Chronic Leg Ulcers' Biomaterials Science

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Chronic leg ulcers (CLUs) are full thickness wounds that usually occur between the ankle and knee, fail to heal after 3 months of standard treatment, or are not entirely healed at 12 months. CLUs present a considerable burden on patients, subjecting them to severe pain and distress, while healthcare systems suffer immense costs and loss of resources. The poor healing outcome of the standard treatment of CLUs generates an urgent clinical need to find effective solutions for these wounds. Tissue Engineering and Biomaterials Science offer exciting prospects for the treatment of CLUs, using a broad range of skin substitutes or scaffolds, and dressings. In this review, we summarize and discuss the various types of scaffolds used clinically in the treatment of CLUs. Their structure and therapeutic effects are described, and for each scaffold type representative examples are discussed, supported by clinical trials. Silver dressings are also reviewed due to their reported benefits in the healing of leg ulcers, as well as recent studies on new dermal scaffolds, reporting on clinical results where available.

Keywords: leg ulcers ; chronic wounds ; diabetic foot ulcers ; tissue engineering ; dermal scaffolds

1. Introduction

Cutaneous wound healing is a complex biological process, reliant on a series of highly regulated and overlapping physiological events including hemostasis, inflammation, proliferation, and tissue remodeling [1]. Disruption at any stage can result in unsuccessful healing and formation of chronic wounds, which are ulcerative skin defects that do not heal in an orderly and timely manner, often failing to heal for more than six months [1]. They frequently remain in the inflammatory stage for an extended period with reduced cell proliferation and deficient response to growth factors [2].

Chronic leg ulcers (CLUs) are full thickness wounds that usually occur between the ankle and knee, fail to heal after 3 months of standard treatment or are not entirely healed at 12 months [3][4]. The frequency of such non-healing ulcers is growing through an increasing ageing population, and factors such as smoking, obesity, and diabetes contribute to the impaired healing. Venous disease is the most common cause of CLUs, accounting for roughly 70% of cases, whilst approximately 20% are caused by arterial disease or mixed arteriovenous disease [5]. Furthermore, peripheral neuropathy accounts for approximately 85% of foot ulcers, which are often complicated by arterial disease [5]. A common complication of diabetes mellitus is the formation of diabetic foot ulcers (DFUs) with an estimated 19–34% of diabetes patients likely to develop DFU in their lifetimes [6].

CLUs present a considerable burden on patients, subjecting them to severe pain and distress, while healthcare systems suffer immense costs and loss of resources. Annual figures by the International Diabetes Federation in 2015 reported that 9.1–26.1 million people will develop DFUs [6]. Approximately 1% of the adult population in developed countries is affected by CLUs and an estimated 3.5 per 1000 individuals affected in the UK, which rises to 20 per 1000 individuals in people over the age of 80 [7][8]. Additionally, the annual national health service (NHS) cost to manage confirmed CLUs such as DFUs was estimated to be between £524.6 and £728.0 million, and for venous leg ulcers (VLUs) between £596.6 and £921.9 million [9][10]. However, these values may be an underestimate since they only consider the confirmed cases, and the NHS also managed patients with lower limb ulcers without a differentiation [9][10]. Moreover, and according to recent published data the worldwide prevalence rate of DFUs was 6.3%. Globally more than 400 million people are suffering from DFUs and the resulting complications and in many cases amputations of extremities, high risk of mortality and morbidity have huge adverse implications on healthcare system and health economics globally [11][12].

In the past, the standard treatment of CLUs involved the use of continuous graduated compression therapy together with dressings [13][14][15]. This treatment method had poor healing outcomes with only 50% of ulcers healing within four months, roughly 20% not healing within two years and about 8% not healing even after five years [16]. Consequently, many patients with unhealed ulcers were admitted in the hospital for treatment, frequently with unsuccessful outcomes leading to lower limb amputation culminating in lifetime disability.

Despite considerable advances in the management of chronic wounds, some of the most promising discoveries still lie ahead. Such advances should lead to the complete anatomical and physiological restoration of the skin. To mention a few, these new approaches and techniques include: (i) cell and gene therapies; (ii) soluble molecules and bioactive factors; and (iii) development of efficient engineered biomaterials at affordable costs and availability.

