From Stem Cells to Immune Cells Populations

Subjects: Immunology

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Although stem cells have been considered promising for the treatment of degenerative diseases by ‘seeding’ them into damaged tissues, it has recently been observed that the regenerative capacity of stem cells is influenced and regulated by the local immune response and in particular by macrophages, which constitute a central component of the damage response and are the coordinators of tissue repair and regeneration. Among the panoply of immune cells involved in the response to both acute and chronic wounds, recent discoveries have highlighted novel and often unexpected roles for certain types of immune cells in promoting a permissive local environment for effective cell replacement and restoration of tissue integrity. Some studies have shown that the control of inflammation is crucial in regenerative therapies: To be effective, regenerative therapies must block and control inflammation to allow tissue regeneration by resident stem cells. Indeed, the presence of inflammation inhibits the regenerative action of tissue-resident mesenchymal stem cells (MSCs). Recent papers suggest that an innovative regenerative strategy could be to polarize macrophages from the M1 inflammatory state to the M2 anti-inflammatory state utilizing immune cells. These reviews conclude that next-generation regenerative therapies need an immune-centric approach instead of the use of stem cells. Thus, depending on the tissue or organ targeted, regenerative strategies could be developed to stimulate macrophage polarization or to recruit subpopulations of pro-healing macrophages. Already, it has been observed that the regeneration of myocardial tissue after ischemia was induced by macrophages that regulate resident stem cells and promote regeneration, suggesting that targeting macrophages could be a new strategy to improve infarct healing and repair. The regenerative and stem-cell-controlling capacity of macrophages has also recently been demonstrated in bone tissue: mesenchymal stem cells act through a paracrine and immune-modulatory and non-differentiative mechanism and the microenvironment and immune system regulate the activity of MSCs regardless of the tissue from which they originate. Based on the role played by several types of macrophages and lymphocytes in the wound-healing response, it is tempting to hypothesize that interventions that reduce the M1 macrophage phenotype and promote M2 may represent a new therapy to induce tissue regeneration.

wound healing diabetic foot immune system monocytes lymphocytes immune centric revolution

1. How to Switch to M2 Regenerative Phenotype?

Macrophages play an important role in wound healing, and the switch to anti-inflammatory M2 phenotypes is necessary for efficient tissue healing. Questions remain regarding monocytes recruitment and macrophage differentiation, specifically whether monocytes are predetermined to differentiate in one specific phenotype, M1 or

M2, or if macrophages polarize from M1 to M2 phenotypes (or vice versa) within the wound. Various approaches have been taken to immune-modulate macrophages to polarize in M2 phenotypes and/or simultaneously M1 macrophages (**Figure 1**). A list of methods to switch to M2, such as immune cell-based therapy, MSC, M2 exosome, and dermal substitute will be discussed.

