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Cell-based Wound Healing: Mechanisms and Treatments

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SKC and KDD designed and prepared the manuscript. Authors SKC, RB and KDD edited the manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The ultimate goal of the treatment of wounds is to restore the damaged skin both structurally and functionally to its original state. Conventional treatment of chronic wounds does not seem to work in several cases, so it is necessary to develop different strategies. Recent research advances have shown the great potential of cell-based therapies in improving the pace and quality of wound healing and skin regeneration. Cell-based therapy is thus considered a new alternative to classic methods of wound healing. This review seeks to give an updated overview of the applications of cell-based therapy in wound management. Even though cell therapy is a relatively new tool, several studies prove these types of cells can be used safely, and they have demonstrated their efficacy in healing wounds in several cases.

Keywords: Wounds; platelet-rich plasma; platelet-rich-fibrin matrix; biomaterials; stem cells.

1. INTRODUCTION

Wound healing is a complex and dynamic process of replacing damaged and missing cellular structures and tissue layers. Wound healing remains a challenging clinical problem and an efficient wound management is essential. Although the process of wound healing is

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continuous, it is typically divided into four phases: (i) haemostasis; (ii) inflammation; (iii) proliferation; and (iv) remodeling [1]. For successful wound healing, all four phases must occur in the proper sequence and time frame. One of the earliest responses to injury stems from the damage that is caused to local blood vessels. It is necessary to stop local hemorrhage immediately, and this is achieved by platelet activation and aggregation, which results in formation of a fibrin clot consisting of a network of insoluble fibrin fibers and it happens in the hemostasis phase of wound healing. It is normally followed by Inflammation which is characterized by the classic signs of heat and redness, pain and swelling, raised temperature, and fever. The phagocytic cells; 'neutrophils and macrophages'; are typically involved in mounting a host response and autolyzing any devitalized necrotic tissue [2]. The proliferative phase essentially involves the generation of the repair materials such as collagen and "extracellular matrix" and into which a new network of blood vessels develop, a process known as 'angiogenesis'. Epithelial cells finally resurface the wound, a process known as 'epithelialization'. In the maturation phase, wound remodeling follows wound repair. In this phase, the vascularity is reduced in the wounded area and there is progressive organization and maturation of the tissues, leading to an increase in collagen strength at the site of injury.

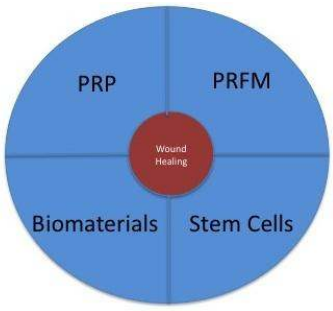

Fig. 1. Different modalities of cell-based therapy

Platelet-rich-plasma (PRP), platelet-rich-fibrinogen-matrix (PRFM), biomaterials and stem cells constitute currently available modalities for wound healing

Many factors can interfere with one or more phases of this process, thus causing improper or impaired wound healing. Wound healing occurs as a cellular response to injury and involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets [3]. Many growth factors and cytokines released by these cell types are needed to coordinate and maintain healing [4]. Thus dysfunction or dysregulation of any of these cellular factors can delay the normal would healing process. Furthermore, many of the microvascular and macrovascular diseases are associated with either dysregulation or dysfunction of many of these factors [5].

In this review we discuss the current cellular therapies using either cell-derived factors or intact cells or using both the modalities for the treatment of wound healing. Fig. 1 depicts the current modalities of cell-based therapy.

