 doi:10.20944/preprints202103.0031.v1

# Nanoceutical Adjuncts as Wound Healing Material: Precepts and Prospects

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**Abstract:**

Dermal wound healing describes the progressive repair and recalcitrant mechanism of damaged skin and eventually reformatting and reshaping the skin. Many probiotics, nutraceuticals, metal nanoparticles have been associated with improved healing process of intra and inter tissue wounds. Despite the vast nature on material based wound healing mediators, the exact mechanism on material-cellular interaction is still point of repent issue particularly in diabetics and pathological condition. The use of bioengineered alternative agents will likely only continue to dominate the outpatient and perioperative management of chronic, recalcitrant wounds as new additional products continue to cut costs and improve wound healing process. This review article provides an update of the various remedies with confirmed wound healing activities by a diverse group of agents from previous experiments conducted by various researchers.

**Keywords:** Dermal wound healing, nutraceuticals, metal nanoparticles, bioengineered alternatives.

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**1. Introduction:**

Wounds or abrasions are an impairment to anatomic structure of the stratum corneum causing a breakdown of the surface and other soft tissues thus altering its normal function. A wound can be a cut, scratch, scrape, punctured skin, hematoma, contusion, avulsions etc. which is an outcome of the physio-pathological process that can occur to any organ by external or internal responses [1]. Wounds disintegrate the local environ within and around the tissue leading to hemorrhage, vasoconstriction, clotting, complement activation, and pre/post inflammatory responses. Healing of a wound is an active process and progression of injury and its timely repair is an intricate and complex one, commencing from wound formation, encompassing several soluble mediators, extracellular matrices along with fibroblast accumulation, epithelial cell migration, replication and reorganization of tissues to finally reparation of its anatomic and functional integrity to thus establish homeostasis [2,3]. Tissue damage is inexorable and may extend from a minor cut or scrape to a complex and intricate impairment. The process of tissue damage to repair follows an unhindered continuum; nevertheless, could sometimes be flawed following an anomalous healing trajectory.

Wounds can be of different categories depending on their etiology, site, causative agent, complexity, infliction, treatment and curative period [4]. A simple bruise or contusion is a result of a blood vessel rupture and appears as black blue marks on the skin surface. Wounds can be open or closed depending upon their underlying tissue exposure or non-exposure to the environment. Avulsion, laceration, cuts, abrasions, punctures, bite, burn and penetrating types are primary open traumatic wounds that are limited to cutaneous and subcutaneous layer of stratum corneum and its intrinsic tissues. Acute traumatic wounds occur when the skin's epidermis/dermis layer is ruptured with a penetrating injury [5,6]. Wounds also differ from each other in their pathophysiology and medical supervision. A bite could be a clean cut in semblance but is prone to contamination, thus requiring extra medico-management [7]. Burn wounds are characterized by excessive loss of blood plasma and augmented capillary penetrability which further might lead to severe bloodstream infection in patients with impaired immune system. Deep burns show a delayed epithelization and restoration and are most prone to bacterial infection. Based on the extent of injury, burn wounds can be of first, second or third order [8,9].

Categorically cutaneous wounds are widely classified either as acute or chronic. "**Acute wounds"** are restored *via* a chronological course of healing *viz.*, inflammation, tissue development and restoration, occurring in a particular orderly fashion. The healing encompasses a complex sequential collective of cell motility and differentiation, new blood vessel formation, structural development of ECM along with scar tissue restoration, modulated by several key mediators like thrombocyte, cell signal mediating glycoproteins, numerous inflammatory cells, matrixins, etc. Such wounds have distinct overlying hemostatic, proliferative and maturation phases with an avascular scar ultimately. Upon injuries caused by accidents, trauma, burns and surgical procedures, give rise to acute wounds

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where the trajectory of wound contraction and tissue epithelization is precisely time dependent [10-12]. However, a lengthy or delayed restorative trajectory may lead to the formation of **"chronic wounds".** Such wounds are often a challenge to treatment with almost no orderly healing and poor tissue repair. Such recurrent protracted wounds result in weakened tissue restoration and are linked to abnormal anatomical or physiological conditions, on example being the diabetic foot ulcers where marginal neuropathy and subsequent anomalies give rise to viable and permeable tissues with dysregulated and continual inflammation sometimes leading to unwarranted deposition of collagen and development of an anomalous scar [13,14]. Handling chronic wounds also leads to a major capital crunch to the healthcare sectors. Non-healing wounds are also subjected to increased proteolytic and metalloproteinase profile which further makes the healing difficult unlike the acute ones, where a proper balance between the growth factors and cellular responses is mediated [15]. Surgical wounds could be any incision, excision or debridement that is a caused result of any surgical procedure. Surgical site infections as it is otherwise termed as, could differ in their size and heal time depending on the extent of wound depth and usually occur within a month of post-operative procedures. Such wounds could be due to burns, cuts in the skin, muscle or even exclusion of an underlying nodule/skin tissue. Infections can also give rise to wounds that need prolonged medicaments for its curing [16]. Diabetic foot ulcers are infectious neuropathic wounds that can progress to sepsis followed by gangrene are common in patients with a fluctuating blood glucose level. Decubitus ulcers, or bedsores, as commonly known, are wounds in the epidermis/dermis layer of the body area which has been subjected to continued and unmitigated pressure like the tailbone, hips, elbows, ankles, etc. Osteomyelitis and Osteoradionecrosis on the other hand, are bone infections that traverse the bloodstream, infecting the nearby tissue exposed to the surrounding or an effect of prolonged doses of radiation cutting off the blood supply at that particular region like in the mandibular bone [17].

Regeneration of new tissues post-wounding happens in an intricate non-linear fashion wherein the participation of cell specific growth and inflammatory factors trigger phlogistic jamboree with the inflamed migratory cells and cytokines being transported in and around the wound site with ECM and collagen accumulation and scarring. These synergistic events combine cell-cell communicators mediators like various peptides molecules, eicosanoids, protein associated molecular pattern receptors, exosomes, non-coding RNA's, etc. [18]. Healing process is independent of the wound characteristics and tangentially traverse a sequence divided into three major phases *viz.,* **inflammation, fibroblastic and maturation** where the cellular variations lead the way to neovascularization, intracellular collagen synthesis, epithelization with wound closure and new tissue repair. Variances in the degree of wound reparation is a consequence of its tissue type and the extent of injury, as for partial thickness wounds, new epithelial regeneration with nominal connective tissue formation is seen whereas the full thickness ones entail synthesis of newer blood vessels, collagen, glycoproteins, proteoglycans, epithelial cells to attain its ultimate contraction [19-20] **(Figure 1).**