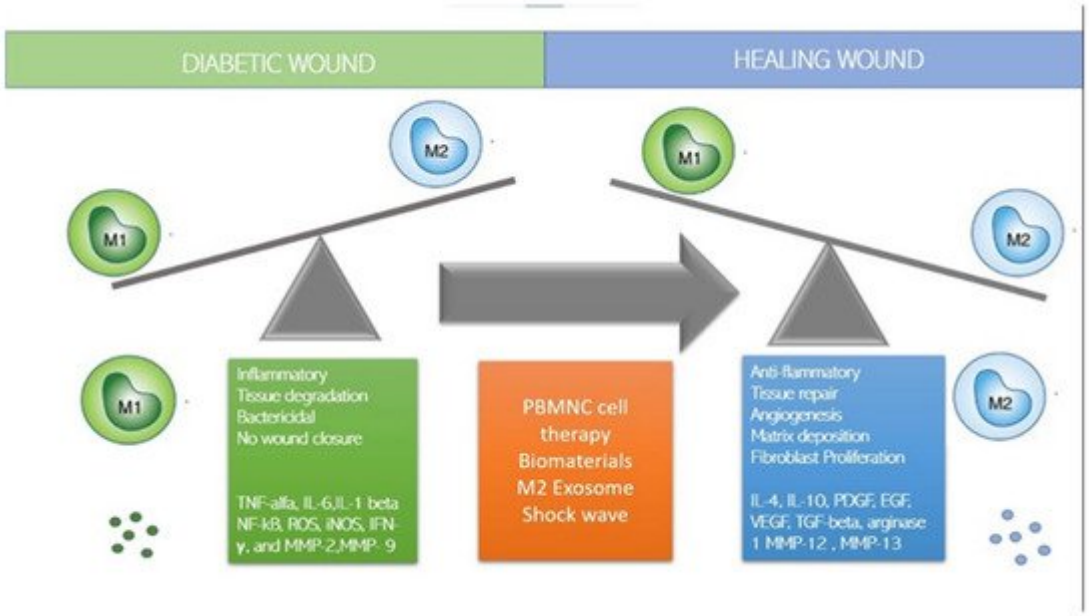

Figure 1. How to switch to M2: strategies.

2. Immune-Cell-Based Cell Therapy

Cell-based therapies are rapidly emerging in regenerative medicine as dynamic treatments that perform multiple therapeutic functions. Monocytes and macrophages, as innate immune cells involved in inflammation control and tissue repair, are increasingly popular clinical candidates due to their robust angiogenic, anti-inflammatory, and regenerative ability. **Table 1** shows a brief description and clinical result of clinical trials based on macrophages or peripheral blood mononuclear cells describe in this review. The treatment of chronic ulcers with blood-derived macrophages activated by hypo-osmotic shock has been used effectively in over 1000 patients in Israel [1]. Previously, Danon et al. in 1997 treated pressure ulcers in elderly patients by injecting macrophages from blood units of young, healthy donors near the wound periphery plus a portion of the cell suspension deposited on top of the wound [2]. Patients were treated with a single implant, or with a second one when delayed healing was present 1 month later, and wound healing was compared with conventional methods (debridement, antibiotics, and wound dressings). In the macrophage-treated group, 27% healed, while only 6% healed in the control group ($p < 0.001$). Moreover, the macrophage-treated group showed faster healing ($p < 0.02$), and no side effects were reported [2]. A second prospective controlled trial was designed to compare macrophage injections from healthy donors (66 patients) to standard care treatments (38 patients) for stage III and IV pressure ulcers in elderly patients. The results showed a significantly higher percentage of completely closed wounds in the macrophages-treated group in comparison to standard care [3]. Interestingly, in the subset of diabetic patients, 65.5% of wounds with the

macrophage treatment healed, while only 15.4% of healing was observed in the standard care group [3]. Magenta et al. recently published an extensive review on autologous cell therapy from different tissue sources (blood, bone marrow, and adipose tissue) to treat critical limb ischemia in diabetic patients, reporting data from basic science to clinical trials [4]. Autologous cell therapy, in particular, Peripheral Blood Mononuclear Cells (PBMNC), based on monocytes/macrophages and lymphocytes represent an interesting strategy to treat non-option critical limb patients and diabetic foot patients [5][6][7][8][9][10]. Rigato et al. on a recent meta-analysis on no-option critical limb ischemia (NO CLI) patients showed that PBMNCs, but not other cell types, were associated with a significant decrease in amputation and increase in amputation-free survival [11]. The same results were observed by Liew et al. in a meta-analysis of 16 randomized trials where PBMNC lowered the risk of major amputation and increased ulcer healing significantly [12]. Three other meta-analyses on autologous cellular therapy including PBMNC on diabetic foot patients showed a benefit of wound healing and reduced amputation associated with $TcPO_2$ increase and reduced pain [13][14][15]. Dubsky et al. have treated 28 patients with diabetic foot disease (17 treated with bone marrow cells and 11 with PBMNC) comparing the result with a control group treated with standard care at 6 months and have reported a statistical increase in TcPO2 with no significant differences between bone marrow cells and peripheral blood cell groups, while no change in transcutaneous oxygen pressure in the control group was observed [8]. In addition, the 6-month major amputation rate was significantly lower in the cell therapy group compared with that in the control group (11.1% vs. 50%), with no difference between bone marrow cells and peripheral blood cells [8]. Interestingly, the same group reported a comparable improvement of CLI major amputation with autologous cell therapy in diabetic foot patients compared with repeated PTA and more effective healing of foot ulcers in the cell therapy group [16]. A user-friendly point of care device based on peripheral blood selective filtration to be used for intra-operatory use in human cell therapy has been developed to produce fresh autologous PBMNC, with evidence in terms of adequate potency in therapeutic angiogenesis in vitro and in vivo [17]. Promising results have been obtained from implanting PBMNC produced by a specific device (Hematrate Blood Filtration system Cook Regentec) in different clinical trials including diabetic patients [9][10][18][19]. Persiani et al. have observed a 9.4% decrease in major amputation in 18 no-option patients with diabetes treated by PBMNC together with an increase in TCPo2 and a pain reduction at 2 years [9]. A similar result in terms of major amputation has also been previously reported on CLI non-option patients, including diabetic and Burgers patients, treated with PBMNC produced by apheresis [5]. Interestingly, it has been demonstrated by a histological examination of incisional biopsies of diabetic non-healing ulcers that autologous PBMNC implants produced by this selective filtration point of care and injected perilesional around diabetic non-healing wounds polarize M1 macrophages in M2. Moreover, the implantation of A-PBMNC promotes relevant changes in the overall molecular setting over time [20]. The consequent cellular and biochemical adaptations favour the establishment of conditions similar to physiological ones that progressively support the regeneration of damaged tissues and finally wound healing measured as inhibition of HIF, NF-KB, and TNF-alpha, progressive polarization of M1 into M2, increase in VEGF, and newly formed capillaries [20]. As the regenerative processes occur, an increase in the vascular network formation is clearly seen [20]. These preliminary data confirm in the ability of fresh, naïve, autologous PBMNC to induce immunomodulation through macrophage polarization and that this results in complete wound healing in a diabetic ulcer. On the contrary, the delivery of macrophages polarized in vitro into M2a and M2c phenotypes and then injected into mouse wounds did not accelerate healing in wild type mice and delayed healing in diabetic mice