2. PLATELET-DERIVED GROWTH FACTORS FOR THE TREATMENT OF WOUNDS

Blood consists of approximately 93% red blood cells (RBC), 1% white blood cells (WBC) and 6% platelets, all suspended in plasma. In platelet-rich plasma (PRP), the RBC count is lowered to 5, since they are less useful in the healing process, while the platelet count is increased to 94%. This leads to a plasma super rich in platelets, at a much higher percentage than would be found in normal blood concentrations i.e. 1,000,000 platelets/mL of plasma. Thus platelet-rich plasma (PRP) is a rich source of growth factors. The growth factors present in PRP are transforming growth factors (TGF-1 and TGF-2), vascular endothelial growth factor (VEGF), 3 isomers of platelet-derived growth factor (PDGF-1, PDGF-2, and PDGF-3), and endothelial growth factor [6]. These growth factors are considered to have the capacity to accelerate chemotaxis, mitogenesis, angiogenesis, and synthesis of collagen matrix and support tissue repair when applied on bone wounds [7]. Furthermore, autologous (derived from the same person) PRP represents a greater similarity to the natural healing process as a composite of multiple growth factors, is relatively safe due to its autologous nature and is produced as needed from patient blood. Conventional therapies such as dressings, surgical debridement, and even skin grafting cannot provide satisfactory healing since these treatments are not able to provide enough necessary growth factors to modulate the healing process. In these types of wounds, the balance between cytokines and extracellular matrix synthesis has been broken. The skin and extracellular matrix are difficult to regenerate and can lead to erosion and deterioration; therefore, primary fresh wounds progress into a chronic state [8]. Furthermore, PRP also contains a high level of leukocytes, which can inhibit infection [9].

Interestingly, after blending with calcium and thrombin, PRP turns into gel, which prevents growth factors and leukocytes from releasing, and maintains their activity for a longer time within the wound.

3. PLATELET-RICH PLASMA FOR THE TREATMENT OF DIABETIC FOOT ULCERS

A diabetic foot ulcer is a wound that develops when high blood sugar levels damage blood vessels and nerves. The damage leads to skin and tissue breakdown. Even a small cut or scratch can become a diabetic foot ulcer.

The number of people with diabetes worldwide was estimated at 131 million in 2000; it is projected to increase to 366 million by 2030 [10]. Previous studies have indicated that diabetic patients have up to a 25% lifetime risk of developing a foot ulcer [11]. The annual incidence of diabetic foot ulcers is ~ 3%. The diabetic patients are subjected to many complications because of foot ulcers, many of them like as chronic wound disease or pressure ulcers (bed sore). Routinely used medical measures for diabetic foot ulcers are depended to nursing care and usually take longer duration of time until pain relief, thus representing a huge burden on any health care system.

The standard current clinical treatment of diabetic ulcer includes local wound care with dressing, virgous and repeated debridement of necrotic tissue, and offloading. Antibiotics will also be given if infection exists. However, the result is still far from satisfaction, and 14%–20% of patients with diabetic ulcer will end up with amputation [12].

Platelet rich plasma (PRP) gel is an efficacious treatment of chronic diabetic foot ulceration [13]. It represents similarity to the natural healing process as a composite of multiple growth factors. It is also safe due to its autologous nature. There have been many studies lately conducted on the efficacy and safety of using platelet rich plasma to treat diabetic foot ulcers, and most of them have concluded that the patients had complete healing. The procedure is safe and there are no serious adverse effects of this therapy. If diabetic foot ulcers are left untreated or treated inappropriately or treated late, gangrene (death of the tissue) may result. PRP gel therapy provides ulcer management option to avoid loss of limb. Table 1 summarizes the clinical studies done so far using PRP.

4. PLATELET-RICH PLASMA FOR THE TREATMENT OF VENOUS AND TROPIC ULCERS

Venous ulcers are wounds that typically occur due to improper functioning of venous valves. They are the major causes of chronic wounds, occurring in 70% to 90% of chronic wound cases [14]. The treatment of venous ulcers results substantial costs. Conventional recombinant growth factor (GF) products, including becaplermin (recombinant platelet-derived GF) have been approved [15] by the Food and Drug Administration, USA, for the treatment of chronic wounds. However, the medication is in a liquid form, and, therefore, can dissipate following wound application. In addition, it is expensive and is unaffordable in developing countries such as India. In comparison, autologous platelet rich plasma (PRP) is a simple point-of-care procedure that helps in accelerating the wound healing by releasing many growth factors like platelet derived growth factors, fibroblast derived growth factors and epidermal growth factors, as chronic non-healing ulcers do not present the necessary growth factors (GFs) and hence do not heal well.

