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Current wound healing procedures and potential care

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Abstract

In this review, we describe current and future potential wound healing treatments for acute and chronic wounds. The current wound healing approaches are based on autografts, allografts, and cultured epithelial autografts, and wound dressings based on biocompatible and biodegradable polymers. The Food and Drug Administration approved wound healing dressings based on several polymers including collagen, silicon, chitosan, and hyaluronic acid. The new potential therapeutic intervention for wound healing includes sustained delivery of growth factors, and siRNA delivery, targeting micro RNA, and stem cell therapy. In addition, environment sensors can also potentially utilize to monitor and manage micro environment at wound site. Sensors use optical, odor, pH, and hydration sensors to detect such characteristics as uric acid level, pH, protease level, and infection – all in the hopes of early detection of complications.

Keywords

growth factors; wound healing; chronic wounds; wound dressings; stem cells; siRNA; sensors

1. Introduction

The skin serves its primary function as a protective barrier against environmental insult. When the structural integrity of the skin is compromised its primary responsibility to the immune system is impacted leading to serious morbidity and mortality [1–3]. With one-third of the adult population currently living with diabetes and 6.5 million cases of chronic skin ulcers yearly, investigation into the processes involved in wound healing have taken on a more prominent role in recent years [1].

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Chronic wounds are frequently being described as an epidemic as they are diagnosed at an alarming rate and causing an enormous burden to the financial structure of our healthcare economy. Due to an aging population and an increased incidence of both diabetes and obesity worldwide, the financial burden of treating chronic wounds has risen dramatically. It is estimated that over \$25 billion is spent each year on the treatment of chronic wounds alone. The costs are even more staggering when one factors in loss of productivity for affected individuals as well as long-term facility and nursing home care [4]. Estimates of the lifetime probability of diabetics developing a chronic foot ulcer are between 10–25% [5,6]. More importantly, diabetes is the leading cause of nontraumatic leg amputations in the United States [6]. While the incidences of diabetic ulcers are sharply on the rise, pressure ulcers in critical care and intensive care patients are also increasing. The prevalence of pressure ulcers within inpatient settings has been reported to be 22%, with as many as 50–80% acquired within the hospital [7]. Diabetic foot ulcers (DFUs) and pressure ulcers represent major sources of morbidity and their care a massive burden financially. Sen and colleagues argue that with the sharp increase in the incidence of diabetes and obesity and with the increasing need for wound care of our veterans, investigation into tissue regeneration in chronic wound repair is vital [4].

Five phases for wound healing is introduced: hemostasis, inflammation, cellular migration and proliferation, protein synthesis and wound contraction, and remodeling [8,9]. Figure 1 [8]. However, mainly only three phases, inflammatory, proliferative, and remodeling [1,2] are presented due to the overlap of phases. These dynamic phases are associated with considerable complexity that involves soluble mediators, extracellular matrix (ECM) formation, and parachymal cell migration [2]. The primary objectives of wound healing involve timely wound closure, prompt pain relief, and an aesthetically acceptable scar. Recent advances in wound healing research have markedly improved our understanding of the processes implicated in tissue repair and regeneration.

In this review, we focus on current treatment methods and potential future treatments based on growth factors and cytokines, small molecules, and stem cells. In addition, we describe potential early detection and management of wound microenvironment using sensor technology.

2. Current Standard Care

2. 1. Acute and Chronic Wounds

There are generally two major classes of wounds: chronic and acute. Acute wounds can be superficial involving both the epidermis and superficial dermis, or full thickness in which the subcutaneous layer is compromised [3]. Examples of acute wounds are surgical incisions, thermal wounds, abrasions, and lacerations with the major associated complication of each being infection. Acute wound healing is regulated by cytokines and growth factors released proximal to the wound [10]. The inflammatory phase associated with wound healing involves neutrophil, macrophage, and lymphocyte migration to the wound producing symptoms of inflammation that last for approximately 2 weeks [11]. As stated previously, wound healing involves several stages and at any point the process can come to a standstill leading to potential dysfunction. If inflammation persists for months or years the wound

becomes classified as chronic and can be associated with numerous pathological alterations including increased protease activity and infection [12]. The proliferative phase follows the inflammatory phase, and is characterized by new tissue formation, granulation and epithelial tissue formation (re-epithelialization) and restores the vascular network. Keratinocytes involves repairing the epidermal barrier while fibroblasts and endothelial cells are responsible for angiogenesis and ECM production. Remodeling phase involves reorganization and contraction of newly formed matrix and can last for several years [8,9,13,14].