The fields of Tissue Engineering and Biomaterials Science offer exciting prospects for the treatment of CLUs, using a broad range of skin substitutes or scaffolds. They consist of a group of biomaterials that provide wound cover for tissue repair and regeneration after an injury that extends deeper than the epidermis of the skin [17]. These scaffolds or substitutes vary in their material composition (biological origin, natural or synthetic polymers, ceramics), permanence (temporary or permanent), intended layer of replacement (epidermis, dermis, or both), and the presence or lack of cells [17]. In terms of the material composition, some of the skin substitutes are of biological origin derived from native extracellular matrix (ECM), therefore are formed of native proteins and ligands that provide the physiological microenvironment for the new tissue to grow [18]. Alternatively, biomaterials that are used to develop novel skin scaffolds commonly include polymers (natural or synthetic) and ceramics (bioactive glasses), which all have their advantages and disadvantages [17]. Accordingly, to minimize or eliminate disadvantages, they are frequently combined to form composites that integrate their various distinctive advantages. Specific conditions are required to develop a functional dermal scaffold: biodegradability as new dermis is formed; and survival for a sufficient period so that cells have ample time to infiltrate and deposit a new ECM, without evoking a foreign body reaction. Furthermore, the scaffold should permit cell proliferation and vascularization, withstand tear forces, retain flexibility, and should be easily handled by surgeons and clinical practitioners [19].

Skin substitutes are not the only type of biomaterials that Tissue Engineering and Biomaterials Science can offer for the treatment of CLUs. Wound dressings are a type of skin biomaterials that are temporarily placed above a wound to facilitate the natural wound healing mechanisms and provide an optimal healing environment. They can be made from a vast array of materials and some of them can include components such as antimicrobial agents [20].

2. Current Research

Despite the availability of various clinically used dermal scaffolds, none were explicitly designed for the treatment of chronic non-healing leg ulcers and the effectiveness in treating such ulcers was in most cases moderate. The materials discussed so far in this review were bioengineered for healing of acute wounds that progress through the normal stages of hemostasis, inflammation, proliferation, and tissue remodeling. However, chronic wounds including CLUs do not normally progress through these stages, hence becoming chronic. It has been shown that the chronic wound microenvironment differs from the one found in acute wounds [21]. Some of these differences include high levels of proteases, senescent cells, or reduced levels of growth factors seen in chronic wounds [21]. These differences should be taken into consideration when designing a scaffold for CLUs. For example, the increased level of proteases will affect protein-based scaffolds degradation—i.e., it will accelerate it—thereby degrading the scaffold before a new dermis is formed. Therefore, the bioengineering of a slowly degradable protein-based scaffold for CLUs would be advantageous. Furthermore, successful wound healing depends upon the reestablishment of stable epidermis as a minimum precondition. Stability of the epidermis depends on regeneration of the basement membrane and vascularization to anchor the outer skin to the body [22]. Various skin replacements satisfy many conditions of wound closure; however, they do not recapitulate the multilayered pattern of the skin and adnexa.

Therefore, there is a further need for the development of novel dermal scaffolds specifically designed to target and treat these wounds, with some researchers currently working on creating such scaffolds, which can be combined with stem cells, therapeutic compounds, antibiotics, or exosomes.

2.1. Platelet-Rich Plasma

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma, consisting of the seven principal protein growth factors including the three isomers of platelet-derived growth factor (PDGFαα, PDGFββ, PDGFαβ), TGFβ1 and TGFβ2, VEGF, and epithelial growth factor (EGF) [23]. These protein growth factors are secreted by platelets to stimulate tissue regeneration and have a valuable therapeutic effect on wound healing [23][24]. Combining PRP and HA in a bio-functionalized scaffold offers several benefits over conventional dressings, including accelerated healing, reduced costs to healthcare systems and a reduction in patient pain [24][25][26].

Burgos-Alonso et al. investigated the efficacy and safety of autologous PRP in comparison with SOC for the treatment of leg ulcers in patients with chronic venous insufficiency, in a primary health-care setting. This was a Phase I–II, open-label, parallel-group, multicenter, randomized pilot study evaluating reduction of ulcer area at five nine weeks after treatment.