[21]. The same study also observed a delayed re-epithelialization and persistence of neutrophils and M2 macrophages in diabetic treated wounds 15 days post-injury, suggesting that the application of ex vivo generated M2 macrophages is not beneficial and contraindicated for cell therapy of skin wounds. It seems instead that to produce a positive clinical outcome in terms of wound healing, polarization should occur in the patients in the wounded tissue which send the right microenvironmental signals to PBMNC. The same groups showed that the implants of Matrigel supplemented with M2a and M2c macrophage subsets in a mice wound model showed an increased number of endothelial cells and tubular structures, while M1-enriched Matrigel did not, suggesting that macrophages polarized towards an M2 phenotype seem to have a higher angiogenic potential compared to other subsets [22]. Accordingly, Di Pardo et al. also observed an increase in VEGF and laminin in the diabetic wound after PBMC implant [20]. A similar result was observed for the first time by De Angelis et al. in no-option CLI patients, including a subset of diabetic patients, after PBMNC implant [10]: histological data confirmed dermal granulation tissue and an increased number of monocytes (CD68+) and newly formed microvessels (CD31+). After the PBMNC treatment in the healed epidermis, the presence of the new vessels was observed, whereas dermal inflammation and monocyte infiltration were reduced. All these data suggest that autologous PBMNC represent a safe, consolidated effective therapy for critical limb ischemia and diabetic foot non-healing wounds. Considering the low invasiveness and the repeatability, PBMNC could represent the new frontier that will replace stem cell therapy.

Table 1. Immune-Cell-based Cell Therapy—Clinical trials on diabetic patients.

	Description	Result	Ref.
Zuloff-Shani et al. 2004	Treatment of chronic ulcers with blood-derived macrophages activated by hypo-osmotic shock in over 1000 patients	Reduction of the healing time, reduction of risk of complications and morbidity. Improvement of the quality of life for long-suffering patients	[23]
Danon et al.	Decubital ulcers of 72 patients (average age 82), were treated by local injection of macrophages prepared from a blood unit in a closed sterile system. The remaining 127 patients (average age 79) were treated conventionally and served as controls. No exclusion criteria were applied.	In the macrophage-treated group, 27% healed, while only 6% healed in the control group ($p < 0.001$). Moreover, the macrophage-treated group showed a faster healing ($p < 0.02$)	[24]
Zuloff-Shani et al. 2010	100 consecutive elderly patients with a total of 216 stage III or IV pressure ulcers, 66 patients were assigned to the autologous macrophages group, 38 patients were assigned to the standard care treatments (38 patients.)	Percentage of completely closed wounds (wound level and patient level) were significantly better ($p < 0.001/p < 0.001$, respectively) in all patients in favor of AMS, as well as in the subset of diabetic patients ($p < 0.001/p < 0.001$).	[25]
Moriya, J et al.	Retrospective study on 42 patients with severe intermittent claudication,	Improvement of ischemic symptoms was observed in 60% to 70% of the patients. The annual rate of	[26]