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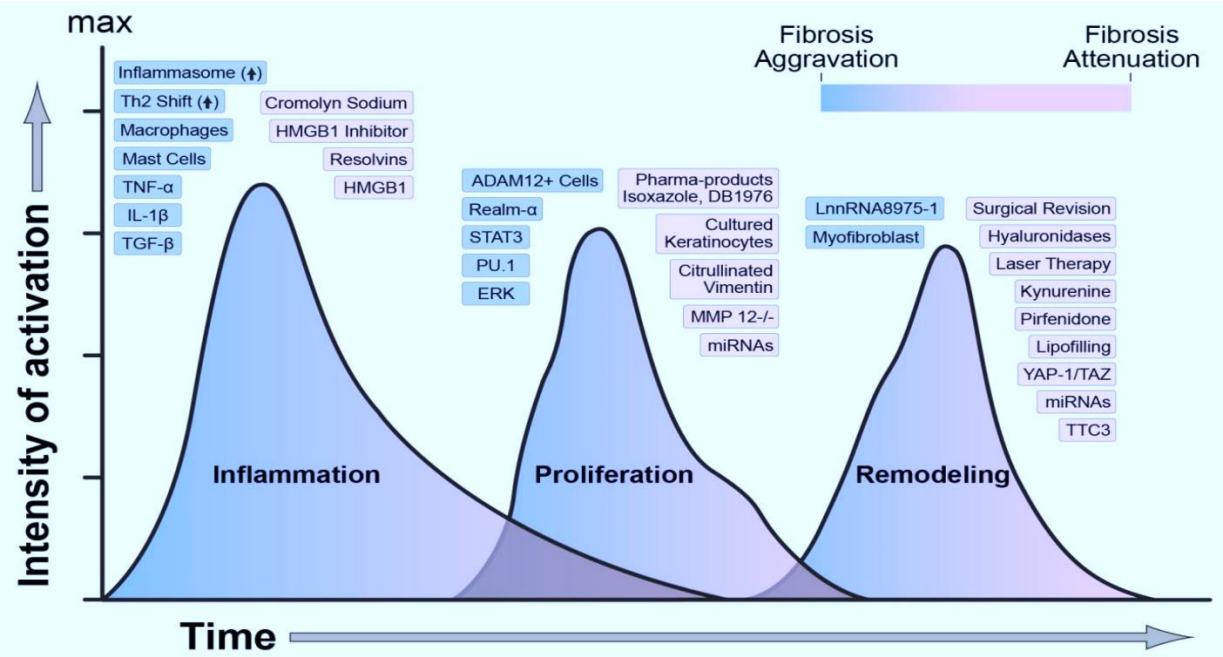


**Figure 1:** Diagrammatic representation of the overlapping phases of wound healing [figure adapted from reference 19]

Under normal functional conditions, complete restoration of the epidermal barrier and a partial restoration of the deep dermis happens which results in scarring where substantial healing over a stipulated period of time with some tissue loss is seen. Molecular and cellular effects are the backbone for an advanced healing. The triggering and activation of keratinocytes and inflammatory cells represent the primary phase of healing where the restoration of normal haemostatic balance is also observed. With the transitional and granulation phase, there is keratinocytes and fibroblasts proliferating/migrating to the wound site, matrix being deposited, newer blood vessels emerging from the pre-existing ones, refurbishing of the ECM and scarring with skin barrier repair respectively. A large number of different cells, chemokines, cytokines and growth factors also contribute to the normal wound closure and epidermal restoration. Nonetheless, with an amiss restorative response, there are usually two possibilities; a chronic wound or a hypertrophic scar [21,22]. In case of wounds that are non-healing, the cellulo-molecular pathobiological mechanisms are weakened and often give rise to hyperproliferation, non-migration of the epidermal layer, abnormal cellular infiltration, development of polymicrobial biofilms and further infection. Chronic wounds are also subjected to unregulated interference of proteinases, senescence of fibroblast cells dearth of stem cells activation and angiogenesis and ECM remodeling that hinders the physiological process of repair by not following an orderly restoration and showing functional disintegrity. Wound when remain temporally unhealed can upsurge the possibilities of vascular inadequacy, diabetes mellitus, and local-pressure effects in compromised nutritional or immunological patients with high risk of chronic mechanical stress, and other comorbidities

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[23]. Hence to sum up, a number of intricate and superfluous mechanisms when complementing one another, simplify wound healing and drive the process of repair onward.

Wound care and management have been an age-old practice in social refinement and dates back from 'Egyptian papyri' to the Crimea battlefields, where healing was accomplished by making bandage dressings of honey, grease, and lint to curb the secondary infection [24]. As already conversed, it is crucial to target and efficaciously treat a chronic wound as it can have an impact on the mortality as well as comorbidity leading to further complications [22]. Looking at the present scenario, there have been much advancements in the field wound care and much more scientifically and industrially feasible advancements have been in the picture. The initial step of any wound treatment is its bed preparation post the underlying cause of the wound has been addressed. Wound bed preparation is done to optimize the process of healing in which the wound is cleaned and made devoid of any debridement (nonviable devitalized wound tissue) to obtain a healthy granulation tissue section. Removal of devitalized tissue is important for a proper bed preparation and can be achieved using mechanical, surgical, enzymatic, autolytic, etc. methods [23].

The most traditional approach for wound healing is the usage of dressings. A dressing should ideally be moist for ready absorption of exudate and to maintain a moisture balance within wounds exterior [25]. The most conventional dressings were the wet-to-dry gauze type where decreased epithelization and a dry gauze surface caused deprived healing and also tissue impairment. Then came moist dressings or 'occlusive' as it is widely known, where an optimal moisture balance quickens cell proliferation and epithelization, averts inflammation and also balances the oxygen tension and optimum exudation in/around the wound. These further enables autolytic debridement thus accelerating healing minus the chances of substantial infections [25]. Clinically proven, moisture retentive dressings have high moisture vapor transmission rates that allow timely healing [26]. Studies by Kannon and Garrett (1995) have shown the clinical efficacy of moisture retentive dressing material in non-healing wounds [27]. Cordts et al. (1992) discussed the efficacy of cost and time compliant hydroactive dressings in treating venous leg ulcers [28]. Films, foams, hydrocolloidal materials, alginic acid, colloidal gels are some of the elementary moisture retentive dressings currently used. Both films and foams are either thin transparent or bilaminate sheets of polyurethane that have easy permeability and thickness apt for skin grafting, surgical and ascetically exudative wound applications where these amphiphilic sheets exhibit an antimicrobial tight packing over exposed bony surface to avoid any fluid leakage [17]. Polyurethane sheets are also a part of hydrocolloidal amenable dressing materials that showcases a strong adhesion onto the wound matrix and promotes autolytic devitalization of the nonviable tissues when in contact with exudate. Easy to adopt, the colloidal counterpart balances the moisture vapor transmission within the wounds. Quite a few scientific studies have shown improved barrier repair using hydrocolloids [29]. Alginic acid and colloidal gels are adsorbent wound dressing constituents comprising of short and long chain polysaccharides that maintains the hemostatic and fluid