They have also evaluated the Chronic Venous Insufficiency Quality of Life Questionnaire score, and the cost of treatment for up to nine weeks. Eight patients were recruited and a total of 12 ulcers were treated with either autologous PRP or SOC. They found an increased quality of life in the patients treated, and a reduction in the time of wound care (once a week vs. three times a week in the SOC group) [27].

Moneib et al., compared the clinical efficacy of PRP in the management of chronic venous leg ulcers vs. conventional treatment. In total, 40 patients with chronic venous leg ulcers were included in the study. Twenty patients were treated with autologous PRP weekly for six weeks (Group A), and 20 patients were treated with conventional treatment (compression and dressing) for six weeks (Group B). They showed a highly significant improvement in the ulcer size post-PRP therapy compared to conventional therapy. The mean change in the area of the ulcer post-PRP and conventional therapy was 4.92 ± 11.94 cm and 0.13 ± 0.27 cm, respectively, while the mean percentage improvement in the area of the ulcer post-PRP and conventional therapy was 67.6% ± 36.6% and 13.67% ± 28.06%, respectively. Their data suggested that PRP is a safe nonsurgical procedure for treating chronic venous leg ulcers [28].

In another randomized controlled, open-labeled clinical trial carried out between 2014 and 2018 an eight-week study protocol was chosen or until 100% wound re-epithelialization was observed. A total of 69 patients (35 in the autologous PRP group and 34 in the control group) were included in the study. Wound size reduction, granulation tissue formation, microbiological wound bed changes and safety were evaluated. The autologous PRP group showed superiority over conventional treatment in wound bed coverage with granulation. No severe adverse events were noted during the study. Both treatment methods were considered equally safe suggesting that topical application of autologous PRP gel could be beneficial in wound size reduction and granulation tissue formation. However, treatment was also associated with more frequent microbiological wound contamination [29].

A recent experimental study involved the in vitro and in vivo assessment of a bio-functionalized scaffold composed of PRP and HA, in patients with chronic diabetic and vascular ulcers [24]. The results of patients receiving the combined PRP and HA treatment (n = 182) were compared to a control group (n = 182) receiving traditional dressings (HA alone). Within 80 days, it was established that patients receiving the combined treatment had 98.4% ± 1.3% re-epithelialization as compared to 87.8% ± 4.1% in the control group ($p < 0.05$). No local recurrence was observed during the follow-up period. The combination treatment showed stronger regenerative potential in terms of epidermal proliferation and dermal renewal as well as an improvement in the healing process in the chronic ulcers compared with HA alone. Overall, the study has shown that combined treatment with PRP and HA could optimize granulation formation and tissue regeneration, provide a more rapid wound closure, an excellent aesthetic improvement, and help preventing infection [24].

2.2. Stem Cells

Human mesenchymal stem cells (MSCs) are ideal for tissue regeneration, due to their various exceptional properties, including self-renewal, multilineage differentiation, and immunomodulation [30][31]. Additionally, MSCs occur in almost all tissues including bone marrow, adipose, and synovium [31][32]. Recently, a randomized clinical trial evaluated the effect of a biological scaffold, seeded with human umbilical cord Wharton's jelly mesenchymal stem cells (WJSCs), in the healing of chronic skin ulcers [33]. Human Wharton's jelly MSCs (hWJSCs) have numerous advantages over other sources of MSCs such as easy availability, non-invasive isolation, higher growth rate, and lower immunogenicity [33]. Moreover, hWJSCs secrete growth factors involved in wound healing—including VEGF, EGF, PDGF, and hepatocyte growth factor (HGF)—and can differentiate into fibroblast, epithelial, and endothelial cells. The biological scaffold used was of acellular human amniotic membrane, which contains ECM components such as collagen, elastin and fibronectin, known to advance the adhesion, growth, proliferation, and migration of differentiated stem cells in wounds [33][34]. In the study, five patients with chronic DFUs were treated with the scaffold seeded with WJSCs for 9 days, every 3 days with a follow-up of 1 month. Results in treated patients, displayed a noteworthy decrease in wound healing time and wound size, with a mean percent healing of 83.43%, and 96.70% after 6 and 9 days, respectively ($p < 0.002$) [33].