Generally, acute wounds tend to heal within 3 weeks while chronic wounds tend to persist for a minimum of 3 months since the time of injury [3]. Chronic wounds can result by destructing all the layers in skin including epidermis, dermis, and underlying subcutaneous fat tissue. Chronic wounds typically result as complications of other disease processes i.e., foot ulcers from diabetes, pressure ulcers resulting from spinal cord injuries, and even as a result of neurodegenerative processes like Pick's disease [4]. Many of the issues related to chronic wound healing focus on the deleterious effects various disease processes have on the mechanisms of biochemical signaling, ECM deposition, and cell migration. For example, hyperglycemia in diabetics is believed to inhibit ECM formation by upregulating matrix metalloproteinases through increased levels of tumor necrosis factor-alpha (TNF- α) and interleukins (IL-1 β) [15]. In addition to ECM dysfunction, diabetic foot ulcers (DFU) are observed to have impaired keratinocyte migration and leukocyte function leading to infection. Furthermore, depleted levels of inorganic phosphate within diabetic ulcers also leads to low levels of adenosine triphosphate (ATP), causing a significant setback to the immune response [15]. Signaling molecules like epidermal growth factor (EGF) functions normally to stimulate proliferation and migration of keratinocytes during wound closure, however, aging, disease, and sun damage inhibit keratinocytes ability to respond to EGF and other growth-promoting mitogens [16,17]. The above mechanisms contribute to impaired wound healing and are the focus of new therapeutic modalities that center on incorporating both ECM and various signaling molecules within chronic wounds in order to promote regeneration and repair.

2.2. Standard wound healing procedures

The current standard of care for chronic wounds consists of swabbing for infection, cleaning, dressing, and in some cases debridement of the wound [5]. For diabetic ulcers, systemic glucose control, debridement of nonviable tissue, and maintenance of adequate extremity perfusion is paramount.

2.2.1. Split-thickness autograft—A gold standard in chronic wound management is the split-thickness autograft. This method of wound closure involves harvesting full thickness fascia from a donor site and grafting it over the compromised region. Comparison studies by Phillips et al., involving patients with chronic leg ulcers found that on average, healing took place over a 6-week period versus the 4 weeks required when using split-thickness allograft material [18]. While cosmetic results of split-thickness autografts are typically superior to other treatment modalities, there are numerous issues that arise with their use. This procedure is often associated with scarring and contracture of the wound site. The amount of

scarring and contracture of the grafted wound relates inversely with the amount of dermis that is delivered in a split-thickness skin graft [19].

Limitations in the quantity of donor skin available and the risk of complications including pain and donor-site infection make split-thickness autografts an imperfect treatment option [20,21]. Split-thickness grafting is severely limited by available donor sites especially in burns that involve greater than 20% total body surface area [22]. Furthermore, skin used for splitskin grafting tends to have only a handful of proliferating cells with proliferative potential inversely related to patient age [16,26]. The need to eliminate the problems associated with splitskin grafts gave rise to cultured autologous grafts.

2.2.2. Use of donor keratinocytes—Cell populations utilize numerous cell signaling molecules and mitogens in order to control their own growth as well as the growth of neighboring cells. The ability of keratinocytes to both respond to as well as secrete their own modulators however is a function of age [16,23,24]. Comparison studies by Stanulis-Praeger et al illustrate significant increases in cell density over a seven-day period in young adult (22–27 years) donor keratinocytes versus that of older adult (60–82 years) donor cells when exposed to varying concentrations of keratinocyte growth factor [16]. Previous studies also illustrate the ability of newborn keratinocytes, harvested from fetal foreskin, to grow in response to factors secreted by other newborn cells [24]. However, adult donor keratinocytes' potential for proliferation is limited. Adult cells, when placed in newborn conditioned media were not stimulated to proliferate, and a similar result was seen when newborn keratinocytes were placed in adult cell conditioned media. "Conditioning" of the medium by keratinocytes represents the cells ability to render their surroundings as growth-stimulatory via secretion of various signaling molecules [24]. Sauder et al., demonstrate that newborn keratinocytes secrete significantly more epidermal cell-derived thymocyte-activating factor, an important growth-promoting mitogen, than adult donor keratinocytes providing further evidence of cell senescence with increasing age [25]. These results demonstrate the inverse relationship between responsiveness of a donor cell line and aging thus leading to increased healing times observed in the elderly population [17,26].