5. PLATELET-RICH-FIBRIN MATRIX (PRFM) FOR THE TREATMENT OF WOUNDS

Platelet-rich-fibrin (PRF) is a material that holds on to growth factors derived from platelets enmeshed in the fibrin network resulting in their sustained release over a period of time that accelerate the wound healing process [16]. In addition, the robust scaffolding structure of PRFM can provide more resistance to physiologic stress, more accurate implantation and may lead to longer persistence and resistance to washout at the site of injection. As opposed to PRP, which involves taking a small amount of patients' blood preoperatively, concentrating the autologous platelets by centrifugation, activating the platelets with thrombin and calcium and finally applying the resultant gel to the surgical sites, the autologous fibrin glue is created from platelet-poor-plasma and consists of primarily fibrinogen. When PRP combined with thrombin and other activators such as calcium is used as an autologous formulation of fibrin glue, the high concentration of platelets promotes wound healing. Table 2 listed the updated clinical studies done using PRFM.

Table 1. Clinical studies summarizing the use of platelet-rich plasma for wound healing

Study	Design	N	Study period	Wound type	Control group	Intervention group
Thomas P. SanGiovanni et al.	Allocation: Randomized Endpoint Classification: Efficacy Study. Intervention Model: Single Group Assignment Masking: Single Blind (Subject) Primary Purpose: Prevention	2000	2.6 yrs	Elective Foot and Ankle Surgery.	Group not receiving autogenous PRP and PPP.	Group receiving PRP and PPP, to surgical site.
Jeng Long-Bin	Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	10	1year	Diabetic Ulcers on Both Feet	Group not receiving autogenous PRP	Group receiving PRP at the site of wound
Wassim Raffoul	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment	45	10 months.	Skin Graft donor site wound	Group not receiving autogenous PRP and Calcium Chloride.	Group receiving PRP sprayed onto wound bed along with10% Calcium Chloride solution
Eduardo A Malavolta, Arnaldo A Ferreira Neto.	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Outcomes Assessor) Primary Purpose: Treatment	54	5.6 yrs	Rotator Cuff Tears	Group receiving conventional arthroscopic repair of rotator cuff without application of PRP	Group receiving conventional arthroscopic repair of rotator cuff with application of PRP.
Richard Holtby, et al.	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Double-Blind Primary Purpose: Treatment	80	3.6 yrs	Rotator Cuff Pathology	Patients will have a rotator cuffrepair without thePRP application.	Group receiving the platelet concentrate extracted from own blood (PRP) will be applied to the surgical site after completion of the repair.
Chris Hyunchul Jo	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Single Blind (Outcomes Assessor) Primary Purpose: Treatment	48	3.3 yrs	Rotator Cuff Tear	Conventional arthroscopic rotator cuffrepair	Arthroscopic rotator cuff repair with PRP

Study	Design	N	Study period	Wound type	Control group	Intervention group
Chris H. Jo,	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Single Blind (Outcomes Assessor) Primary Purpose: Treatment	74	2yrs	Rotator Cuff Tear	• Conventional arthroscopic rotator cuff repair	Arthroscopic rotator cuff repair with PRP
Aditya K Aggarwal	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Investigator, Outcomes Assessor) Primary Purpose: Prevention	40	1yr	Blood Loss Pain	Group doesn't received PRP.	Platelet-rich plasma was applied over the wound including the capsule, medial and lateral recesses.

Table 2. Clinical studies summarizing the use of platelet-rich-fibrin-matrix and biomaterials for wound healing

Study	Design	N	Study period	Wound type	Control group	Intervention group
Robert Burks et al.	Observational Model: Cohort Time Perspective: Prospective	37	4 yrs	Rotator Cuff Tear Arthropathy	Arthroscopic repair of rotator cuff tears with similar size characteristics without PRFM.	Platelet-rich fibrin matrix (PRFM) augmentation to at-risk arthroscopic rotator cuffrepairs.
Anthony P Sclafani et al.	Endpoint Classification: Efficacy Study	15	15 months	Nasolabial Folds	Aged 25- 75 years with moderate to severe nasolabial folds.	Biological: Platelet rich fibrin matrix 0-2 cc of autologous platelet rich fibrin matrix injected intra and subdermally to effect nasolabial fold.
Robert G. McCormack et al.	Allocation: Randomized Endpoint Classification: Efficacy Study, Intervention Model: Parallel Assignment	116	~3 yrs	Meniscus Lesion	Complete vertical longitudinal tear > 10 mm in length, tear located in the vascular portion of the meniscus, classified as either red-red or red-white zones, a stable knee, or a knee that is stabilized with a concurrent ACL reconstruction, skeletally	With PRP and Without PRP

