientific Evidence and Roadmap Toward Clinical Practice

Nicolo C. Brembilla[1,2], Hubert Vuagnat[3], Wolf-Henning Boehncke[1,2], Karl-Heinz Krause[1,4], Olivier Preynat-Seauve[*,4,5]

[1]Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[2]Division of Dermatology and Venereology, Geneva University Hospitals, Geneva, Switzerland
[3]Program for Wounds and Wound Healing, Care Directorate, Geneva University Hospitals, Geneva, Switzerland
[4]Laboratory of Therapy and Stem Cells, Geneva University Hospitals, Geneva, Switzerland
[5]Department of Medicine, Faculty of Medicine, University of Geneva; Geneva, Switzerland

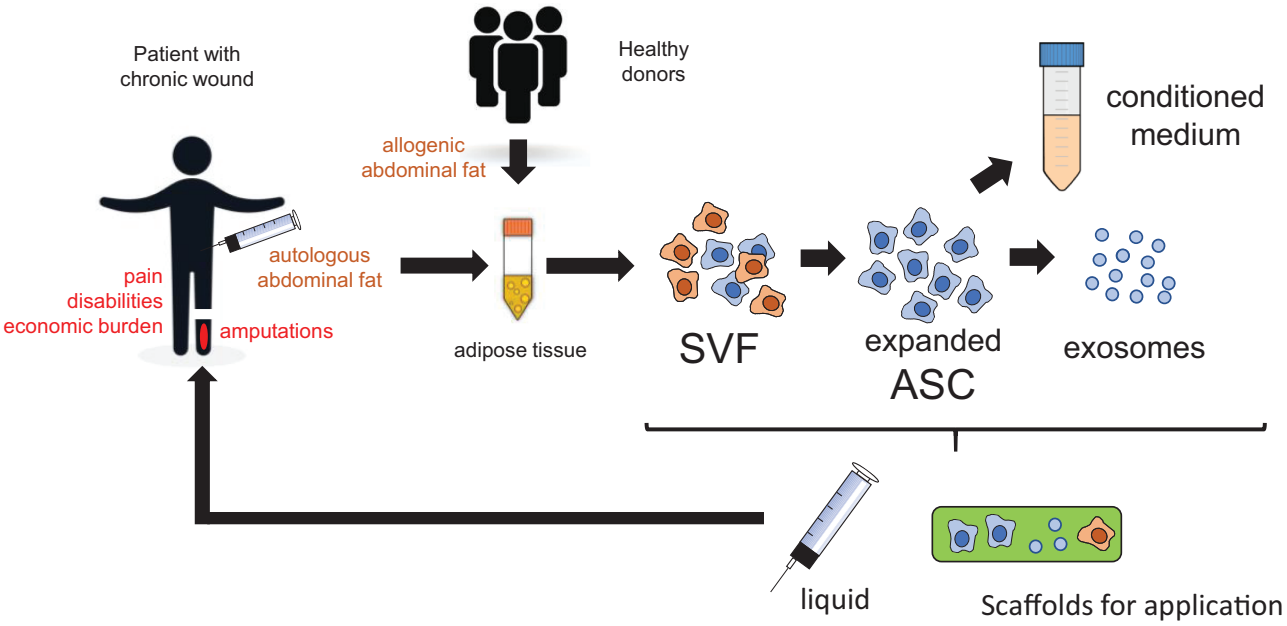
[*]Corresponding author: Olivier Preynat-Seauve, PATIM, 1 rue Michel Servet CH-1211 Geneva 4, Switzerland. Tel: +41223794139; Email: olivier.preynat-seauve@hcuge.ch