2.2.3. Cultured epithelial autografts (CEA)—Designed to reduce pain and hasten the healing of chronic wounds, cultured autologous keratinocyte grafts were first utilized clinically by O'Connor et al., in the treatment of burns in 1981 [27,28]. The method involves the harvesting of cells with a punch biopsy, expanding the cells *in vitro* by supporting them with 3T3 fibroblast cells in a growth-stimulatory medium consisting of EGF and cholera toxin, harvesting sheets of epithelia with the enzyme dispase, and ultimately grafting sheets of the cultured epithelia onto the wound bed [29,30]. A donor site punch biopsy can be expanded 1000 fold in 3–4 weeks [31]. Unlike allogenic grafts, cultured autologous grafts are beneficial for both acute and chronic wounds, and provide a permanent skin replacement without the risk of graft rejection. Cultured epidermal autografts have been utilized through numerous methods in order to enhance wound repair. Some of these methods include application of confluent sheets of cells applied directly to the wound bed or onto a pre-prepared wound base made of allograft dermis [32]. Pre-confluent cultured epidermal grafts

can also be sprayed directly onto the wound and have the advantage of being in a hyperproliferative state [22].

The success or “take” rate of the graft is limited by the attachment of the graft to the basement membrane, which is needed for survival, proliferation and differentiation of the graft [33–38,39]. Clinically assessed, the “take” rate of cultured autografts has been studied in a number of different disorders. Leg ulcers observed 30% success, giant hairy cell nevi 20–90%, and in the presence of infection an average rate of “take” was observed to be 40% [36–38]. Hefton et al., observed complete ulcer healing using cultured epidermal cells within 28 days in four ulcers that had failed to resolve over a prior two-month period being treated with standard dressings and split-thickness grafts [28]. With increased use of this method clinically however, the limitations of cultured autografts as a mechanism of wound repair have become obvious.

As stated above, “take” rates for cultured epidermal autografts vary from 0%–85%, which may be a reflection of their fragile composition [31,39]. After stratification, the cultured epidermal grafts are only 4–6 cell layers thick making them susceptible to infection and digestion by enzymes including collagenase, which are present within the wound [31]. In addition, the “take” rate may also be decreased by disruption of the basal cell layer when exposed to disperse.

While CEA offers a permanent solution for wound repair, the time required to culture and prepare sheets of cells for grafting greatly limits their value. A biopsy site the size of a stamp can take up to 3–4 weeks of preparation before grafting, with even lengthier times expected in the elderly [20]. Thus preparation time hinders immediate grafting of burns, leg ulcers, and blistering disorders with cultured autografts. Additionally, the time lapse during preparation leads to increased risk of sepsis and future graft loss secondary to bacterial colonization. The success of a graft is limited by its attachment to the basement membrane. The dermal component of the wound bed is vital for the formation of anchoring fibrils necessary for the proper attachment of the graft [40]. Madden et al., observed a substantial increase in “take” rate when wound sites maintained an intact dermal bed and when wounds had been prepared with a cadaver dermal allograft [41]. Addressed in more detail in the composite graft section, cadaver graft can be treated chemically to remove epidermal components, leaving behind an acellular dermal matrix as an “anchor” for cultured epidermal autograft in full thickness wounds.

Spontaneous blistering at the graft site has also been observed in burn patients treated with cultured keratinocytes, likely secondary to broad dermal loss [32,42,43]. Poor cosmetic results due to significant contracture within the graft site have also been reported to be an issue. Some studies have found graft sites contracting to 50% their original size compared to 95% typically observed in split-thickness grafts [44]. Contraction of the graft may also be a product of its preparation. *In vitro* studies have found that cultured epithelial grafts shrink, on average, to about one-fourth their original size when removed from the cell culture dishes, however the reason for this phenomenon is not certain [29]. Finally, De Luca et al., found that the success rate of cultured autologous grafts was inversely proportional to the age of the patient [45]. As stated previously, the intricate cell signaling that is necessary for

survival and growth of keratinocyte colonies is a function of age. Older patients provide donor cells that are less responsive to conditioned media, thus decreasing both their doubling rate and survival time.

An obvious drawback to the method described above is the polarity of cell growth within a culture dish. Stratification of the cultured epithelium leads to multiplying cells residing on the surface of the cell culture dish thus obviating the need to maintain this polarity when transplanting the graft into the wound bed [29]. If disaggregated cells were obtained from the surface of the epidermis, a large fraction would be incapable of multiplication. Cultivation of cells on a transplantable surface allows for the maintenance of graft polarity in order to maximize the number of multiplying cells [46].