Study	Design	N	Study period	Wound type	Control group	Intervention group
					mature patients 18-60 years of age.	
Andrew D Pearle et al.	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Double-Blind Primary Purpose: Treatment	136	-NA-	Rotator Cuff Tendon injuries	Patients 45 years of age and older who have failed conservative treatment for rotator cuff pathology. Patients in this study will have full thickness rotator cuff tears that are classified arthroscopically as medium (1 to 3 cm) or large (3 to 5 cm) and that are treated with arthroscopic repair.	Placement of Platelet Rich Fibrin Matrix During Arthroscopic Rotator Cuff Surgery
Park Nicollet Institute	Allocation: Randomized Endpoint Classification: Efficacy Study Intervention Model: Single Group Assignment Masking: Double-Blind Primary Purpose: Treatment	110	~5 yrs	Rotator Cuff Tear	Evidence of a rotator cuff tear as determined by clinical examination and diagnostic imaging (MRI), Patient undergoes arthroscopic rotator cuff repair, Age 18 or older	Double row, PRFM procedure :double row
Stephen Honeybul et al.	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	~10	~2 yrs and 5 mo	Surgically-Created Resection Cavity	All adult patients (age > 18 years) who have had a decompressive craniectomy, with a defect size of less than 80 mm in diameter	Repair of cranial defects by tissue engineering and repair of cranial defects by tissue engineering
Brant K Oelschlager et al.	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model:	150	4 yrs	Hernia Paraesophageal Hernia	• Documented symptomatic paraesophageal hernia	Hernia repair

Study	Design	N	Study period	Wound type	Control group	Intervention group
	Parallel Assignment Masking: Open Label Primary Purpose: Treatme nt					
Florias A Morfesis et al.	Observational Model: Cohort Time Perspective: Prospective	50	1 year	Inguinal Hernia	Patients with inguinal Hernia	Open inguinal hernia repair with mesh
Pornanong Aramwit et al.	Allocation: Randomized Endpoint Classification: Saf ety/Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatme nt	30	1 year	Allocation: Randomized Endpoint Classification: Safet y/Efficacy Study Intervention Model: Parallel A ssignment Masking: Open Label Primary Purpose: Treatment	Split thickness skin graft, the donor sites of split-thickness skin graft locate on the thigh.	Silk fibroin with bioactive coating layer dressing, Bactigras wound dressing
Daniel Altman et al.	Allocation: Non-Randomized Endpoint Classification: Saf ety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatme nt	250	6 mo	Pelvic Organ Prolapse	• Pelvic organ prolapse stage 2 or more according to the POP-Q staging system, symptoms related to pelvic organ prolapse including bulging, pelvic heaviness and vaginal protrusion	• Symptoms related to pelvic organ prolapse including bulging, pelvic heaviness and vaginal protrusion
Enrique Gomez Barrena et al.	Endpoint Classification: Saf ety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	30	2 yrs	Delayed Union After Fracture of Humerus, Tibial or Femur	• Traumatic isolated closed or open Gustilo I and II humerus, tibial or femur diaphyseal or metaphyseal-diaphyseal fracture status delayed union or non-union	Implantation of bone substitute plus autologous cultured mesenchymal cells Implantation surgery of a synthetic bone substitute associated with autologous bone marrow cells expanded
Luis Meseguer	Allocation: Randomized	12	3 yrs	Pseudoarthrosis	• Pseudarthrosis of tibia established any	ABM seeded onto a porous TCP and DBM