Abstract

Chronic wounds, ie, non-healing ulcers, have a prevalence of ~1% in the general population. Chronic wounds strongly affect the quality of life and generate considerable medical costs. A fraction of chronic wounds will heal within months of appropriate treatment; however, a significant fraction of patients will develop therapy-refractory chronic wounds, leading to chronic pain, infection, and amputation. Given the paucity of therapeutic options for refractory wounds, cell therapy and in particular the use of adipose-derived stromal cells (ASC) has emerged as a promising concept. ASC can be used as autologous or allogeneic cells. They can be delivered in suspension or in 3D cultures within scaffolds. ASC can be used without further processing (stromal vascular fraction of the adipose tissue) or can be expanded in vitro. ASC-derived non-cellular components, such as conditioned media or exosomes, have also been investigated. Many in vitro and preclinical studies in animals have demonstrated the ASC efficacy on wounds. ASC efficiency appears to occur mainly through their regenerative secretome. Hitherto, the majority of clinical trials focused mainly on safety issues. However more recently, a small number of randomized, well-controlled trials provided the first convincing evidence for the clinical efficacy of ASC-based chronic wound therapies in humans. This brief review summarizes the current knowledge on the mechanism of action, delivery, and efficacy of ASC in chronic wound therapy. It also discusses the scientific and pharmaceutical challenges to be solved before ASC-based wound therapy enters clinical reality.

Key words: adipose stem cells; clinical translation; stem cell transplantation; stromal cells; tissue engineering.

Graphical Abstract



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Significance Statement

This concise review provides an update on the evidence for the benefit of adipose-derived stromal cells in skin wound healing, their mechanisms of action, mode of delivery, and the current challenges in implementing the treatment procedures clinically.

Introduction

Chronic wounds are a major consequence of chronic vascular deficiencies induced by several diseases including diabetes and aging. Severe ulcers form particularly in feet and legs due to a combination of poor arterial circulation, chronic irritation from friction and pressure combined with a reduced ability to feel pain in the feet due to nerve damage. They are chronically painful and often result in clinical depression and a high risk of amputation. Chronic wounds have a prevalence of ~1% in the general population and, in addition to the strong impact on the quality of life, generate considerable medical costs. Despite the variety of therapies available to clinicians, treatment of chronic wounds suffers from a lack of gold standards and is ineffective for a fraction of non-healing patients. Numerous preclinical and clinical studies demonstrated the potential of adipose-derived stromal cells (ASC) to treat refractory chronic wounds thanks to their regenerative properties. Despite emerging promising controlled trials, several challenges have also been highlighted. This article summarizes the knowledge on mechanism of action and delivery of ASC and discusses the current scientific and pharmaceutical challenges to be solved before ASC-based wound therapy becomes a clinical reality.