2.4. Wound dressings

There are a multitude of wound dressings in the market, all of which function to preserve hydration within the wound in order to optimize regeneration, protect against infection, and avoid disruption of the wound base [3,8,9]. The most currently available wound dressings are made using chitosan, hyaluronic acid, collagen and silicon [47–52]. In addition, other biomaterials that are currently being investigated for wound dressings consist of alginates, heparin, cellulose, and gelatin [53–57].

Limitations in autologous epidermal graft cultivation have given rise to novel techniques in growing cultures [29,46]. Utilization of transplantable membranes for the growth of cultured epithelia would also eliminate the need for dispase thus protecting the integrity of basement membrane proteins. There are a number of such “vehicles” based on above mentioned polymers [47–57]. The use of carrier mediums for the transfer of confluent sheets of cells to the wound bed would decrease graft contraction, blistering and ulceration at the wound site, and would ultimately increase the stability of the graft.

2.4.1. Hyaluronic acid dressings—Hyaluronic acid (HA) is a natural polysaccharide found in the extracellular matrices of mammalian tissue. Its composition is made up of disaccharide units consisting of glucuronic acid and N-acetyl-glucosamine [58,59]. HA is an appealing polymer for use in tissue engineering investigation as its biodegradation and biocompatibility support cell growth and proliferation. It has been used extensively as a drug delivery system for numerous therapeutic modalities and as a biopolymer for structural support and stem cell delivery in bone regeneration [58]. Recent success in its use as an *in vitro* culture template for proliferation of keratinocytes has made it an exciting new product for future use clinically in wound regeneration. Studies by Turley et al., found that the biodegradation of HA produces by-products that aid in epithelial cell proliferation and migration [47]. Prior studies found that enzymes involved in the degradation of HA stimulate cell proliferation, providing further evidence that HA must be broken down in order to promote cell growth [48,59]. The molecular structure of HA itself also facilitates the movement of cells within the ECM providing a substrate for cell migration and proliferation thus enhancing dermal repair [60]. Interestingly, HA may also play even more significant role in angiogenesis and the inflammatory response, further supporting cellular growth [61,62]. However, there was no significant difference between the thicknesses of the

epidermis treated with vitronectin growth factor alone and vitronectin growth factor together with HA delivery vehicle. While the addition of HA did not enhance all the cellular responses to vitronectin growth factor examined, it was not inhibitory [63].

The molecular weight of HA (MWA) is also an important factor when considering its role in wound regeneration [64–68,33]. Specifically, studies by Campo and colleagues found that breakdown products of low molecular weight HA are pro-inflammatory leading to increased tissue damage [64]. Interestingly, high molecular weight HA (HMWA) may inhibit the nutrient supply to regenerating epithelial tissue by blocking the formation of capillary networks [62]. While HMWA may limit wound regeneration, some studies revealed that in the presence of medium molecular weight HA (MMWA), wound repair could be enhanced [65]. Additionally, previous studies have found that MMWA enhances wound closure through up-regulation of junctional adhesion molecules at the epidermal diffusion border [65].

The limitations in wound repair with HA grafts are likely related to co-morbidities found in high-risk patients. Arterial occlusion and wound site infection have a negative impact on graft survival and wound repair. A study with 14 patients by Lobmann *et al.*, found that 79% of DFU's treated with HA template grafts fully healed between 7 and 64 days post-procedure [68]. Interestingly, 3 of the grafts that failed to survive had been grafted in patients with considerable arterial occlusive disease or with concomitant infection [68].

Composite Laserskin graft is a hyaluronic acid derivative consisting of micropores that support cell growth. Its use as a template for cultured epithelial cell grafts has been studied extensively. The efficiency of seeding the template with cultured epithelial cells is dependent upon the use of a fibroblast feeder layer. Lam *et al.*, performed studies comparing the efficacy of seeding composite laser skin grafts with cultured keratinocytes alone and with an allogenic fibroblast cell layer [31].