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equilibrium by means of calcium-sodium interchange in the system. Their three-dimensional crosslinking polymeric bonds in the liquid gels offer an additional precedence for dry necrotic wound beds [29]. Vacuum aided negative pressure therapy is a crucial technology compatible for diabetic, pressure ulcers, traumatic, surgical wounds, skin grafts etc. It is supposed that this therapy fastens wound repair by sustaining moisture around the wound edge, reducing edema, stimulating angiogenesis and granulation tissue deposition. Soares and coworkers (2013) carried out a randomized controlled experimental analysis wherein they found that negative pressure therapy not only facilitated the reduction in bacterial load but also quickened wound contraction unlike the conventional moist gauze dressings [30]. A high-quality, independent evidenced healthcare database has stated that the therapy works amazingly in postoperative diabetic foot ulcers [29]. However, the lack of data has left an ambiguity in this treatment efficacy and further experimental evidences are a prerequisite for its wide usage. Biologically engineered skin equivalents mimetize the stratum corneum structure and trigger several tandem reactions to replicate healing as it happens in a normal biological skin surface. Categorized into epidermal, dermal, and dermo-epidermal combination skin constructs, these are highly effective in treating diabetic and venous ulcers. Topical adjuvants, autologous skin grafting and hyperbaric oxygen treatments have also been explored for disease specific management of chronic wounds.

## 2. Wound Healing Management

### The Process of Wound Reparation and its Cellular Crosstalk Underneath

Wound healing upon an acute damage begins with thrombogenesis where there is a cramming of immune cells and platelets that permeate the injury site to release several chemokines, cytokines and growth factors [19]. Thereafter, a huge chunk of inflammatory phagocytes is recruited at the wound site upon a steep rise in the cytokine concentrations, thus inducing inflammation [5,15]. These chemotactic cell signalling molecules take part in the inflammatory phase by exuding a cascade of bioactive molecules responsible for clotting, swelling, fibrous tissue formation, ECM deposition and remodelling, vasculogenesis, epithelialization and contraction [15,16,19]. Monocytes and macrophages are also important players of inflammation and tissue repair as these accumulate at site in response to a cascade of integrins and initiate proliferation and regulation of several other growth factors.

#### *Inflammatory cellular response and its transition from inflammation to reepithelization and refurbishment*

Whether it is skin, soft tissue, bone or any organ, the the response to damage/trauma is no unalike. When acute wounds heal, there is a time modulated and well-organized refurbishment of dermis/epidermis tissue barrier which remains unfinished with chronic wounds where predominantly a scar or a keloid is formed with delayed healing due to the unbalanced release of cytokines and growth factors at the wound site [31].

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The inflammatory and proliferative phases commence within 24 to 48 hours post-damage, with the penetration of neutrophils followed by macrophages (cresting approximately till 5 days), fibroblasts (7-9 days), and lymphocytes (cresting approximately on day 7) into the site of wound. Once haemostasis is attained, thrombocyte aggregation and vasoconstriction triggering decreases blood loss with hypoxia, increased glycolysis, pH variations and coagulation in the wound bed. Wound bed being the interim wound matrix framework for exodus of diverse cellular players channelizes platelet degranulation and activation of complement pathway to stimulate inflammation [32,33]. Keratinocytes, fibroblasts, mucosal and dermal epithelial barrier, platelets, immune cells are the primary cellular players that coordinate the multifaceted cellular and molecular mechanistic function of tissue repair.

Contemporaneous with the hemostatic/coagulation phase, inflammatory phase is designated as the 'early phase of healing' where the innate immune response is activated [34]. When the body senses an injury, the typical dermal cells *viz.*, keratinocytes, fibroblasts, dendritic cells, monocytes, macrophages are subjected to certain molecular pattern 'threat' signals either from the host cellular stress responses or from the guest pathogenic moieties like bacterial polysaccharides [35]. The immune response through its pattern recognition receptors (the toll-like-receptors or TRL's) smartly identifies these 'threat' signals to then activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a crucial transcription factor in immune response, apoptosis, inflammation and serine/threonine-specific protein kinase involved in guiding diverse cellular retorts such as osmotic dysfunction, heat shock and proinflammatory cytokines, to express of various genes, cytokines, chemokines, antimicrobial peptides, etc. to recruit and disseminate the inflammatory cellular response[34,35]. As well, more than a one cellular, molecular immune triggering key players specific to each phase drive the whole process of repair and will be described as and when they come into the 'healing scene'.

Normally, with acute wounds, the inflammatory phase spans for first 5 days and terminates when the cellular response stimuli have subsided, although the innate and adaptive immune cellular responses persist to function during all the stages of repair [31,32]. As the inflammation subsides, the proliferative phase sets in where new tissues comprising of the collagen and other ECM components are restored (re-epithelialization) with simultaneous wound contraction through a well-knit vascular network and granulation tissue formation. Re-epithelization is one of the most crucial phases during any epithelial or dermal wound repair and occurs immediately to day one after the wound induction, when the wound-edge keratinocytes start migrating. Basal keratinocytes are rapidly migrated to conceal the wound after 48 hours post-injury and this migration is triggered by the aid certain cell adhesion assemblies; the desmosomal and hemi-desmosomal membranes that activate the calcium dependent kinases, which in turn reorganizes the cytoskeleton to drive migration [36]. Keratinocytes, prior to their further migration to initiate wound repair, embrace new wound specific cell-fibrin rich matrices and also alters their normal cell matrix adhesions. The

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switching on/off regime of several integrins is extremely important in order for the cells to continue the process of wound migration. For example, in mice models, the keratinocyte-specific knockout of b1-integrins can cause severe impedance in the re-epithelization phase [37].

The enzymes collagenase, elastase and hyaluronidase also determine the cutaneous healing potential. Repair signaling molecules: nitric oxide together with epidermal growth factor (EGF), KGF, IGF-1, and nerve growth factor (NGF) [38] also stimulate the process of re-epithelization. Blood vessel repair is also a crucial step in epithelial tissue restoration. Vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and thrombin [39] begin the process of angiogenesis by activating the endothelial cells that are the storehouse of proteolytic enzymes. These enzymes dissolve the ECM basement membrane and allow the endothelial cells to seep out of the existing damaged vessels for proliferation and migration to form newer blood vessel networks, arteries, venules and further sprout to give rise to Rouget cells and non-straited muscle cells [40]. Moreover, vascular regeneration with bone marrow (BM) stem/progenitor cells or vasculogenesis is the formation of blood vessel in the rudimentary embryo produced *de novo* by the endothelial cells. Occasionally paired with angiogenesis, it is primary stage of formation of the vascular network prior to mature blood vessel making [31,40].