Chronic Wounds With Therapeutic Failure: the Unmet Need

Wound healing is a complex biological process. Most clinicians divide it in 4 overlapping phases[1]: Hemostasis is the first phase directly linked to the "trauma" induced bleeding. Over a period of a minutes to hours, the aggregated platelets release mediators that initiate the second inflammatory phase. Through inflammation, the wound site proceeds to autolysis of the dead tissues, defends itself against bacterial infection, and moves in a few days into the third proliferative phase. Angiogenesis is induced during this much longer phase (3-4 weeks) to restore and increase the blood flow in the newly forming tissue. Under the action of growth factors, dermal fibroblasts multiply to produce extracellular matrix, and keratinocytes proliferate to create the newly formed epidermis, leading to the complete wound closure. It usually takes a several months or years to create a skin tissue with its final composition and strength, which is the fourth or remodelling phase. Although there is no strict definition, chronic wounds[2] (also known as "difficult to heal", "non-healing" or "hard-to-heal" wounds) are referred to ulcers which fail to heal in a timely and orderly manner.[3] Chronic wounds encompass a very large number of different aetiologies. In industrialized countries, the leading causes, both in number and in costs are venous leg ulcers, diabetic foot ulcers and pressure ulcers.[4] Vascular defects, creating excessive local pressure or absence of oxygen supply, and hyperglycaemia, affecting the healing process, are the 2 major factors predisposing to chronicity.[5] Although chronic wounds represent a major health concern, they are not yet fully recognized as a major medical entity. Global medico-economic studies are yet scarce but show a high impact on both quality of life and health economics.[6] A 2014 estimate in the US on Medicare spending for all wound types ranges between $28.1 and $96.8 billion USD. In the UK, it was estimated in 2018 that 3.8 million patients with wounds were managed for an annual cost of 8.3 billion GBP.[7] A concern arising from this study is the sharp increase of 71% in the prevalence of patients treated compared to 5 years before. To treat chronic wounds, clinicians will seek to identify and optimise the natural phases of healing. This is made easier by following some basic therapeutic principles: (i) diagnosing and treating the exact cause that lead to the wound, but also any other conditions that could interfere with healing; (ii) keeping the wound at an optimal moisture level so that the different cells and biological factors can operate optimally; (iii) protecting the wound from further trauma; (iv) keeping the wound bed free from necrotic material and infection; (v) reducing oedema around the wound, as it prevents oxygen from diffusing and nutrients from reaching the wound bed while retaining pro-inflammatory cytokines and proteolytic environment. Basic modern wound dressings, such as hydro cellular foams, gelling fibers, alginates and hydrogels, aim to provide this favorable environment for healing. In addition, they can be impregnated with antimicrobial agents (silver, polyhexamethyline biguanide, honey, and others). By following these basic clinical principles, most wounds, even complicated, will heal. Each patient will benefit of an individualized treatment plan and thorough revaluations. These will allow the detection of a small percentage of wounds that will evolve toward chronicity. If this is the case, the clinician must go back to the basic principles, specifically checking whether the causative agent has been corrected (eg, whether pressure was relieved in pressure ulcer, whether revascularisation is effective in an arterial ulcer). A wide range of more advanced therapies is offered to the clinicians[8,9]: skin autograft, negative pressure wound therapy, collagen-derived products, Matrix Metallo Proteinases (MMP) inhibitors, artificial cellular and acellular matrixes and platelet-rich plasma have now been used for a few decades. In this context, new approaches capable of better restoring the natural healing process are warranted. Cellular therapies based on the use of ASC represent a promising option to meet this need.

Mechanism of Action and ASC Delivery on Wounds

ASC (also referred as adipose-derived stem cells, mesenchymal stem cells from the adipose tissue or mesenchymal stromal cells) are defined by their phenotype (negative for CD45, CD235a, CD31, and positive for CD34, CD90, CD73, CD105, and CD44), in vitro attachment and expansion on plastic culture plates, and multipotent differentiation toward adipocytes, chondroblasts, and osteoblasts upon appropriated stimuli.[10] Other sources of stromal cells than the adipose tissue have been demonstrated to be effective in the treatment of chronic wounds (ie, cord blood, bone marrow). This review focuses specifically on ASC because adipose tissue is considered the most convenient and a less invasive source of