Laserskin's micropores are laser produced perforations that are 40 µm in diameters which can deliver keratinocytes which are roughly 20 µm thick. A 10 cm by 10 cm sheet of Laserskin (along with a fibroblast feeding layer) can plate about 4 million keratinocyte cells [69]. Laserskin, when accompanied by allogenic fibroblasts, is a highly effective human skin substitute in terms of wound resurfacing. In a comparison study by Lam and colleagues, the seeding efficacy of human keratinocytes on plain Laserskin was 75% while Laserskin with the fibroblast layer boasted a 95% efficacy. The difference was even more pronounced in rat keratinocytes which increased from 46% to 88% with the addition of the feeder layer. Preliminary clinical data supported that composite laser skin grafts with a fibroblast cell layer was a powerful tool with respect to its durability, biocompatibility, graft take rate, low infection rate, and seeding efficacy [31].

2.4.2. Biobrane dressings—Biobrane is a biosynthetic material made of silicone film attached to porcine collagen cross-linked nylon matrix [70]. Clinically, it is used as a dressing for burns, however there are isolated case reports of its use in ulcers and blistering disorders [71,72]. Biobrane is attractive for use in burns as its makeup allows blood and sera within the wound to form a clot, naturally affixing it to the wound bed while re-

epithelialization occurs. The outer silicone layer augments the wound environment by reducing water loss through evaporation.

The unique composition of Biobrane gives it numerous advantages with regard to standard dressings. The material is typically used for coverage of partial-thickness burns that contain no associated debris or eschar since it is unable to debride dead tissue from the wound [73,74]. In fact previous studies indicate that the depth of insult has an inverse relationship to its adherence to the wound bed [75]. One study found that only 21.7% of deep wounds covered with Biobrane maintained a natural adherence compared to 100% adherence rates observed in shallow wounds [76]. In a controlled study involving partial-thickness burns it was observed that Biobrane decreased total healing time from 15 days to 10.6 days while also decreasing treatment costs [77]. Importantly, Biobrane outperformed or was equivalent to competitive dressings in most treatment parameters. These included re-epithelialization rates, pain reduction, comfort, and ease of storage [70].

There are limitations to Biobranes for clinical use. In a controlled 30 subject clinical study involving partial thickness burns, Biobrane was not shown to decrease infection rates when compared to silver sulfadiazine and in 3 cases removal was necessary due to infection [78]. Additional studies found Biobrane to be more expensive and led to increased infection rates when compared to standard xeroform gauze [49]. Concerns regarding Biobranes propensity for infection are growing. A recent study involving 21 patients observed a 57% infection rate with the use Biobrane versus 9.5% observed in Scarlet Red (the old standard of care for donor site wound) [50]. The increased rates of infection seen with Biobrane are likely due to partial adherence to the wound bed leading to bacterial overgrowth. Ehrenreich and colleagues argue that the increased rates of infection seen with Biobrane are likely a product of their misuse as treatment of inappropriately chosen wounds [70].

Novel approaches to wound treatment with Biobrane are being investigated. Studies combining CEA and Biobrane technology have yielded promising results. Frew and colleagues treated three burn patients with pre-confluent cells seeded onto a Biobrane template [79]. The results were encouraging as re-epithelialization was complete by 9, 10 and 16 days post grafting with graft preparation time reduced [79]. As discussed previously, utilization of pre-confluent cells not only allows for diminished preparation time, thus limiting potential infection, but also allows the use of hyperproliferative cells for grafting [22]. Importantly, seeding Biobrane with cultured epithelial cells can potentially improve care by combining the advantages of CEA with a natural dressing. Furthermore, Biobranes transparent material allows for direct visualization of the wound during the healing process, minimizing unneeded dressing changes that could impede regeneration. The use of a template also alleviates graft contraction and increases the stability of the graft itself.

Advanced Wound Bioengineered Alternative Tissue (AWBAT), performance improved product similar to Biobrane, has been cleared by Food and Drug Administration (FDA) in 2010 [80–82]. In this product, the limitations in Biobrane has been improved by making the silicone membrane more porous and excluding the use of toxic cross-linking agents to make covalent bond between collagen peptide to the silicone-nylon composite.

2.4.4. Chitosan based dressings—Chitosan is a linear copolymer derived from crustacean's exoskeletons such as shrimp and crab. Chitosan is biocompatible, biodegradable, and non toxic with cell cultures *in vitro* and *in vivo* animal experiments [83–87]. In addition, chitosan has been shown to have intrinsic antimicrobial properties on bacteria and fungi, and to be haemostatic. Chitosan based implants have shown a minimal body reaction. Chitosan has largely been investigated due to its favorable properties suitable for many medical applications including wound healing [88,89]. Moura et al., developed the diabetic wound dressings using 5-methyl pyrrolidinone chitosan (MPC) which delivers neurotensin, a neuropeptide that acts as an inflammatory modulator in wound healing. They found that these dressings can promote wound healing for DFU, Figure 2 [88]. Different forms of chitosan including membranes, microparticles, scaffolds, and hydrogels were investigated for potential wound healing applications [88–94].