The proliferative phase consists of the granulation tissue development from the interim wound bed formed during the haemostatic phase. The granulation tissue comprises of a pool of fibroblasts, white blood cells, phagocytic cells, blood vessel networks and collagen bundles that recuperates the structural and functional integrity of the damaged tissue [41]. Fibroblasts exhibit significant role in preserving skin's homeostatic balance and for orchestrating granulation tissue formation. Upon its migration into the interim dermis wound bed, these activated fibroblasts proliferate and secrete MMP's for simultaneous degradation of wound matrix [41] and remodelling of ECM ensuring wound closure. Myofibroblasts are capable of cell adhesion, growth, division, apoptosis with growth factor bioavailability by binding, sequestration, and initiation during the repair process [42]. For prolonged wounds, the bone marrow derived fibrocytes circulate around the damaged region and endorse its healing by recruiting fibroblasts, cytokines, other growth factors to hasten angiogenesis [42].

Clinically the maturation phase commences post the development of granulation tissue where myofibroblasts are driven by TGF- β for ready expression of $\alpha$-smooth muscle actin (SMA) and contraction of wound [43]. During maturation, the Collagen III (major component of the ECM) are replaced by Collagen I having higher tissue strength. Myofibroblasts then undergo caspase-mediated cell death upon completion of remodelling. A decline in the new blood vessel network is observed and a mature avascular environ [31]. Based on the depth of the wound, the wound recovery is estimated; like hair follicles and sweat glands detest complete recovery after serious trauma with only 3/4$^{th}$ of the original structure and strength of the tissue achieved [31].

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Irrespective of the wound site, there is always a steady but equilibrated simultaneous synthesis and degradation of extracellular matrix and matrix metalloproteinases (MMP's) respectively that preserves the structural coherence of the restoring tissue. The first line of defence upon any injury is the stimulation of epithelial cells adjacent to the wound that repopulates the cut edges and skin appendages and initiates the upregulation of numerous gene clusters in and around the edges [44]. Such evidences have been revealed by mouse transcriptome analysis experiments. The early prime genes are the Activator protein 1 (AP-1), a heterodimeric transcription factor of Jun and Fos family protein moieties (that include the Jun proteins c-Jun, JunB, and JunD, as well as the Fos proteins c-Fos, Fra1, Fra2, and Fosb respectively) along with Cys2His2 zinc finger transcription factor Krox-26 which partake in the transcription machinery by activating several hundred other genes [46]. Subsequently, the upregulation of these cells outpours in proliferation and epithelium migration of keratinocytes at the scab-wound granulation tissue junction [43]. Likewise, modifications are also necessary in cell-cell junctions which if not functioning properly might lead to delayed healing like the desmosome-keratinocyte junctions that acquire calcium dependency instead of being serine/threonine kinase dependent and contribute to unsteady cell adhesions and ultimately to late wound repair [16]. It is the MMP's that come into the 'foreground' to link the integrin-collagen as the epidermal barrier gets restored [44].

*Crosstalk of keratinocytes and fibroblasts during healing*

Reports confirm that mesenchymal-epithelial cross talk when mediated by autocrine/paracrine regulatory mechanisms initiate keratinocyte-fibroblast interaction, development and differentiation which controls the MMP expression and therefore, help achieve skin homeostasis [41,46]. Keratinocytes, in a typical skin, are accumulated within the dormant epidermal tissue adjoined by the desmosomal-hemidesmosomal cell junctions. Studies on keratinocytes sheets cultured in a keratinocyte-conditioned medium has been seen to accelerate wound epithelization and healing when used for transient wound covering *in vitro* [41]. Experimental demonstrations also revealed that lyophilized keratinocyte cell lysates exhibit mitogenesis markers for endothelial cells, fibroblasts and keratinocytes [46]. In both wound repair and re-epithelization processes, the keratinocytes and fibroblasts play a vital role. The mutual communication between the epithelium and mesenchyme for keratinocyte stem cell phenotype differentiation has been long established [46] and growth factors are the essential hook for epidermal proliferation. The keratinocyte seeded mesenchymal feeder cell cultures direct the fibroblasts to initiate the secretion of keratinocyte growth factor (KGF)/fibroblast growth factor-7 (FGF7), IL-6, and GM-CSF [47]. Modulation of fibroblast proliferation and paracrine mediated extracellular matrix formation in keratinocyte-conditioned medium demonstrated an upsurged fibroblast replication and reduced collagen formation during the repair process. Goulet *et al.* reported soluble factors mediated increase in DNA synthesis in a keratinocyte-fibroblast co-culture medium [48]. Interruption in the keratinocyte-fibroblast coordination might also alter dermal fibroblast function. Postponements in the process of epithelialization escalates the incidence of fibrotic conditions.

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In fact, when keratinocytes cover the wound, only 22% of the structurally site coordinated wounds develop fibrosis, within the first few weeks, which reached to 78% when re-epithelialization happens after 21 days. Thus, it is understood that when epithelization is non-occurring, the extracellular matrix still continues its deposition until a paracrine signal is from epidermal cells to the fibroblasts to slow down the healing of wound is received [49]. McKee *et al*. verified using microarray analysis that, in a keratinocyte-fibroblast co-culture system, a large number of genes in the fibroblasts, that code for multiple growth factors, cytokines and their receptors, ECM, adhesion receptors, MMPs, and cell cycle regulators, are meticulously being controlled by keratinocyte-derived factors *in vitro*, signifying that the same extent of intercellular communiqué might also occur in vivo leading to the reconstruction of tissue integrity post-wounding [50].