Study	Design	N	Study period	Wound type	Control group	Intervention group
Olmo et al.	Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment				cause with at least 9 months.	cells collection under sedation. 114 mL are obtained and processed through a ficoll gradient. Autologous bone marrow (ABM) cells seeded onto a porous tricalcium phosphate ceramic (TCP) and demineralized bone matrix (DBM)
W. Payne et al.	Allocation: Randomized Intervention Model: Single Group Assignment Masking: Open Label	13	~3 yrs	Diabetic Foot Ulcers (DFU) Venous Stasis Ulcers (VSU)	• Diabetic foot ulcer (DFU) or venous stasis ulcer (VSU) of the leg, with certain restrictions on size, duration and underlying health	OASIS wound Matrix OASIS (an acellular biomaterial that supports tissue repair with a scaffold-like matrix having a natural structure and composition)
Hannu T Aro et al.	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Single Blind (Outcomes Assessor) Primary Purpose: Treatment	180	~5 yrs	Bone Neoplasm	• Autologous bone graft (Stratum I) and allogeneic bone graft (Stratum II), primary or recurrent benign bone tumor or tumor-like condition that requires operative treatment by means of tumor evacuation and defect filling, Pathological fractures of patients in Stratum I are treated by means of conservative	Bioactive glass Surgical implantation: Beta-tricalcium phosphate (ChronOs) Surgical implantation Autograft Surgical transplantation from iliac crest Bioactive glass Surgical implantation Beta-tricalcium phosphate (ChronOs) Surgical implantation Allograft (frozen femoral head) Surgical transplantation

Study	Design	N	Study period	Wound type	Control group	Intervention group
					treatment for three months before tumor surgery	
Daniel Altman et al.	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Single Blind (Subject) Primary Purpose: Treatment	400	3 yrs	Vaginal Prolapse	Prolapse of the anterior vaginal wall ≥POPQ-stadium II Prolapse specific pelvic symptom	Anterior colporrhaphy Standardised colporrhaphy of the anterior vaginal wall Anterior PROLIFT Transvaginal mesh surgery of the anterior vaginal wall
Santiago Medialdea	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	20	~5 yrs	Corneal Ulcer	Patients will be subjected to the usual treatment of their condition, consisting of amniotic membrane transplantation	Anterior lamellar nanostructured artificial human cornea. Implantation of an anterior lamellar nanostructured artificial human cornea with allogeneic cells from dead donors and biomaterials

6. APPLICATION OF BIOMATERIALS FOR THE TREATMENT OF WOUNDS

Regenerative tissue engineering has the potential to revolutionize reconstructive surgeries using prefabricated tissue or responsive biomaterials with patient-specific geometry [17]. However, up until now, there are no models of an artificial skin that completely replicates normal uninjured skin. However, natural biopolymers such as collagen and fibronectin [18] have been investigated as potential sources of biomaterial to which cells can attach.

With its wide distribution in soft and hard connective tissues, collagen is the most abundant of animal proteins. In vitro, natural collagen can be formed into highly organized, three-dimensional scaffolds that are biocompatible, biodegradable, nontoxic upon exogenous application, and endowed with high tensile strength. These attributes make collagen the material of choice for wound healing and tissue engineering applications.

Table 3. Sources of allogenic and autogenic stem cells

Allogenic	Autogenic
Embryonic	Bone marrow
Fetal	Adipose tissue
Human umbilical cord	Skin
Human umbilical cord blood	
Bone marrow	

Unopposed activation of inflammatory macrophages (M1) is assumed as major cause for persistent inflammation [19]. Thus biomaterials capable to modulate macrophage activation and to promote inflammatory resolution from pro-inflammatory to less or anti-inflammatory represent a promising approach for treatment of non-healing wounds [20]. S. Frenz and his colleagues [21] had shown that collagen matrices containing high-sulfated hemagglutinin (HA) modulate phenotype and function of human pro-inflammatory M1 macrophages to make them less inflammatory. Furthermore, biomaterials have recently been shown to be effective against diabetic wounds [22]. Using spherical nucleic acid-gold nanoparticle (SNA) gene suppression to modulate GM3S expression and purified GM3 as a biochemical supplement, A Shehu and his colleagues from Northwestern University [23], were able to show enhanced insulin-mediated glucose transport and increased keratinocytes proliferation at the wounded site. Ganglioside GM3 has previously been shown to be accumulated in the diabetic skin and blocks insulin signaling. Table 2 also listed the updated clinical studies done using biomaterials.