stromal cells. There is a consensus that ASC act on wounds primary through the production of a regenerative secretome[11] rather than cell differentiation. Exploited through the use of a conditioned media,[12-14] purified exosomes,[15] or cell therapy,[11] the ASC secretome contains ECM proteins (collagens, laminin, fibronectin),[16] and many MMPs that are key players in the healing process.[17] In wounds, excessive levels of some MMPs have been correlated with chronicity,[18] probably altering the equilibrium between ECM production and degradation. It is still not clear whether the concomitant production of ECM and MMPs by therapeutic ASC contributes to their therapeutic effect on chronic wounds where a disequilibrium in remodelling is observed. ASC have an immunomodulatory role: when the local microenvironment is not-inflammatory, ASC tend to promote inflammation thanks to the production of inflammatory factors: IL-1beta, IL-6, IL-8, MCP-1. In the presence of inflammatory factors, they rather have inhibitory functions through the secretion of IL1-RA, IL-10, TGF-β.[19,20] In a therapeutic context where wound chronicity is frequently associated with a blockage at the inflammatory phase, ASC could act through their anti-inflammatory functions rather than through a pro-inflammatory/angiogenic activity. A large body of evidence suggests that the angiogenic properties of ASC[21] play a key role in wounds. ASC primarily stimulate angiogenesis through paracrine secretions of angiopoietins, VEGF, FGF-2, PDGF, TGF-β, and HGF,[22-24] while also supporting angiogenesis through their own differentiation toward endothelial cells.[25] In addition to angiogenesis, ASC produce cell growth factors (such as FGFs, EGF, KGF, IGF) promoting the proliferation of dermal fibroblast and keratinocytes[26] leading to the dermo-epidermic reconstruction. They also secrete anti-fibrotic molecules[27] that could positively affect chronic wounds, express pattern recognition receptors against pathogens, such as Toll-like receptors,[28] and exert an anti-microbial activity.[29] ASC have been described to reduce pain associated with chronic wounds, but a direct action on the nervous system has never been demonstrated. Their beneficial effects are more likely due to their anti-inflammatory properties and/or ability to close wounds. Although not considered crucial, the multipotent capacities of ASC could play a role in their therapeutic action on wounds. ASC can indeed differentiate into dermic fibroblasts accelerating granulation tissue formation[30,31] or keratinocyte-like cells having the capacity to form a stratified epidermis.[32] ASC are enriched in the stromal vascular fraction (SVF) of the adipose tissue, which can be directly used for therapeutic intervention. Nonetheless, numerous studies use in vitro purified autologous or allogeneic ASC[33] or their secreted exosomes.[34] Table 1 describes and compares the advantages and limitations of the different ASC sources that do/do not use a scaffold to form a gel or a more solid structure that can be applied as a patch. Fat harvesting area influences the quality of ASC

Table 1. Comparison of the different ASC sources available for the treatment of chronic wounds

	Advantages	Limitations
SVF	Quick production	Dilution of ASC
	Simplified reglementation (not ATMP)	Uncontrolled activity
	Contains other cells involved in healing	Lack of standardization
	Automated production available	
	Reduced costs	
	Living cells: prolonged activity in vivo	
	Controlled clinical trials showing efficacy	
Allo-ASC	Standardization > SVF	Heavier reglementation than SVF (ATMP)
	Costs < auto ASC	Possible immune rejection
	One donor for multiple controlled freezable productions	Increased safety controls (viral)
	More controlled activity than SVF	No engrafment
	Living cells: prolonged activity in vivo	
	Controlled clinical trials showing efficacy	
Auto-ASC	Standardization > SVF	Heavier reglementation than SVF (ATMP)
	Safety controls < allo ASC	Too high costs of production
	No immune rejection	One donor for one treatment
	Possibility of long-term engraftment	
	More controlled activity than SVF	
	Living cells: prolonged activity in vivo	
	Controlled clinical trials showing efficacy	
Conditioned media	Freezable for large scale production	Short-term activity in vivo
	Costs < auto ASC	No controlled clinical trials available
	One donor for multiple controlled and freezable productions	
Exosomes	Standardization > SVF	Costs of production (better if allo)
	Living material: prolonged activity in vivo	Heavier reglementation than SVF (ATMP)
	Costs < auto exosomes if allo	No controlled clinical trials available
	More controlled activity than SVF	

Abbreviations: ASC, adipose-derived stromal cells; ATMP, advanced therapy medicinal products; SVF, stromal vascular fraction; Allo ASC, allogeneic ASC; auto ASC, autologous ASC.