*Crosstalk of Innate and Adaptive immunological response during healing*

With time, innate and adaptive defense systems have not only been explored in the expanse of wound repair and regeneration but also have been crucial in managing the complex cascade of cellular and molecular events that pertain to wound healing [51,52]. Cellular crosstalk, synthesis and secretion of growth factors, cytokines, chemokines, etc. are hallmarks of both non-specific(innate) and immune effector (adaptive) cells that control re-epithelization and repair process. Innate and Adaptive immune responses share a concurrent relationship and currently experimental indications for their use as novel therapeutics is being explored [51]. Similarly, it is necessary to throw light on the mechanistic approaches that keratinocytes, immune cells adapt for successful healing which can very well be a new treatment option. Impaired healing occurs when there is untimely and unbalanced production of the enzymes, growth hormones, chemokines triggering inflammation, ulceration and edema formation [52]. Therefore, it is of utmost importance to acquire about the cellular biology and wound immunology along with interactions of keratinocytes with immune cells to fairly contribute to the mechanism of reepithelization of a damaged tissue. Nonspecific immunity is the initial line of defense which exhibits instantaneous action in response to any trauma and eliminates the chances of host infection. This non-specific cell reaction depends on certain molecular pattern structures that are highly conserved in microorganisms and are termed the pattern recognition receptors (PRRs) [53]. Both PAMPs and DAMPs participate in healing. While PAMs are bacterial survival structures like bacterial endo/exotoxins, double stranded DNA, murein; DAMPs belong to cytoplasmic and nuclear protein components (high mobility group box 1 [HMGB1] proteins, heat shock proteins [HSPs], S100-β homodimer proteins, and purine metabolism [53,54], released during cell stress response, necrosis, acute inflammation, apoptosis, etc. Straino and co-workers (2008) have reported the chemotactic activity of HMGB1's on epithelial and fibroblast cells *in vitro* which is seen to accelerate epithelial tissue formation in diabetic rats when administered topically [55]. PRR's are solely expressed in antigen-presenting professional and non-professional cells and categorized into four major classes of toll-like receptors (TLRs), C-type lectin receptors; and retinoic acid-inducible gene-I-like receptors, and NOD-like receptors (NLRs). Toll-like receptors (TLRs) are single-pass

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membrane-spanning receptors majorly expressed on the sentinel cells and is among the extensively studies PRR. Upon activation, TLRs consequently also stimulates the NF- kB and MAPK cellular pathways with the help of a cascade of adaptor protein signalling molecules; the myeloid differentiation factor 88 (MyD88) and MyD88 adaptor-like protein (MAL/ TIRAP), TRIF-related adaptor molecules which further activates and produces cytokines (IL-1, IL-6, IL-8, IL-12) and TNF-$\alpha$ [56]. Cytokines makes sure that other small chemotactic cytokine molecules are triggered from the adjacent cells which causes the migration of inflammatory cells to the site of injury to help the innate immune response to set in [57]. Maturation of antigen presenting accessory cells also occurring via the TLR-activated inflammatory mediator cells bring about T-cell maturation and T-helper type 1 (Th1) polarization, thus employing the acquired immune response to come into the play and initiate the process of wound repair [56]. Adenosine A2AR receptors, secreted in all human cells, act through the seven transmembrane G-proteins that can modulate cAMP to diminish inflammation and thus protect the tissues from inflammatory impairment. The A2AR receptor controls the TLR-mediated cytokines and chemokines to be formed in order to accelerate wound closure. Clinical evidences have suggested that such adenosine receptor agonists enhance wound epithelization and contraction in MyD88+/+ mice [57,58]. These also possess a modulatory activity on sentinel cells and have been theorized to exhibit wound restoration activity. Besides, the immune system modulating CpG oligodeoxynucleotoides stimulated by TLR-9 signalling pathway, shortens the time of re-epithelization and the development of granulation tissue [59]. Keratinocytes also contribute to the innate immune response and trigger TLR cell pathways to source early skin healing. Not only do they provide structural sustenance but also resists several skin pathogens and controls inflammation at the injury site. The cells produce certain antimicrobial peptides (eg. human β-defensins (hBDs)) that very well persuades differentiation and migration of keratinocytes to the wound edge. The acquired immune response unlike innate possesses an immunological memory which makes its response to any immunologic trial very quick and prolonged. However, there occurs a link between the two immunological responses and preliminary experimental establishments have shown both these immunities co-exist and confer to wound healing [46,48]. Even though certain cells activate, functionalize and link both these immune responses like the plasmacytoid dendritic cells, gamma delta T lymphocytes and Langerhans cells, which are also the prime participants in wound healing, it is necessary to explore these mechanisms further for a clearer picture.

*Crosstalk of innate immune response and epithelial cells during healing*

Epithelial cells present in the skin and mucosa are defensive shields against harsh environments and microbial infection. When these cells are subjected to contusions, there immediate task lies in the renewal of this injured epithelium with the help of certain complex reactions that signals the immune cells (neutrophils, monocytes, phagocytic cells) to begin the repair. Therefore, there is a whole cascade of multi-layered events that happens between the epithelial and the immune cells that also contribute to wound repair and homeostatic balance

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within the tissues in complicated disorders like from inflammatory bowel disease and ulcerative colitis which causes recurrent mucosal inflammation and damage. Epithelial cells exhibit significant migration and proliferation activities for wound regeneration in both duodenal and cutaneal surfaces. It is also partly recognized that the complex three-dimensional and chronological relationship between the various professional phagocytic cells and the crosstalk between these innate immune cells duodenal and cutaneal epithelial cells initiate tissue healing [52].

Like cutaneal wounds, duodenal ones also follow the same cellular and molecular path of coagulation, infiltration of immune cells towards wound edge, followed by grouped migration of epithelial keratinocytes, their proliferation and maturation and finally restoration of barrier function.MMPs are the torch bearers of wound maturation phase. The MMPs, during the wound repair process, in the intestine, sever and control ECM components in the epithelium. These also subtract the injured structural peptides from the wound to add newly formed collagen. Additionally, MMP-7 (of matrixin family) is responsible for renewal of the epithelial cells in the human intestinal mucosal barrier [60,61]. The series of cellular events in turn activates leucocytes, multipotent stem cells, PRRs, and intracellular calcium pockets that orchestrates healing. Signals from Rho GTPases Rac1 and other wound repair-related proteins also aid in epithelial repair; the G proteins stimulate F-actin and integrin mediated cell-matrix adhesions that are associated with epithelia movement and wound closure [62,63]. The epithelial environment also balances many such remodelling signalling molecules like the annexins and serum amyloid A1 that promotes adhesive kinase and ECM activation in mice and human mucosal barrier. TNF-$\alpha$ and TGF- $\beta$ are among the cytokines that in consort with 'Wnt glycoproteins' indorse epithelial intestinal tissue repair [64].

## Interplay amid the key players involved and their effect in deferred wound repair

It is crucial to keep a check on the start and intensification of inflammation and proliferation phases in order to maintain a timely healing response. It is also well acknowledged that any interruption in the healing process may result in scarring, secondary microbial infections, peri-wound edema, hematoma, necrosis, dehiscence, etc. Hence, a fair comprehension on the underlying cellular and molecular mechanisms and the interplay among various key factors associated with healing is vital. Moreover, the effect of these factors on non-healing refractory wounds is also something that cannot be unkempt. This section elaborates the role of key players in timely healing of typical wounds and also their role in refractory complicated non-healing wounds.