7. APPLICATION OF STEM CELLS FOR THE TREATMENT OF WOUNDS

Cell therapy can be defined as a set of strategies that use live cells with therapeutic purposes. The aim of such therapy is to repair, replace or restore the biological function of a damaged tissue or organ. Thus, the use of stem cells in cell therapy has been investigated over several areas of medicine [24-26]. Stem cells are undifferentiated cells capable of auto renewing and differentiating into progenitor or precursor cells of one or several specific cell types. After the initial use of embryonic stem cells, the focus has been set on autologous mesenchymal stem cells over the past years. Adult stem cells are the most used in regenerative medicine; they are relatively easy to obtain through *in vitro* culturing and their use does not raise any ethical concerns as in the case of embryonic cells, although the proliferative ability and differentiation potential of adult stem cells are not as high [27]. Table 1 shows the sources of allogenic and autogenic stem cells. Adult stem cells can be collected from almost any tissue; nevertheless, bone marrow (BM) is possibly the most common source. Several studies point out that cells obtained from the BM contribute to the regeneration or repair of many tissues, including the myocardium, bone, tendons, cartilage, and skin [28].

The BM is composed of a heterogeneous cell population, including fibroblasts, adipocytes and mononuclear Cells (MNCs). BM-MNCs cells include hematopoietic stem cells (HSCs), mesenchymal stem Cells (MSCs), endothelial progenitors, and cellular precursors [28]. HSCs are responsible for all blood cell lines (erythrocytes, platelets and white cells). MSCs are a group of stem cells originated at the mesodermal germinal layer and they are found in very small quantities in the bone marrow (about 0.001-0.01% of mononuclear cells). In addition, these MSCs can differentiate into osteoblasts, adipocytes, and chondrocytes. Once a wound occurs, both HSCs and MSCs mobilize from the BM to the wound site, where they manage and regulate cell proliferation and migration during the inflammation phase of cicatrization [29]. Both cell types feature a high degree of plasticity, and are able to contribute with cell progenitors for hematopoietic and non-hematopoietic tissues.

Table 4. Clinical studies summarizing the use of stem cells for wound healing

Study	Design	d	Study period	Wound type	Control group	Intervention group
Diethelm Tschoepe Herz- und Diabeteszentrum et al.	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment	30	4 yrs	Diabetic Foot	Group doesn't receiving stem cells.	Group receiving bone marrow cells administered intramuscular or intra arterial or expanded bone marrow cells administered a intramuscular or intra arterial resulting in five distinct groups.
Edmond Ritter	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment	30	1 yr	Wounds	Control group will receive collagen matrix for wound coverage and standard wound care that will include chemical/surgical debridement	Patients will receive amniotic stem cell (NuCell) embedded in collagen matrix for wound coverage, and standard wound care that will include chemical/surgical debridement.
Carl Schulman et al.	Allocation: Non-Randomized Endpoint Classification: Safety Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Health Services Research	20	~3 yrs	Skin Burn Degree Second	Superficial, Intermediate or Deep 2nd Degree Burn Wounds	Allogeneic (MSC's) Application to the Burn Wounds. The first group of 5 will be started on the lowest dose. If there are no adverse reactions, the second group of 5 will receive a higher dose. This will be repeated for the third and fourth groups with each receiving a higher dose.
Robert Soler et al.	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment	15	~3 yrs	Osteoarthritis, Knee Knee injuries Joint Diseases	Gonarthrosis grade II-III of Kellgren and Lawrence, chronic knee pain with mechanical	Autologous MSC knee implantation, Isolation and "Ex-Vivo" expansion of