	Description	Result	Ref.
	ischemic rest pain, or non-healing ischemic ulcers caused by peripheral arterial disease, including thromboangiitis obliterans, and who had not responded to conventional therapy that included nonsurgical and surgical revascularization (no option).	major amputation was decreased significantly by treatment. The survival rate of younger responders was better than that of non-responders.	
Huang, P.P et al.	150 patients with peripheral arterial disease were randomised to mobilized PBMNC 76 cases or BMMNC 74 cases implanted, follow up for 12 weeks. Primary outcomes were safety and efficacy of treatment, based on ankle-brachial index (ABI) and rest pain	Significant improvement of the ABI, skin temperature and rest pain was observed in both groups after transplantation and was better I PBMNC group. However, there was no significant difference between two groups for pain-free walking distance, transcutaneous oxygen pressure, ulcers, and rate of lower limb amputation	[27]
Liotta, F et al.	Autologous Non-Mobilized Enriched Circulating Endothelial Progenitors obtained from non-mobilized peripheral blood by immunomagnetic selection of CD14+ and CD34+ cells) or BM-MNC were injected into the gastrocnemius of the affected limb in 23 and 17 patients with no option critical limb ischemia.	After 2 yrs follow-up, both groups showed significant and progressive improvement in muscle perfusion (primary endpoint), rest pain, consumption of analgesics, pain-free walking distance, wound healing, quality of life, ankle-brachial index, toe-brachial index, and transcutaneous PO2	[28]
Dubsky, M et al.	28 patients with diabetic foot disease (17 treated by bone marrow cells and 11 by peripheral blood mononuclear cell) were included into an active group and 22 patients into a control group without cell treatment.	The transcutaneous oxygen pressure increased significantly ($p < 0.05$) compared with baseline in both active groups after 6 months, with no significant differences between bone marrow cells and peripheral blood cell groups, while no change in the control group was observed. The rate of major amputation by 6 months was significantly lower in the active cell therapy group compared with that in the control group (11.1% vs. 50%, $p = 0.0032$), with no difference between bone marrow cells and peripheral blood cells.	[29]
Persiani, F. et al.	50 diabetic patients affected by CLI underwent PBMNCs implant (32 patients underwent PBMNCs therapy associated with endovascular revascularization, 18 patients, non-option CLI)	The follow-up period was 10 months. In the PBMNC group + revascularization TcPO, pain VAS Scale improved. In PBMNCs therapy group, the mean TcPO2 improved from 16.2 ± 7.2 mmHg to 23.5 ± 8.4 mmHg ($p < 0.001$), and VAS score means decreased from 9 ± 1.1 to 4.1 ± 3.3 ($p < 0.001$). Major amputation was observed in 3 cases (9.4%), both in adjuvant therapy group and in PBMNCs therapy. (16.7%) (P ¼ 0.6) as the therapeutic choice (PBMNCs therapy group).	[30]