*(a) Scavenger white blood phagocytic cells- the macrophages:* Macrophages exhibit diversification in their functional phenotypes and retort differently to varying micro-environs of wound repair. The scavenging white blood cells are the only prime players that 'work on' all the

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phases of repair (ref). Under normal skin conditions, macrophages are involved in maintaining a hemopoietic and homeostatic equilibrium within the system. Post-injury, monocytes accumulate around the site of the wound and macrophage cells with an altered phenotype are concurrently activated and influenced by PRR moieties and natural killer cell-derived interferon-gamma. These then differentiate to M1 (classically activated macrophages) which release nitric oxide to curb intracellular pathogens, stabilized host cell and promotes antitumor T-helper cells producing immune response [65,66]. The M2 (alternatively activated macrophages) set, possessing anti-inflammation, glucose regulating and healing activity is driven by the Interleukin family (mainly IL-4 and IL-13) [66,67]. Toll-like receptors team up with IgG complexes to stimulate the macrophage cells to produce immunosuppressive IL-10 and TGF-β1 [65]. During hemostasis, the M1 type initiates phagocytosis, apoptosis, foraging of cell remains and induces Interleukin pro-inflammatory mediators and TNF-$\alpha$ to trigger the leukocytic cells [68]. As the process progresses towards the inflammatory and proliferation phases, M1 is transitioned to the M2 set of cells producing decoy/regulatory receptors for agonist ligands of IL-1 family along with growth factors that promote fibroblast differentiation, ECM remodelling and formation of new blood vessels [65]. Thus, the M1/M2 changeover is extremely significant for assuming the persistence of inflammatory phase and for preserving the sense of balance to tissue restoration [66,68]. M2 phenotype is also induced by several glucocorticoids, prostaglandins, glucose-lipid modulators and some cytokines as well. But then in case of complex non-healing wounds, the functional modulation of M1/M2 macrophage subset is fragmented. With chronic wounds, disruption in the M1/M2 phase may be due to iron overload within the macrophages that pushes them into an uncontrolled pro-inflammatory M1 activation state, which has been observed in case of venous ulcers and delayed skin repair [67]. Macrophages efficiently endure a changeover from pro-inflammatory to healing-correlated properties that is necessary for effectual wound repair and this swop is due to the presence of peroxisome proliferator-activated receptor (PPAR)γ. The upregulation of PPARγ's and the simultaneous mitochondrial matrix leads to accelerated wound closure and epithelization. This PPARγ upregulation is repressed by IL-1β under diabetic conditions as studied in mice and human wound models [65]. Furthermore, the myeloid-specific PPARγ in genetically modified mice models exhibited that a protracted inflammatory phase and deferred healing can take place when there is a loss of PPARγ from the macrophage veneers [65]. Reduced levels of inducible nitric oxide synthase expression (marker for M1 phenotype) which is responsible angiogenesis and neural development during deep wounds, and elevated levels of arginase-1(marker for M2 phenotype) which lead to shortened healing time of cutaneous wounds was observed in db/db experimental mice models of type 2 diabetes. This very imbalance in both the enzyme levels further caused chaotic anti-inflammation and high levels of IL-4 and 10. Improper epithelization and delayed wound healing can occur during the later phases of injury by the accumulation of advanced glycation end products (AGEs) that triggers the macrophages to secrete unwarranted levels of TNF-$\alpha$ [66]. Transitory change of phagocytic cells from pro-inflammation to healing allied phenotypes is the key for complete wound repair. This impaired phenotypic alteration in

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macrophages is linked to up-regulation and increase of PPARγ and mitochondrial content levels respectively [69]. Also, experimental evidences showed that loss of PPARγ in macrophages was could with 'ease' lengthen wound inflammation and interrupt repair in myeloid-specific PPARγ knockout mice [69].

*(b) Endothelial cells:* Endothelial cells (EC), platelets and enzymatic breakdown of fibrin in blood clots are some the prime factors that control haemostasis. Endothelial cells are the indirect reservoir of blood supply to the newly formed cells and tissues and supports its development and subsistence and regulate inflammatory reactions in the cells [2,15]. The crucial processes such as clotting, regulation of blood flow, transport of plasma proteins into the tissues are functions of resting endothelial cells which impedes inflammation. Adequate levels of nitric oxide production have an influence on the proper functioning of endothelial cells. Endothelium activation is important in the process of inflammation during repair. Endothelium undergoes two types of activation; in type I, the guanine nucleotide binding protein (G protein) facilitated receptors trigger G-protein $\alpha$q subunits and instruct the cells to augment blood flow and plasma proteins into the tissue indorsing the stimulation and binding of neutrophils which finally leads to erupts into the site of inflammation. In type II, tumor-necrosis factor (TNF) and interleukin-1 (IL-1) facilitates the augmentation of blood flow and helps in the permeability of plasma proteins and simultaneous recruitment of leucocytes. Type-II activated endothelial cells also function in recruiting neutrophil mediated monocytes and T helper cells for inflammatory reactions to take place. During the course of non-healing wounds, higher glucose or AGE levels could make the cells to suffer higher apoptotic cell death, upregulated secretion of intracellular adhesion molecules: CD54 and CD106, increased production of reactive oxygen species and malonaldehyde, lower levels of dismutase. This as a result, activates the MAPK and NF-κB pathways and recruits the congregation of leukocytes onto the injury site [5,10,15]. Hyperglycemic environment does induce the production of reactive oxygen species (ROS) in the endothelial cells by means of either of the pathways, *viz.*, polyol pathway, AGE/RAGE pathway, sorbitol pathway, etc. [15]. An overaccumulation of ROS in the bloodstream can restrict vasodilating factors: nitric oxide (NO) and prostaglandin I2, and upsurges vasoconstrictors: preproendothelin-1 (PPET1) and thromboxane which initiate an inflammatory reaction to promote white blood cell adhesion and trigger TNF-$\alpha$ secretion. Such situations could be a reason for deferment in the process of wound repair especially in diabetic foot ulcers [19].