Study	Design	d	Study period	Wound type	Control group	Intervention group
	Masking: Open Label Primary Purpose: Treatment			Rheumatic Diseases Cartilage Diseases	characteristics	Mesenchymal stem cells (MSC) obtained from each patient's bone marrow under GMP conditions at Xcelia-División de Terapias avanzadas del Banc de Sang I Teixits. After 21 days, approximately 40 millions of autologous MSC will be implanted in the knee by articular injection.
Issa F. Khouri et al.	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	30	3 yrs	Lymphoma	Patients with mantle cell lymphoma, T-cell lymphoma, and diffuse large b-cell lymphoma within 6 months post autologous transplantation and without relapse.	Drug: Carfilzomib Drug: Dexamethasone
Andrew Goldberg et al.	Endpoint Classification: Safety Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	10	~2 yrs	Achilles Tendinitis, Right Leg Achilles Tendinitis Achilles Degeneration Achilles Tendon Thickening Tendinopathy Achilles Tendinitis, Left Leg	Participants with chronic midportion AT with symptoms for longer than 6 months	Autologous Mesenchymal stem cells

Study	Design	d	Study period	Wound type	Control group	Intervention group
Jerome Roncalli et al.	Endpoint Classification: Safety Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	10	5 yrs	Chronic Myocardial Ischemia Left Ventricular Dysfunction	NYHA (Heart failure) Class II-IV or Angina pectoris CCS Class III or IV, Chronic coronary artery disease with left ventricular function below 35%	Mesenchymal stem cell 60x106 MSCs Trans-endocardial intramyocardial injections (n=14-16)
Jose D Araújo et al.	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment Masking: Open Label Primary Purpose: Treatment	10	~1 year	Critical Limb Ischemia Ischemic Ulcers	Patients with lower limb ischemia	Stem cells transplant
Vaclav Prochazka et al.	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment	96	2 yrs	Critical Limb Ischemia	Patients suffering from chronic and critical limb ischemia	BMAC application in Critical Limb Ischemia
Joshua M Hare et al.	Allocation: Randomized Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment Masking: Double Blind (Subject, Investigator) Primary Purpose: Treatment	30	~4 yrs	Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction.	Ischemic left ventricular dysfunction secondary to myocardial infarction	Allogeneic hMSCs

Furthermore, recent studies reveal that BM cells, most notably MSCs, play a major role in skin regeneration and vascularization [30-33]. MSCs influence the wounds' ability to progress beyond the inflammatory phase and not regress to a chronic wound state. The mechanism of action of these cells is that they directly attenuate inflammatory response so that they decrease secretion of the pro-inflammatory cytokines while increasing the production of anti-inflammatory cytokines [34]. These anti-inflammatory properties make them particularly beneficial to chronic wounds by advancing the wound past a chronic inflammatory state into the next stage of healing. Nowadays, it is recognized that MSCs have antimicrobial activity [35]. Furthermore, growth factors derived from MSCs promote dermal fibroblast proliferation, angiogenesis, and collagen deposition. Diverse mechanisms have been proposed to explain the regenerative properties of MSCs. One such mechanism is MSCs migrating into the wound site and differentiate into cells with the phenotype and function required to repair the damaged tissue. This theory constitutes a remarkable foundation upon which cellular theory stands.

The skin is home to many different types of stem cells including epidermal stem cells, melanocyte stem cells (which make pigment-producing cells), and epithelial and mesenchymal stem cells that reside in hair follicles. Together, these stem cells are responsible for making the multitude of different skin cells that are important for perpetually renewing normal and healthy skin [36]. In response to general injuries the number of circulating stem cells in the body increase. Hair follicle stem cells and epithelial stem cells are thought to interact with bone marrow stem cells, which are recruited to a wound during the inflammatory phase of wound healing, and together these stem cells bring about speedy wound closure and tissue repair [37]. To date, it is not clear whether stem cells from the bone marrow might also contribute to the healing process by actually making new skin cells.