	Description	Result	Ref.
De Angelis, B et al.	Prospective, not randomized study based on a treated group who did not respond to conventional therapy (n = 43) when implanted with A-PBMNC cells versus a historically matched control group. Patients of both groups were suffering from CLI Fontaine scale IV with chronic ulcers	The A-PBMNC-treated group showed a statistically significant improvement of limb rescue of 95.3% versus 52.2% of the control group ($p < 0.001$) at 2 years. The A-PBMNC group also showed reduction in pain at rest, increased maximum walking distance, and healing of the wound and an overall improvement in the quality of life. Post-treatment radiological studies showed an improvement of vascularization with the formation of new collateral and by histological findings.	[31]
Dubsky et al.	31 patients with DFU and CLI treated by autologous stem cells and 30 patients treated by PTA were included in the study; 23 patients with the same inclusion criteria who could not undergo PTA or cell therapy formed the control group.	Amputation-free survival after 6 and 12 months was significantly greater in the cell therapy and PTA groups compared with controls ($p < 0.001$ and $p < 0.0029$, respectively) without significant differences between the active treatment groups. Increase in TcPO2 did not differ between cell therapy and PTA groups until 12 months but TcPO2 in the control group did not change over the follow-up period. More healed ulcers were observed up to 12 months in the cell therapy group compared with the PTA and control groups (84% vs. 57.7% vs. 44.4%; $p < 0.042$).	[32]
Scatena et al.	The study included 76 NO-CLI patients with DFUs. All patients were treated with the same standard care (control group), but 38 patients were also treated with autologous PBMNC implants.	Only 4 out 38 amputations (10.5%) were observed in the PBMNC group, while 15 out of 38 amputations (39.5%) were recorded in the control group ($p = 0.0037$). The Kaplan–Meier curves and the log-rank test results showed a significantly lower amputation rate in the PBMNCs group vs. the control group ($p = 0.000$). At two years follow-up, nearly 80% of the PBMNCs group was still alive vs. only 20% of the control group ($p = 0.000$). In the PBMNC group, 33 patients healed (86.6%) while only one patient healed in the control group ($p = 0.000$).	[33]
Di Vieste et a[35]	Case report of a 59-year-old patient with type 2 diabetes mellitus who had a gangrene of the right toe. After an ineffective angioplasty, it was decided to use a PBMNC therapy.	The patient underwent to amputation of the first necrotic toe and three PBMNC treatment sessions with complete surgical wound healing and limb rescue	

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aged cells [34].
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supported by data showing that the suppression of lymphocytes T proliferation induced by MSC in co-cultures is achieved only after the supplement of adequate amounts of IFN-γ and TNF-α [36]. By producing a large number of immunomodulatory molecules such as TGF-β, hepatic growth factor (HGF), nitric oxide (NO), indolamine 2,3-dioxygenase (IDO), L-10, IL-6, IL-1 receptor antagonist (IL-1Ra), heme-oxygenase-1 (HO-1), prostaglandin E2 (PGE2), and pro-angiogenic factors VEGF, angiopoietin-1, placental growth factor (PGF), HGF, basic fibroblast growth factor (bFGF), TGF-β, PDGF, and IL-6, MSCs regulate immune response and vasculogenesis, crucially contributing to the enhanced repair of injured tissues in various organ [37]. The transplantation of autologous MSCs effectively repaired corneal wounds, and macrophage depletion completely abrogated MSC-based beneficial

effects, confirming that the cooperation between MSCs and macrophages was required for successful vascular regeneration [37]. MSC-injected survivals is dependent on the phenotype and function of tissue-resident macrophages [37][38]. As observed in myocardial infarction and spinal cord in murine models of injury, anti-inflammatory M2 macrophages offer a favourable environment for the engraftment of MSCs [38]. Moreover, the polarization of M1 macrophages to M2 phenotype is critical for the long-term survival of MSCs in healing tissues, suggesting that a reciprocally positive feedback loop exists between M2 macrophages and MSCs [38]. In a similar fashion, Tregs enhance the survival and engraftment of MSCs in ischemic tissues, and Tregs may even improve the angiogenic properties of MSCs by improving VEGF production [39]. It is important to consider the special characteristics of chronic wound environments, such as low oxygen tension, and how they may influence cell functions. It has been observed that a hypoxic environment diminished macrophage plasticity in response to MSCs [40]. Moreover, in vitro studies showed that macrophages cultured in normoxic conditions with MSCs produced high levels of IL-10, however, while in hypoxic conditions (1% O_2), the release of the inflammatory cytokine was strongly reduced [40]. In vitro assays showed that MSC from diabetic patients' adipose tissue demonstrated reduced proliferative capacity and decreased VEGF paracrine release, with lower expression of the stemness gene SOX2 [41]. In keeping, the MSC from Stromal Vascular Fraction (SVF) of diabetic patients did not rescue limb ischemia and this reduced its effect and has been correlated to a significant depletion of CD271+ cells compared to non-diabetic patients [41]. Accordingly, Cianfarani et al. also showed that MSCs from diabetic mice released lower amounts of hepatocyte growth factor and insulin-like growth factor-1 and that the supernatant of diabetic ASCs manifested in a reduced capability to promote keratinocyte and fibroblast proliferation and migration, probably due to a reduced ability for macrophage polarization in M2 [42]. Moreover, the density of adipose-derived cells (ASC) was lower in the adipose tissue of diabetic rats compared with non-diabetic rats and did not promote wound healing in diabetic rats, suggesting that caution is necessary regarding the clinical use of diabetic adipose tissue for the treatment of diabetic wounds [43]. ASC from diabetic patients also exhibited a reduction in VEGF secretion and an impaired angiogenic capacity [44]. Overall, these data suggest that the therapeutic cell therapy potential from the adipose tissue of diabetic patients (SVF, MSC, Adipose-derived stem cells ADSC) is dampened when compared with cells isolated from nondiabetic patients because diabetes alters MSCs' intrinsic properties and impairs their function [45].