*(c) Granulocytes, fibroblasts and the keratinocytes:* Granulocytes, specifically the **Neutrophils** are the primary shields of innate immunity and respond to host infection or harmful mediators. These 'suicidal killers' adopt one of the three approaches to subside wound injury mediated inflammation and initiate tissue repair. Firstly, neutrophils act as specialized phagocytes, removing tissue debris at the injury site. Secondly, mature neutrophils activate the release of certain growth and pro-angiogenic factors to directly begin regenerate and revascularize the wounded tissue. Thirdly, neutrophils undergo apoptosis and are cleared up by macrophages

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[70] by a feed-forward mechanism of the release of tissue-repairing cytokines to accelerate tissue renovation. Neutrophils primarily act as decontaminators during the normal repair process. But an abnormalcy in their numbers in and around the wound site over time may contribute to the pathogenesis of non-healing wounds as seen in patients with high blood glucose levels. Neutrophil serine proteases can damage ECM as well as certain essential repair proteins e.g., clotting factors, complement systems, cytokines and immunoglobulins [2] and build up oxidative stress in the cell [15,19]. Reports have showed that neutrophils are susceptible to apoptosis in hyperglycemic patients where a decrease in the neutrophil longevity and their fast clearance from the site of infection may lead to prolonged infection phase. An *in vitro* study on diabetic rat models depicted the abundancy of AGE's in skin tissues that hindered the binding of neutrophils to the surface receptors and triggered a number of cytokines to induce oxidative stress. This cytokine triggering and ROS production in turn affected the heal time [19, 57].

**Fibroblasts** or the structure stromal cells are prime active regulators of wound healing and pro-inflammatory events [70,71]. These cells match up with local stomal environment to regulate the level and kinetics of inflammation by interacting with the infiltrating inflammatory cells via CD40 receptors to activate NF-κB complex and direct the fibroblasts to regulate the infiltration and function of immune cells by stimulating IL-6, IL-8, cyclooxygenase-2 [71]. Inflammatory cells undergo apoptosis when cytokine production becomes deficit and the inflammation is brought down [71] and fibroblasts participate in regulation of apoptosis by the aid of type I IFNs [70]. It is observed that skin upon inflammation has amplified expression of stromal derived factor (SDF-1) and fusin on infiltrating T-helper cells which interact together and might lead to the inapt retention of immune cells in the skin [93]. Overall, fibroblasts do affect the inflammatory-proliferative phase transition by 'repair' and 'removal' role. However, chronic wounds compel fibroblasts to exhibit altered functionalities. *In vitro* experiments by Wang et al. on the proliferation of fibroblasts indicated apoptotic cell death and deterioration in proliferation of fibroblasts in the presence of certain glycation end products [70]. A dose reliant drop in the fibroblast proliferation, collagen and hyaluronic acid secretion with anomalous cytokine and matrix metalloproteinase expressions were also observed in a glucose rich AGE medium. Fibroblast mediated vascular endothelial growth factor (VEGF) remains impaired under hypoxic environments, MM-9 are overexpressed and trigger the pro-degradative activity in diabetic mice model studies [72]. Diabetic fibroblasts fail to produce nitric oxide that is responsible for higher levels MMP-8 and 9. Hinderances in NO production curtail the cell to proliferate and restore the damaged tissue [73].

**Keratinocytes,** players of the proliferation phase of healing do so by secreting proteins to rebuild the basement membrane and cause re-epithelization. However, this regulation process goes haywire when a few unwarranted factors play in. A significantly higher NF-kB regulation of inflammatory response in keratinocytes was observed in diabetic rats in a study conducted by Takao and co-workers [74]. It is studied that the keratinocytes undergo an inverse concentration dependent activation via the AGEs and higher concentrations of AGE

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inhibit keratinocyte proliferation by blocking the changeover from S to G2/M cell phase and by inhibiting NF-kB signalling pathway and promoting apoptosis of keratinocytes. Greater neural deposition of AGEs might increase cytoskeletal proteins which can impair the transport of plasma to have an influence on intracellular signalling and phosphorylation ultimately lead to degradation of axons [74]. Many a times, over accumulation of AGEs in the nerve nutrient vessels causes the nerve vessel to narrow down an constrict and concurrently associates with a signal transduction receptor on the endothelial cells to lessen iNOS production and blood flow resulting in dysfunction of the nerves [41].

## 4. Prospective agents of wound healing

### Conventional therapies implemented for healing

*(a) Skin grafting techniques*

Tissue grafting has been explored since a long time now, with initial use of autografts going back as far as 6$^{th}$ century (ref). Skin grafts come into play when the tissue loss or injury is chronic. Based on the graft thickness they could either be split thickness or full thickness skin grafts [75]. Typically, split thickness grafts use the epidermis and the papillary dermis of an adult healthy skin for repair [75]. Spilt thickness grating is known to be the gold standard for a variety of cutaneous wounds (ref) but comes with certain limitations. Split thickness procedures fail to repair If the skin loss is more than 1/3$^{rd}$ of the total area of body skin [75]. While meshing can increase the surface area at the graft sites, but balancing the meshing ratio which ideally should be more than 3:1(graft: wound area) is hard as it is prone to contracting during repair [76]. Post grafting symptoms of ache, redness and inflammation are also observe eth such skin grafts. Contrary to it, full thickness grafts use both the epidermal and complete dermal layer and are advantageous in the repair of soft tissue defects. A full thickness skin graft can handle chronic injuries well, with less skin shrinking and more aesthetically natural looking post-healing unlike split thickness ones [76]. Full thickness grafts, however, need a fully vascularised bed for grafting and is affected by donor skin unavailability [77]. Lately, the efficiency of autologous skin grafts has been improved by combining it with scaffolds, gels, therapeutic agents, etc. to accomplish massive full thickness injuries [75]. Allotransplantation or homografts are obtained from different people of similar species and are often beneficial in traumatic wounds where a transient graft covering to alleviate the recipient's wound bed until autografting is done [78]. Homografts are immediately available, increases donor supply and extended storage before use thus giving them an upper hand in the grating method. Regrettably, allotransplants are often subjected to viral contaminations such as human immunodeficiency virus, cytomegalovirus and hepatitis [78] and also might induce

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strong recipient inflammatory immune reactions leading to the interference of T and B lymphocytes to ultimately reject the homograft [79]. Recently, experiments have shown that implementing mixed chimeric molecules with donor's bone marrow could subdue recipient graft rejection in clinical therapies [79] like the *in vitro* assay on RA-iTreg cells (retinoic acid) that exhibits immunosuppressive T-cell proliferative activity and also prevents T-cell cytokine activity in mice models [80]. Xenotransplants on the other hand, are obtained from heterologous species with the most frequently used being porcine xenografts, which are ready for use, but can cause secondary infection from other dissimilar species. Usually used with burn wounds where <25% of total skin area is affected, xenografts reduce the implementation surgical excisions and saves time
[81].