The HemCon® Bandage is a FDA approved chitosan dressing capable of stopping blood loss after injury and can be used as a barrier for bacteria [51,52]. One study evaluated the efficacy of HemCon dental dressing with the patients having 2 or more surgical oral sites. The study focused on how early hemostasis affects on postoperative care and surgical healing outcomes following oral surgical procedures [51]. They found that these dressings significantly shorten bleeding time and improved surgical wound healing compared to the placebo group.

The local treatment of wound dressings based on chitosan-collagen complex on the thermal skin burn in rats increased healing rate by accelerating the formation of granulation and fibrous connective tissue compared to the controls [52]. The dressing based on chitosan derivatives was used to sustained deliver neurotensin (NT), a neuropeptide that acts as an inflammatory modulator in wound healing. The dressing prepared with 5-methyl pyrrolidinone chitosan has shown rapid wound healing (50% wound area reduction) in diabetic wounds in mice [88].

3. Potential care for wound healing

3.1. Delivery of growth factors and other molecules

Platelets secrete different types of adhesive proteins, growth factors and cytokines to activate and recruit the neutrophils, macrophages, and fibroblasts [95–97]. These cells bind to the ECM and differentiate into macrophages and secrete interleukins (IL-1 α , IL-1 β), and tumor necrosis factor-alpha (TNF- α). In addition, these cells secrete fibroblast growth factor 2 (FGF 2), insulin growth factor (IGF-1), and transforming growth factor beta-1 and -2 tumor growth factor (TGF- β 1, TGF- β 2) to activate collagen synthesis and vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) to activate the angiogenesis.

Many growth factors and cytokines involve in the process of wound healing and regeneration. The topical gel comprised with a recombinant PDGF, becaplermin, approved by the FDA to treat lower limb ulcers in diabetic wounds. However, there is a concern about usage of more than three tubes of this product due to the formation of a cancer [98,99]. Yao et al., prepared the recombinant FGF loaded onto an absorbable collagen sponge to treat the

patients with chronic traumatic ulcers. The patients were treated with FGF/collagen increased the complete wound closure by 68% after 3 weeks and shortened the time to achieve complete wound closure by 24% compared to the placebo group [96]. In a similar study, the patients with diabetic foot ulcers were treated with topical application of gel comprised with human epidermal growth factor, wound healing time and closure, was significantly shortens and high, respectively, compared to the placebo group [100]. The carriers for the growth factors should be able to deliver growth factors in a sustained manner for extended time [101,102] in contrast to burst release, Figure 3 [101].

In chronic non healing wounds inflammatory mediators can be identified and targeted to improve wound healing [103–108]. Inhibition of these proinflammatory molecules is considered to be an effective way to promote wound healing. The small interfering ribonucleic acid (siRNA) can be used as a drug for chronic wounds through sequence-specific gene delivery. Proinflammatory genes such as mothers against decapentaplegic homolog 3 (SMAD3) and tumor suppression gene (p53) can be silenced at the wound microenvironment [106,107]. Another potentially new approach is targeting microRNA, the endogenous small non-coding RNA molecules, at the wound site as a molecular therapeutic intervention [109–117]. The main functions of micro RNA are to regulate post-transcriptional gene expression by binding to their target messenger RNAs (mRNAs), leading to mRNA degradation, suppression of translation or even gene activation [113]. The different wound healing phases and a summary of the most relevant micro RNAs identified, that are involved in wound healing impairment in diabetes, Figure 4 [113].

3.2. Stem cells in wound repair

Stem cells have a unique capability to differentiate into various tissues in the body. Stem cells derived from various sources, such as bone marrow, peripheral blood, umbilical cord blood, have been used for the healing of acute and chronic wounds [118–121]. Mesenchymal stem cells derived from bone marrow (BMSCs) either autologous or allogenic have been largely used in chronic wounds [118–121]. These cells have been demonstrated enhance of wound healing, increase of blood vessel formation, and granular tissue formation.