In addition, the bone marrow of diabetic patients showed a deep remodelling, consisting of a strong reduction in micro-vessels and sensory neurons, as well as fat accumulation, which creates an unfavourable microenvironment for resident stem cells, which in turn compromises the regenerative efficacy of bone marrow cells which could become harmful vectors of inflammation and anti-angiogenic molecules in diabetic patients [46][47]. This is an important issue that emerging autologous therapies should keep in consideration regarding diabetic non-healing wounds.

4. Extracellular Vesicles (EVs) and Exosome (Exo)

EV is a generic term for membrane-contained particles naturally released by cells, not containing a nucleus. EVs are traditionally divided into subtypes based on the vesicle sizes: exosomes (50–150 nm diameter), microvesicles

(100–1000 nm diameter), and apoptotic bodies (50–4000 nm diameter). Exosomes are formed after the fusion of the membrane of the endosome with the plasma membrane, while both microvesicles and apoptotic bodies are generated by direct outward blooming from the cell surface [48]. MSC-derived extracellular vesicles (MSC-EVs) can transfer functional proteins and nucleic acids, including microRNAs (miRNAs) and messenger RNAs (mRNAs) to other cells without cell-to-cell contact. Recent studies have demonstrated that MSC-EVs reduce M1 polarization and/or promote M2 polarization in a variety of settings such as cardiovascular, pulmonary, digestive, renal, and central nervous system diseases [48]. An in vitro study revealed that MSCs derived from adipose tissue through exosome release induce M2 polarization [49]. He et al. recently showed that the early depletion of macrophages also delayed wound repair after MSC injection, confirming that MSC-mediated wound healing requires macrophages [50]. In the same paper, the authors demonstrated that MSCs from bone marrow infused systemically could translocate to reach the wound site, promote M2 polarization, and enhance wound healing [50]. The authors also observed that exosomes derived from MSCs induced macrophage polarization while the depletion of the exosomes of MSCs reduced the M2 phenotype [50]. Infusing MSCs without exosomes produced a smaller number of M2 in the wound site and delayed repair [50]. The paper also showed that miR-223, derived from the exosomes of MSCs, regulated macrophage polarization by targeting transcription factor p-Knox 1 [50]. These important findings provided evidence for the first time that MSC provokes M2 polarization and could accelerate wound healing by releasing exosome-derived microRNA. Li et al. confirmed that macrophage-derived exosomes exercised anti-inflammatory effects through the inhibition of the secretion of inflammatory enzymes and cytokines and provided the healing of diabetic wounds by significantly quickening angiogenesis and improving repair [51]. Another study confirmed that M2-derived exosomes (M2-Exo) induce a complete switch of M1 to M2 [52]. The subcutaneous injections of M2-Exo into the wound edge decreased the local populations of M1 and increased the M2 population and accelerated wound healing by improving angiogenesis, re-epithelialization, and collagen deposition. Accordingly, in a diabetic rat model, it has been observed that exosomes that are overexpressing transcription factor Nrf2 hasten wound healing by inducing vascularization [53]. Exosomes derived from macrophages may represent a novel therapeutic strategy in the treatment of diabetic wound damage.