Furthermore, MSCs from bone marrow (BM-MSCs) in patients with chronic wounds can achieve the closure of the wound and tissue reconstruction. The clinical benefits of systemic administration of MSCs were also observed in a study carried out by Lu et al. [38] performed on diabetic patients with critical lower limb ischemia. Patients were injected with autologous BM-MSCs or bone marrow mononuclear cells (BM-MNCs) in one of their legs. In the contra lateral leg they were injected with saline serum as control. The results at 24 weeks after transplantation showed an improvement in pain and a significant increase of the healing rate of the ulcer. BM-MSCs therapy may be better tolerated and more effective than BM-MNCs for increasing lower limb perfusion and promoting foot ulcer healing in diabetic patients with critical limb ischemia. However, in other types of wounds, such as pressure ulcers, BM-MNC administration produced beneficial results for wound closure. All these results suggest that cell therapy with BM-MSCs or BM-MNCs applied either topically or systemically yields clinical benefits for the treatment of chronic wounds. Furthermore, some remarkable results can be achieved in the treatment of chronic wounds by combining BM-MSCs with tissue engineering, i.e. application of cells on an adequate support/scaffold which ensures cells remain viable and may efficiently migrate in the wound bed. Table 4 summarizes the updated clinical studies using stem cells.

8. CLINICAL APPLICATIONS OF ADIPOSE-TISSUE-DERIVED MSCS (ASCS) IN WOUND HEALING

As previously discussed (Table 3), MSCs can be harvested from different tissues. Some of these alternative sources are very promising, because bone marrow harvesting is rather invasive and painful. In 2001 adipose tissue derived MSCs (ASCs) from lipoaspirates was identified and characterized. A promising and cost-effective source of autologous mesenchymal stem cells is subcutaneous adipose tissue. The content of ASCs per gram tissue is five-fold higher than in bone marrow. They are less invasive to harvest, migrate to the wound site through paracrine effects and catalyze wound healing via paracrine mechanisms as well as fusion and differentiation, for example, into keratinocytes or fibroblasts; two essentials cellular components of skin. On the other hand, BM-MSCs reside in the BM stroma, but only a very small percentage of the nucleated cells that compose the BM are actually MSCs, whereas the amount of ASCs is approximately 500-fold greater when isolated from an equivalent amount of adipose tissue. ASCs are relatively homogeneous based on their surface immunophenotype, displaying similar, but not identical, surface antigens to those found in BM-MSCs. *In vitro*, ASCs can differentiate along multiple pathways, including adipogenic, chondrogenic, adipogenic, and myogenic [39-40]. Furthermore, ASCs secrete an array of cytokines and growth factors simila to those released by

BM-MSCs. All these features make ASCs an interesting alternative for cell therapy, and hence they are currently being used for a variety of clinical treatments, including wound healing. As yet, there are very few clinical trials using ASCs for wound healing. A small pilot study with 20 participants looked at using ASCs for chronic wounds caused by radiation treatment for cancer [41]. ASC therapy transformed the radiation-damaged tissue into normal tissue and it was hypothesized to be the ability of ASCs to promote blood vessel formation. This early success has paved the way for additional trials testing the safety and efficacy of ASCs for wound healing. However, the use of ASCs in wound healing applications is still limited by a lack of substantial clinical data. Therefore, further studies are needed to validate the usage of ASCs for their routine clinical application.

9. CONCLUSION

Wound repair constitutes an intricate process where different cell types and molecules act in an orchestrating way. Any disruption in the process, could lead to inappropriate healing and/or healing failure leading to chronic wound or ulcer development. Current therapies such as surgery, dressings, topical negative pressure or skin substitutes are not always effective. Cell therapy using cell-derived factors and intact stem cells has emerged as a promising tool in those cases where conventional treatments failed. However, prospective studies with greater follow-up periods are necessary to verify the long-term safety and efficacy of PRP. Furthermore, regardless of the interesting results presented in this review, the use of MSCs for cell therapy often requires *in vitro* expansion that can delay the treatment especially for the larger wounds, augments contamination risks due to extensive culturing and can increase the cost for therapy as well. Importantly, because the precise underlying mechanism that allows this beneficial effect of stem cells in wound healing is not completely understood, future studies focused on the role of adult stem cells in wound healing are needed in order to understand the safety and to further improve the efficacy of this therapy. In spite of some limitations, cell-based therapies using both cell-derived factors and intact cells promise as an effective treatment modality for wound healing.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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