Otero et al. evaluated the feasibility, safety, and initial clinical outcome of autologous bone marrow-derived cells (BMDC) therapy associated with standard treatment in patients with VLUs. They conducted an open-label, single-arm, prospective pilot clinical trial in four patients with six chronic VLUs. Bone marrow was harvested, processed, and then administered by multiple injections into the ulcers. All patients received standard treatment and non-healing characteristics of the VLUs were confirmed at study entry. Both ulcer size and wound pain were significantly reduced 12 months after BMDC treatment, and a long-term follow-up showed that treatment was safe and well tolerated. Despite the low number of patients studied, these data have shown that autologous BMDC treatment could be a useful, feasible, and safe procedure to enhance ulcer healing [35].

Adipose tissue (AT) is a safe and promising tool to treat non-healing venous leg ulcers (VLUs). Zollino et al. conducted a phase II randomized clinical trial for the treatment of recalcitrant chronic leg ulcers using centrifuged AT containing progenitor cells. From an initial cohort of 38 patients, 16 patients affected by non-healing VLUs were randomly allocated to the experimental arm (treatment with AT) and control arm (no treatment). Each group had five men and three women. The primary outcome measures were healing time and safety of the cell treatment. Secondary outcomes were pain evaluated by numeric rating scale (NRS), complete wound healing at 24 weeks by Margolis Index and wound-healing process expressed in square centimeters per week. The various immunophenotypic and functional characteristics of AT-derived stem cells were correlated with the clinical outcomes. The healing time was significantly faster in the AT treated patients (17.5 ± 7.0 weeks) versus 24.5 ± 4.9 weeks recorded in the control arm. No major adverse events were recorded [36].

2.3. Hydrogels

Hydrogels and flowable gels are being investigated by numerous research groups as potential materials to treat CLUs. Hydrogels can be natural, synthetic, or hybrid and comprise a three-dimensional network of cross-linked polymers with a hydrophilic structure, which enables them to hold large amounts of water [37][38]. In clinical practice, hydrogels preserve a moist wound environment by supplying water molecules to dehydrated tissue stimulating faster wound healing [38].

A recent study involved the utilization of an engineered hydrogel, known as FHE@exosomes (FHE@exo) hydrogel, for promoting chronic diabetic wound healing and complete skin regeneration [39]. FHE@exo is an injectable, self-healing, and antibacterial polypeptide (poly-ε-L-lysine, oxidative HA and Pluronic F127) based hydrogel containing stimuli-responsive adipose-derived mesenchymal stem cells exosomes (AMSCs-exo) [39]. In vivo results demonstrated that the FHE@exo hydrogel notably strengthened the healing of diabetic full-thickness wounds, through amplified wound closure rates, rapid angiogenesis, re-epithelization, and collagen deposition within the wound site [39]. Another study assessed the safety and efficiency of a novel recombinant human type I collagen (rhCollagen) based flowable wound matrix, in patients with CLUs [40]. A single-arm, open-label, multicenter trial was conducted on 20 patients with CLUs, undergoing rhCollagen flowable gel treatment. Wounds were photographed and preliminary surgical debridement was performed prior to rhCollagen application. Patients received a single application of rhCollagen to the wound bed, followed by weekly assessments of the wound—including wound size, granulation, and epithelialization—and application of polyurethane dressing. After four weeks, there was 94% median wound area reduction, with fifteen ulcers showing ≥70% wound closure, nine of which attained complete closure. However, one of the study limitations was that no control group was included to compare with wound healing or with a more traditional product. The authors decided to first assess a short-term applicability and safety and to assess comparative outcomes and long-term follow up in the next trial [40].

The current standard treatment of CLUs has poor healing outcomes, presenting a considerable burden on patients while healthcare systems suffer immense costs and loss of resources. Tissue Engineering and Biomaterials Science offer exciting prospects for the treatment of these wounds using a broad range of skin substitutes or scaffolds, and wound dressings.

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