The harvesting of BMSCs from living tissue is painful and leads to have donor site morbidity. Therefore, other types of MSCs are being investigated to use for wound healing and repair. Adipose derived stem cells (ADSCs) have been attracted much attention due to their early isolation, relative abundance and multipotency [122]. ADSCs can be harvested by liposuction procedure eliminating the tissue damage [122–124]. ADSCs also release angiogenic factors which stimulate angiogenesis required for healthy wound repair [123–125]. ADSCs seeded on acellular derived matrix enhanced wound healing, promote angiogenesis and vascularization in a full thickness cutaneous wounds in a murine model [126]. ADSCs were able to reduce scar formation in wounds due to the cellular elements and numerous cytokines [125].

MSCs were isolated from the umbilical cord Wharton's jelly which eliminates the complications of harvesting of MSCs from bone marrow [127–129]. A poly(vinyl alcohol) hydrogel (PVA) membrane with MSCs from Wharton's jelly was tested to promote wound

healing in two dogs showing non-healing large skin lesions by the standard treatments. Both animals have shown a significant progress in skin regeneration after the MSC-PVA treatment [127]. MSCs were isolated from the umbilical cord Wharton's jelly improved wound healing through multifaceted paracrine mechanisms [128,130], and showed less inflammation, thinner granulation tissue formation with minimum scar in the treated wounds in goat in comparison with control wounds [129]. Shohara et al. investigated that mouse excisional splinted wounds receiving the human umbilical cord perivascular cells (HUCPVC) shown significantly faster wound healing compared with the human skin-fibroblast (hSFb)-treated and phosphate buffered saline (PBS)-injected controls, Figure 5 [130].

3.3. Environmental sensors and wound microenvironment

The enormous costs associated with these materials has pushed new research endeavors to focus on wound and graft environment sensors as a means of decreasing cost and improving care [131]. Research has shown that delays in wound treatment and unnecessary changing of wound dressings impedes wound healing, leads to complications, and is an enormous financial burden [6,132]. As stated previously, the wound environment impacts graft "take" rates as well as wound repair itself. Given this information there are obvious benefits to being able to adjust wound care and treatment based on cues from the damaged region including decreased hospitalization times and decreased number of amputations, both leading to decreased financial costs. These same principles can be adopted by tissue grafting, in which unnecessary re-grafting and bandaging sharply increases costs and delays wound repair. Sensors that are able to detect uric acid levels, pH, protease levels, and infection would aide greatly in guiding therapeutic regimens [3]. Advances in tissue engineering have given rise to advancement in biological sensor technology, allowing individual sensors to be placed on wound dressings in order to detect significant alterations in the wound environment.

One of the most important complications of wound healing is infection. In addition, the current signs of obvious infection including purulent exudate, smell, and pain are often late signs, many times obviating the need for wound dressing and/or graft replacement, and antibiotics. Misuse of bacterial growth inhibitors including silver compounds can also impede wound healing [133]. Therefore, the need for early detection of infection in order to guide the proper use of antibiotics and antibacterial products would greatly reduce healthcare costs and expedite wound healing. Optical sensors using porous silicon has lead to the ability to detect early gram-negative bacteria infection within the wound via a redshift in light [134]. Odor sensors giving rise to malodorous cues when exposed to by-products of bacterial metabolism has also aided in the detection of infection [135,136]. Specifically, the odor sensors ability to detect infection without the need to remove bandages to visualize the sensor would greatly aide in wound healing by decreasing disturbance to the wound site. Sensors used in the detection of temperature changes representing infection before superficial changes in the skin become apparent have also been designed [137,138]. Hattori et al., investigated epidermal electronics system with thermal sensors and actuators to promote accurate and quantitative data relevance to the management of wound healing, Figure 6 [138].

The use of pH indicators as a marker of wound healing is extremely complex, especially for chronic wounds due to the broad range of values observed throughout the inflammatory and remodeling processes. That being said, however, pH monitors can be embedded within wound dressings in order to monitor pH range during wound regeneration [6]. Finally, the hydration status of a wound has long been established to play a crucial role in wound healing. Some authors argue that optimal hydration is the most important factor for favorable wound healing results [139]. Too much moisture leads to maceration of the wound and not enough moisture can lead to the wound bed drying out [140]. Clinical studies have found that fluid within the wound promotes keratinocyte proliferation and fibroblast and endothelial cell growth leading to increased wound regeneration [141,142]. Therefore monitoring wound moisture status has potential benefits. The evaluation of wound hydration status can be detected with alternating current measurements, thus guiding treatment [143]. The ability to incorporate these sensors into future investigations centered on the evaluation of graft and wound repair is vital to the improvement of current regenerative technology.