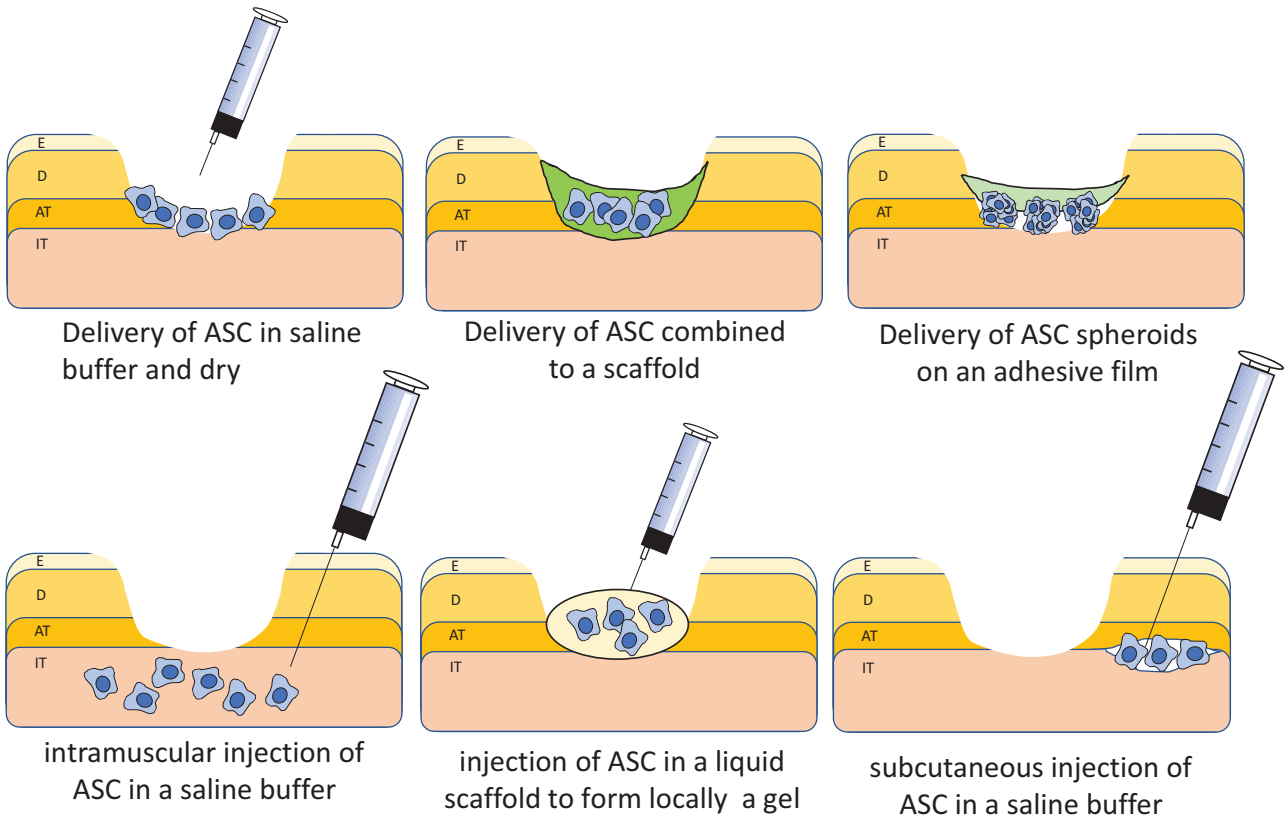

Figure 1 Different delivery modes reported for ASC on wounds. E: epidermis, D: dermis, AT: adipose tissue, IT: internal tissue.

production. Compared to abdomen, thighs showed increased viability and yield.[35] The dose required for ASC treatment on wounds, which can be measured in cell concentration for suspensions or in cell density for topical applications, cannot be easily defined. The dose, currently not formally established by appropriate studies, must allow sufficient and prolonged action/stability of the ASC secretome on the healing process. ASC has been spread as a cell suspension directly on the wound cavity or injected subcutaneously or in muscles near the wound. However, the latter route of administration is not optimal because it relies on multiple and painful injection sites that cannot be adequately controlled spatially. A few studies showed that clusterization into spheroids enhanced the therapeutic effects of ASC,[36,37] fostering wound closure and increasing their engraftment with less cell apoptosis. Some authors introduced ASC spheroids on adhesive films directly covering the ulcer.[38] The inclusion of ASC within scaffolds may provide a bio-compatible 3D microenvironment closer to the in vivo situation, with stronger cell-to-cell and cell-to matrix interactions. ASC in a physically stable scaffold can be locally concentrated in the entire surface of the wound and maximize their effect on the healing process. Scaffolds should possess several features, such as bio-compatibility, non-toxicity, resemblance to the ECM and optimal porosity to support ASC growth and activity. Many scaffolds combined with ASC have been tested on wounds: chitosan,[39] fibrin,[40] hyaluronic acid,[41] collagen sponge,[42] collagen peptide scaffolds,[43] decellularized tissues,[44,45] atelocollagen,[46,47] amniotic membranes,[48] platelet gels,[49] and mixtures of different scaffolds.[50] These biomaterials are not only physical 3D support but can be biologically active on wounds and/or ASC,[51] resulting in improved healing properties, regulations of proliferation, adhesion, and the secretome composition. Figure 1 summarizes the different modes of ASC delivery described to date in wound care.