5. Dermal Substitutes

Fully acellular dermal substitutes are used in DFU treatment because of the high safety profile and beneficial outcomes as reported in the literature. Ideal scaffolds and tissue substitutes including skin matrices should be non-immunogenic, regenerative, protective, durable, and biocompatible. On the basis of the innovative macrophage-centred approach, they also should have a good capacity to induce M2 polarization [54]. Their therapeutic outcome originates from and is dependent on their source, method of preparation, and further modification. The decellularization method and tissue source can deeply affect the wound microenvironment when the substitute is implanted. Cross-linking or the possible addition of other substances can affect the wound environment and the clinical outcome as well [55]. The chemotactic attractiveness of human fibroblasts to collagens I, II, and III have been studied for many years and is well recognized. Monocytes' adhesion to collagen types I and III showed a noticeable effect on the secretion of different mediators, including growth factors, cytokines, and enzymes, which in turn play a key role in normative wound healing [56]. Predictably, the diverse surface morphologies and integrated

active components can induce an effect on the macrophage's phenotype. Consequently, it is extremely important to study the immunomodulatory effects of dermal substitutes, especially when implanted on chronic and/or diabetic wound. It has been observed that particular geometrical parameters could direct human macrophage polarization [57]. Fibrous collagen scaffolds with box-shaped pores and precise inter-fibre spacing from 100 µm down to only 40 µm facilitate primary human macrophage elongation accompanied by differentiation towards the M2 type [57]. **Table 2** show commercially available dermal substitutes evaluated for their immunomodulatory and M2 polarization ability.

Table 2. Dermal Substitutes tested for immunomodulatory and macrophage polarization ability.

	Primary Material Composition	Source and Other Components	Refs.
Nevelia	Porous resorbable double layer matrix 2 mm thickness made of stabilized native collagen type I and a silicone sheet 200 mm thickness mechanically reinforced with a polyester fabric. The extraction procedure and the freeze-drying process allow the structuring of the collagen into a matrix with optimal hydrophilicity, pore structure and pore size (20–125 µm)	Bovine, Native collagen Type I. No glycosaminoglycan (GAG) added to improve cell attachment and proliferation. Glutaraldehyde Cross-linking	[58] [59] [60]
Integra	Bilayer system for skin replacement made of a porous matrix of fibers of cross-linked bovine tendon collagen and glycosaminoglycan (chondroitin-6-sulfate) that is manufactured with a controlled porosity and defined degradation rate. The Integra pore size of 20 to 125 µm allows influx of cells.	Bovine Tendon Type I Collage Shark cartilage -derived chondroitin-6-sulphate (GAG). Glutaraldehyde Cross-linking	[61] [62] [63]
PriMatrix	Acellular dermal tissue matrix. comprising of both type I and type III collagen derived from fetal bovine dermis. This matrix is processed in a way to maintains the extracellular matrix in its native and undamaged state while removing all lipids, fats, cells, carbohydrates and non-collagenous proteins.	Fetal Bovine collagen type I and type III collagen. No cross-link	[62]
Oasis Wound Matrix	Lyophilized, decellularized porcine small intestine submucosa (SIS). Matrix is derived from a single layer of porcine small intestinal submucosa (SIS) technology. The technology provides an intact three-dimensional extracellular matrix which allows for host cell migration. The SIS is freeze-dried and sterilized with ethylne oxide gas in preparation for clinical use	Porcine small intestine submucosa (SIS). No cross-link	[62]
Allomend	Decellularized donated human dermal tissue, with significant removal of cellular debris (including DNA and RNA), proteins and antigens. The process does not require the use of detergents or enzymes, thereby mitigating the	Human dermal tissue No cross link	[61] [62] [63]