Summary

Wound healing including acute and chronic wounds, is one of the major clinical challenges in the world. Even though split-thickness autograft considered as a gold standard in chronic wound management, several limitations exist in these autografts including severe donor site morbidity and pain. Other wound healing methods such as skin dressing and growth factor delivery also need significant improvement in order to heal wounds in suitable manner without delaying. New wound healing strategies are emerging including SiRNA delivery, and targeting mRNA molecules and receptors in the wound microenvironment. Stem cells isolated from different tissues such as bone marrow, adipose, and umbilical cord Wharton's jelly also have high potential to use in acute and chronic wound healing. In addition, to reduce the enormous costs associated with these materials has pushed new research endeavors to focus on wound and graft environment sensors as a means of decreasing cost and improving care.

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Highlights

- Currently available wound dressings are mainly made of natural polymeric materials.
- The carriers for the growth factors should be able to deliver them in a sustained manner.
- New wound healing strategies are emerging including siRNA delivery and targeting mRNA molecules.
- Stem cells derived from various tissues have a potential to heal acute and chronic wounds.
- Sensors and actuators are being investigated to improve the management of wound healing.

Fig. 1.

The acute wound-healing cascade. The progression of acute wound healing from hemostasis to the final phases of remodeling is dependent on a complex interplay of varied acute wound-healing events. Cytokines play a central role in wound healing and serve as a central signal for various cell types and healing events [8].

Fig. 2. Histopathological analysis of (A) hematoxylin and eosin (H&E) and (B) Masson's trichrome staining of control and diabetic mouse skin, untreated or treated with MPC, NT and NT-loaded MPC foam (magnification 100×). Representative images of three skin stainings were analyzed. (a) In diabetic wounds granulation tissue is retained in the dermis with overgrowing fibroblast proliferation on day 3 post-wounding (H&E, 200×). (b) Infiltrating polymorphonuclear leukocytes and lymphocytes in the granulation tissue in control mice on day 3 post-wounding (H&E, 200×). (c) Persistent inflammatory cells (neutrophils and

lympho-plasmocytic cells) in PBS-treated diabetic mice on day 10 post-wounding (H&E, 200×). (d) Fewer inflammatory cells in granulation tissue, compared with (c), in MPC-treated wounds on day 10 post-wounding (H&E, 200×). (e) Less deposition of collagen in PBS-treated diabetic mice on day 10 post-wounding (Masson's trichrome, 200×). (f) The granulation tissue is formed mainly of thin collagen fibers parallel to the epidermis (Masson's trichrome), [88].

Fig. 3. Release of Insulin-like growth factor-1 (IGF-1) from chitosan microparticles prepared by coacervation method at different environmental conditions [101].

Fig. 4. Schematic representation of the different wound healing phases and a summary of the most relevant micro RNAs thus far identified, that are involved in wound healing impairment in diabetes. Arrows indicate wound up-or down regulation [113].

Fig. 5.

Transplanted HUCPVC accelerated wound healing. (A) Mouse excisional wound-splinting model. (B) Representative photographs of the wounds on days 7 and 14 after HUCPVC transplantation. (C) Measurement of wound closure at different time-points (HUCPVC, n = 6; hSFb, n = 6; PBS, n = 10). The percentage of wound closure was calculated as: (area of original wound – area of wound at time of analysis)/area of original wound × 100. (D – O) Histologic analysis of the wound 14 days after transplantation. (D, H, L) HE staining. (E, F, I, J, M, N) Staining with an anti-human type I collagen MAb. (G, K, O). Staining with an

anti-mouse CD31 MAb. Scale bar = 100 μ m. Controls were PBS-injected [130]. Data are presented as means $\pm$ SD. *P<0.05; **P< .01; ***P<0.001.

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Fig. 6.

Use of an EES on human subjects in a clinical setting. a) EES laminated on the skin (forearm) after sterilization. b) Microscope images of the skin with 30 separate processes of mounting and removing an EES. c) Microscope image of the skin after the medical tape removal (1) and image of the tape surface (2). d) Illustration of the materials interface between the EES and skin e) Illustration of the medical tape and skin. f) Fluorescence images of viability of skin cells grown on an EES (left) and the results of control experiments on standard cell culture materials (right). Most of the cells on the EES remain

viable (“red” cells). g) Clinical setting for wound monitoring in a typical exam room. h) EES laminated on wound and contralateral (control) sites. i) Assessment sequence and estimated time [138].

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