Preclinical and Clinical Studies Evaluating the Use of ASC on Skin Wounds

Many preclinical studies in animals have reported the in vivo efficacy of ASC on chronic wounds, using different sources, animal models, and modes of administration. Several preclinical reports have shown that ASC increase wound healing in vivo through the promotion of angiogenesis, collagen deposition, and re-epithelization.[52-54] Several uncontrolled trials in humans demonstrated the safety of ASC on wounds and reported encouraging data with regard to their efficacy.[55] However, the most important and recent advances were performed through emerging controlled trials. Table 2 summarizes the completed controlled clinical trials to date and the currently registered trials in clinicaltrials.gov. Most studies used SVF and only one controlled trial evaluated allogenic ASC in a scaffold. This latter controlled trial[56] tested the efficacy of a treatment based on an allogeneic ASC/hydrogel sheet on 44 patients with chronic wounds. A complete wound closure in the treatment group versus the control group was observed in 73% vs 47% of cases at week 8, and 82% vs 53% at week 12. No adverse events related to wound dressing were noticed, providing a very encouraging study for the use of ASC in humans. In another controlled trial, 100 patients with chronic wounds received a subcutaneous injection of autologous SVF near the wound.[57] Before treatment they reported poor epithelization, heavy inflammation, and immature granulation tissue. In the study group and compared

Table 2 Reported controlled and currently registered clinical trials.

Disease	Auto/allo	Controlled	ASC sources	Delivery mode	Participants	PMID	Efficacy
CW	Auto	Yes	SVF	sc injection	100	34596433	yes
AW	?	Yes	not reported	sc injection	346	35368914	yes
CW	Auto	Yes	SVF+PRP	topical	40	27071140	yes
VLU	Auto	Yes	SVF+HA dressing	sc injection	16	30583949	yes
DFU	Auto	Yes	SVF-SVF/PRP vs control	sc injection	334	32633854	no
DFU	Allo	Yes	ASC+hydrogel	Topical	59	30679183	yes
Disease	**Auto/allo**	**Controlled**	**ASC sources**	**Delivery mode**	**Participants**	**ClinicalTrial.gov ID**	
VU	Auto	Yes	SVF	sc injection	36	NCT02961699	
DFU, VU PU	Auto	No	SVF	sc injection	25	NCT02092870	
DFU	Allo	No	ASC	topical	46	NCT03865394	
CW	Auto	No	SVF	sc injection	10	NCT02590042	
CW	Auto	No	SVF/fat	na	40	NCT03882983	

Abbreviations: CW, chronic wounds; AW, acute wounds; VU, venous ulcer; DFU, diabetic foot ulcer; PU, pressure ulcer; sc, subcutaneous; SVF, stromal vascular fraction; HA, hyaluronic acid; na, not available; ?, not reported.

to the control group, re-epithelization, mature granulation tissue formation, collagen deposition and angiogenesis was shown, confirming the mechanism of action of ASC on various healing phases. Authors reported a complete healing in 92% of treated patients versus 60% of control patients, with a reduced time for complete closure of 50%. A controlled study was also performed to evaluate the efficacy of ASC in 336 patients with skin wounds from 2016 to 2021.[58] Authors topically introduced ASC in a saline buffer directly on the wound. Granulation was significantly improved in the ASC-treated group compared with the control group receiving standard dressing with no serious adverse event. As of today, 5 clinical trials are registered in clinicaltrials.gov to evaluate the efficacy of ASC therapy on skin wounds.