	Primary Material Composition	Source and Other Components	Refs.
	possibility of harmful residuals in the tissue. The decellularization process also inactivates microorganisms through cellular disruption. USA only, not available in Europe		
DermaMatrix	Cadaveric human allograft treated with a disinfectant solution that combines detergents with acidic and antiseptic reagents. USA only, not available in Europe	Human dermal tissue No cross link	[61] [62] [63]
Dermacell	Decellularized regenerative human tissue matrix allograft processed using proprietary technology that removes at least 97% of donor DNA without compromising the desired biomechanical structure or biochemical properties. [65] USA only, not available in Europe	Human dermal tissue No cross link	[61] [62] [64]

ing that a GF+ cells also have al roles in the immune response. CS at an increasing dose range of 100–1000 µg/mL was found to significantly increase the phagocytic activity and ROS production as well as the secretion levels of NO, TNF-α, IL-6, and IL-10 by monocyte/macrophage lineage (RAW264.7) [66]. Witherel et al. studied the responses of monocyte-derived macrophages isolated from blood to four different commercially available biomaterials in vitro: OASIS® Wound Matrix, which is an extracellular matrix from porcine small intestinal mucosa; INTEGRA® Bilayer Matrix, a dermal bilayer of cross-linked bovine tendon type I collagen and chondroitin-6-sulfate plus a layer of polysiloxane; AlloMend® Acellular Dermal Matrix, a decellularized matrix composed mainly of collagen and elastin; and PriMatrix® Dermal Repair Scaffold, decellularized fetal bovine dermis rich in type I and II collagen [55]. The OASIS® and INTEGRA™ matrices downregulated the expression of M2a anti-inflammatory markers CCL22 and TIMP3, suggesting a probable inhibition of extracellular matrix secretion and fibrosis, which are crucial events for wound closure. OASIS® was also the biomaterial responsible for the greatest increase in M1 genes expression. The authors suggest that INTEGRA® inflammatory response could be related to glutaraldehyde cross-link and suggest that both OASIS® and INTEGRA™ seem to be a poor option for chronic wounds [55]. PriMatrix® as well showed a downregulation of the anti-inflammatory genes CCL22 and TIMP3 and overexpression of both the pro-inflammatory cytokine TNF-α and CD163, associated with M2c. AlloMend® only induced an effect of the upregulation of CD163, and it was considered the biomaterial with the lowest influence on macrophage response. Agrawal et al. compare DermaMatrix®, AlloDerm®, Integra®, and DermACELL® M1/M2 polarization in an animal model [67]. Macrophage surface markers CD68 (all macrophages), CCR7 (M1 phenotype), and CD206 (M2 phenotype) were used to characterize an M1–M2 profile by an immuno-histological assay. All dermal substitutes showed a bell-shaped curve for the distribution of CD68+ macrophages, except Integra®, which showed an increasing trend of macrophages with time [67]. Moreover, DermACELL® had the highest entry of macrophages, while Integra® had the smallest [67]. AlloDerm® showed that the macrophages were mostly M1 at 7, 14, 21, and 42 days post-implantation, while Integra® showed a mixed M1/M2 population of macrophages at all time-points: The trend for the M1:M2 ratio was skewed towards M2 on day 7, towards M1 on days 14 to 21, and again towards M2 on day 42 for Integra® [67]. A recent study showed that the implant of a porcine urinary bladder matrix (UBM) is associated with the modulation of wound inflammation in diabetic patients, measured as mRNA associated with M1 and M2 macrophages [68]. Recently, Montanaro et al. show investigate how the dermal substitute Nevelia®, which is a dermal substitute consisting of a three-dimensional porous matrix of type 1; purified, stabilized, bovine-origin

collagen; and a layer of reinforced silicone may influence the inflammatory infiltrate and macrophages polarization [69]. The study randomly enrolled 15 diabetic patients with chronic foot ulcers, 5 treated only by standard of care as control group, and 10 treated with Nevelia®. Biopsy was performed at baseline and after 30 days and histological, immunohistochemical, and immunofluorescence analysis was performed to evaluate the number of M1 and M2 macrophages. Dermal substitute group showed a general macrophage activation and a greater and significative polarization toward M2 subpopulation at 30 days, compared with control. The increase in M2 phenotypes population was also confirmed by confocal microscopy. Moreover, after 6 months, 6 patients (60%) of the Nevelia® were completely healed, while only 1 patient (20%) healed in the control group, suggesting that this dermal substitute induce tissue reparative processes through macrophage activation and M2 reparative polarization in diabetic lesions [69]. The positive clinical outcome of this dermal substitute was previously observed in 41 patients with chronic diabetic wounds [70]. In addition, Nevelia® dermal substitute was observed to polarize in M2 also in an in vitro model [71].

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