From Bench to Clinical Implementation: Scientific Challenges

In recent decades, the scientific and medical community has made significant progress in understanding the functional properties of ASC. Clinical applications, as described above, are beginning to become a reality. However, the road is still long and complex, and several aspects related to biology of ASCs still need to be understood in order to achieve widespread application of these therapies. First, the source of the cells. In general terms, adipose tissue is an advantageous choice in terms of safety, collection, culture, and ethical concerns compared with other sources such as bone marrow. Adipose tissue includes 2 distinct subtypes, white and brown, and its composition may vary depending on the anatomical location. Of course, the characteristics of ASCs also vary according to the type and anatomical region of adipose tissue harvested, with white subcutaneous abdominal adipose tissue currently considered the best source for yield and quality.[59] Second, researchers have shown that the technique of fat harvesting[60] and the method used to isolate and expand ASC largely influence their basic characteristics and functionality.[61-63] These considerations extend to the culture condition used, which is a critical prerequisite for clinical translation. Studies using animal-derived growth supplements, such as animal serum, are common, but this has critical limitations and safety concerns for regulatory authorities. Several alternative formulations free of animal products have been developed to enable good medical practice—compliant production.[64] The storage condition of ASC adds to the list of debated topics: some authors have shown that cryopreservation can interfere with their immunomodulatory properties while others argue that their basic functions remain unchanged by the freezing process.[65,66] The general lack of standardization is undoubtedly one of the major obstacles and challenges that researchers must overcome to get the green light from regulatory authorities and ultimately bring ASC to patients in routine clinical practice. Establishing a commonly accepted ASC definition and providing manufacturing guidelines would also enable better cross-interpretation of clinical trials, once again accelerating the possibility of routine clinical adoption.

Another important issue is the immunogenicity of ASC. ASCs, and mesenchymal stem cells in general, have long been considered hypoimmunogenic because of the absence of HLA class II, low expression of HLA class I and co-stimulatory molecules such as CD40 and CD80. However, some studies have highlighted that ASCs can become more immunogenic during cell expansion in vitro, depending on culture conditions,[67,68] and may induce cellular and humoral responses when used in an allogeneic setting.[69,70] Complement activation properties have also been reported.[71] These aspects need to be better addressed for each specific clinical application, as alloreactivity may lead to decreased viability of ASC. However, the extent to which this affects therapeutic efficacy remains unknown, particularly in local application for wound care, where the cells can exert their beneficial effects in a particularly short time. The possibility of using allogeneic ASC goes beyond simply discussing possible alloreactivity, but needs to be considered for broader commercial and scientific implications. For example, age, sex, health status, and weight are all factors that have been shown to have a potential impact on the regenerative potential or proliferative capacity of ASC. Thus, the donor status becomes a critical parameter that can directly influence the success of therapy. This issue will never be resolved in an autologous setting, and autologous ASC therapy could potentially be so patient-dependent as to prevent comprehensive clinical adoption. The use of prevalidated

"standardized" allogeneic stem cells would therefore also be advantageous from the point of view of efficacy. In this regard, and of great significance, a solution of expanded allogeneic ASC was approved in 2018 by the European Medicines Agency under the trade name Alofisel for the treatment (local injection) of complex perianal fistulas in adult patients with Crohn's disease. This is a milestone in regenerative medicine, as it demonstrates the willingness of regulatory authorities to be open to these innovative cell-based therapeutic strategies. In a randomized phase III trial that preceded the drug's approval,[72,73] about one-third of patients showed anti-donor antibody production at week 12. Of note, the immunogenicity of allogeneic ASC had no effect on in vivo safety or efficacy at week 52.

From Bench to Clinical Implementation: Drug Development Issues

The scientific promise hold by ASC-based therapy in wound care will only translate into reality if such therapies are successfully adopted into clinical routine. Indeed, despite growing investment and enthusiasm from controlled clinical trial, at the light of the scientific limitations described above, the market does not yet seem ready for large-scale adoption.[74] Industries do not see a sufficient return on investment for cell therapies and are only timidly entering in this new market. Public entities, ie, universities and hospitals, effectively master the manufacturing process, but lack the vocation for scale-up to enable widespread clinical application. First, these treatments are based on live and rare cells. A single "production batch" is capable, at best, ie, an allogeneic therapy, of satisfying only a few dozen patients, when compared with the millions of doses produced by a single batch of a traditional drug. This aspect is exacerbated in autologous approaches, where one batch allows for the generation of a single treatment. In addition, automation is still minimal and most of the work relies on manual processes that require highly skilled operators.[75] This results in very limited productivity and high production costs. Another problem is a particularly complex supply and logistics chain. Speaking generally, in both autologous and allogeneic approaches, the raw material is taken from a human being. This means that the harvesting procedure must be performed in an appropriate hospital facility by qualified personnel. Once produced, the treatment does not go through the traditional distribution chain, as is the case with most traditional drugs. In fact, the final treatment cannot be consumed independently by the patient, to date, but must be administered to the patient by trained personnel in an appropriate facility. This is an important issue, as the lack of adequate education and training of hospital staff on this new family of drugs may jeopardize the success of the therapy itself. Inappropriate handling of live-cell products by the hospital personnel has been indeed associated with increased ASC mortality and aggregation, leading to the failure of precursor formulations of Alofisel during phase III studies, despite successful completion of phases I and II.[76,77] The living nature of both the sampled material and the final treatment means that all transport must be carried out under controlled conditions. Logistical complexity is again greatest in autologous applications, since the same patient is both the source for sampling the material to generate the treatment and the recipient of the therapy. This result, among other things, in the inability to plan the production process, which begins when the patient presents to the clinic. While not comparable to a classic "off-the-shelf" drug, the logistical complexity in the case of allogeneic therapy is reduced by the ability to disconnect the beginning (the sampling of human tissue from a donor) with the end of the chain (the treated patient). Production and logistical constraints increase production costs, which in turn affect the final price of the treatment. Mesenchymal stem cell therapies will most likely rank at the high end of the cost range in the multitude of treatment offerings for chronic wound management. This high upfront cost must be weighed against the total costs that would otherwise be incurred with one-or more-standard therapies over the time required for manage the wound. It is worth noting that the treatment and care of chronic wounds account for more than 3% of total health expenditure in developed countries.[78,79] Most of these costs are due to the long treatment/hospitalization times of hard-to-heal cases. Even a moderate increase in the efficacy rate (ie, reduction in treatment/hospitalization time) in this subgroup of patients will thus have a large impact on total costs. Well-designed pharmacoeconomic studies will thus be the trump card in convincing payers, who are usually reluctant to reimburse high costs if their benefit is not clearly demonstrated. And this will not be easy to achieve, since the broader economic and social benefits that ASC-based therapies would produce could not be directly realized by payers, and the slow long-term savings in the health budget may not be attractive to payers when compared with the high upfront costs.

Concluding Remarks

Recent evidence, particularly in controlled studies, suggests that ASC could be a superior treatment option for patients with chronic wounds. An allogenic source of expanded ASC could allow a more controlled manufacturing setting and guarantee a higher access to therapy. Although the available results are encouraging, further controlled studies are still needed, particularly to explore the impact of allogeneic ASC/scaffold combined products and the possibility of long-term storage in secured cell banks for subsequent off-the-shelf topical application. Regulatory barriers to the use of allogeneic cells remain a major unresolved challenge to bring these therapies to large-scale clinical adoption.

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Conflict of Interest

The authors indicated no financial relationships.

Author Contributions

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Data Availability

No new data were generated or analyzed in support of this research.

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