*(b) Wound dressings*

A dressing is considered ideal if it confers complete wound shielding, eliminates excess exudate, possesses antimicrobial efficacy, maintains a balance between optimum hydration and oxygen, is easy to handle has non-anaphylactic properties [25]. Some of the most frequent dressing materials used for wound healing are described here. Conventionally cotton gauze, lint, plasters, bandages and cotton wool were used as primary or secondary dressings for wounds [25]. Most of the dressings bared a problem of frequent changing, contamination from the wound fluid, imbalance of wound moisture, difficult to remove post application, incomplete antimicrobial protection. Then cotton and polymeric bandages were used to treat dry wounds and those with mild exudation. For example, nonocclusive dressings like the Xeroform™ is made up of petroleum based fine mesh gauze with 3% of bismuth tribromophenate for treating preliminary exudating wounds. Fabric based non-allergic dressings saturated with paraffin and olive oil such as Bactigras, Jelonet, Paratulle etc. are commercial are non-adherent and gamma sterilized dressings suitable for superficial clean wound. The setbacks that the traditional dressings have like providing an occlusive hydrated wound healing environment have given way to modern alternatives *viz.*, contemporary formulated dressings. The contemporary cotton gauge dressings incorporate chitosan-silver-zinc oxide nanocomposites for efficient moisture retention and anti-bacterial efficiency [25, 26]. During the late 20$^{th}$ century, **human amniotic membranes** were used for dressing for exudate and fluid laden burn wounds. Used as a non-cellular medium for adherence of mesenchymal stem cells and these served as a vital platform for skin equivalent development. Though such dressings provided temporary pain relief, balanced the optimum hydration in wounds, was time and cost effective but the chance of infection spread

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was high [82]. Among **polysaccharide** dressings, chitosan and chitin are the most explored ones for clinical therapeutics because their non-toxicity, biocompatibility, high durability, antibacterial efficiency and suitability to be applied onto open wounds [25]. Their limitations include low tensile strength and elasticity. **Algal extract-impregnated dressings** have good absorbency, are hemostatic, and anti-microbial in nature and thus are useful in exuding wounds [83]. Use of chitosan-alginate amalgamated dressings can improve the mechanical strength and stabilize the dressing. **Hyaluronic acid**, a linear polysaccharide, incorporated into dressings are compatible with burn, chronic and surgical wounds [26] where it gives a structural sustenance to enable the nutrient diffusion, clear wound debris by their interaction with the CD44 molecules and balances hyperhydration during new tissue regeneration [26]. In addition, hyaluronic acid dressings activate keratinocyte to migrate and proliferate wound site for its ready repair [84]. However, they are highly dissolvable and have less residence time *in vivo*. **Microbial cellulose** obtained from *Acetobacter* can precisely be made into a dressing and be useful for prophylaxis of extremely chronic injuries that require recurrent dressing change [25,26]. Unlike from other phytocelluloses, microbial ones show substantial pliability, strength, biocompatibility and good absorbency, but then their anti-microbial action limits its medical applications. And could be improved to a certain extent by incorporation of nanoparticles like zinc oxide nanoparticles. **Hydrocolloid** based dressings are occlusive dressings for pressure ulcers [85]. They maintain an optimum water and oxygen balance within the wounds but fail to hold large amount of exudate for which their frequent changing is necessary to evade maceration of tissues [85]. **Foam** dressings are bilaminar structurally with a hydrophilic end with moderate exudate absorbency for wounds with exposed bone. Foam dressings can be rightly called as the new substitute to conventional dressings for treating venous pressure ulcers in preventing hospital-acquired pressure ulcers in critically ill individuals. They, yet, do not have much adherence to wound bed and hence are not indorsed for heavy exudative wounds [86]. **Adhesive transparent film** dressings are suitably conglomerated with hydrogels that allow optimum wound hydration, maintain skin integrity and easy monitoring of the wounds [26]. Research to expand its antimicrobial effectiveness, has led to its combination with chlorhexidine that displays high adherence and declines catheter-related infection to improve vascularization.

*(c) Natural and Phytochemicals therapy*

Natural and plant-based products have been the traditional ancestral therapies that was used in skin wound care and management prior to the rise of pharmaceutical and

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clinical alternatives. For centuries these products due their potent anti-microbial, anti-inflammatory, anti-analgesic, cell stimulating characteristics, these have been implemented as traditional medicine for acute as well as chronic wounds. Owing to the existing incidence of diabetes, severe cardiac and vascular implications, chronic wound interventions seek much attention, which makes the use of natural therapies for healing applications of specific interest. Natural amalgams encompass a widespread assortment of substances, antioxidants, phenols, terpenes, flavones and many more such organic and inorganic constituents that act as specific targets in the healing process [87]. These constituents have been clinically tested for its efficiency through *in vitro* and *in vivo* models. Since wound repair is a complex cascade of biochemical events, it is of utmost importance to stimulate a reparation process without any microbial infections. Hence traditional therapeutic agents and plant based natural products have shown exemplary outcomes. Further scientific investigation on the progress of various extraction and purification methods, their precise mechanism of action, safety and quality control assessments etc. is obligatory. Traditional therapies are cost complaint and beneficial for primary wound care and management, but inconsistency in their batch-to-batch results, sudden immunologic reactions, adverse after-effects can restrict their implication in multidisciplinary wound management. Nonetheless, a combined traditional and modern therapy approach can target repair faster with least side effects such as silver impregnated nanofibers, aloe vera extract embedded alginate hydrogels, propolis wound dressings, honey based post-operative bandages, etc. could likely expand modern medicine.

*(d)Mechanical adjuncts and physical agents*

Despite several attempts to equilibrate the cellular, biomolecular events during wound repair and preserve an optimal hydrated healing environ, there are times when wounds become chronic non-healing. A series of mechanical adjuncts and physical agents in use do contribute to such wound reparation processes and provide constructive and adjunctive functions. Hydrotherapy, UV-C radiation, vacuum assisted closure, hyperbaric oxygen and electrical stimulation are a few to name. **Hydrotherapy**, being one of the oldest adjuvant therapies is effective for burn wounds where a continuous rotation of water and air eliminates debris, toxic components and dilutes microbial colonization [88]. Hydrotherapy is advantageous for individuals with venous stasis dermatitis, pyoderma gangrenosum, peripheral artery disease teeth lacerations and rarely diabetes mellitus that are sensitive wounds. Th method effectively upholds an optimal moisture in and around the wound surface for better revascularization and dermal regeneration. With a number of advantages, there

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comes a few disadvantages. A particular pressure of the water circulation id needed at the wound surface for rinsing of granulation tissue which might impair the developing granulation tissue, restrict epidermal cell migration and cause skin maceration [88]. Also, bacterial infections can emerge if the moisture circulation is prolonged and proper drying of the wound is not done. ***Pulsed lavage therapy*** has currently become a replacement to hydrotherapy in terms its use of an irrigating solution maintained at a particular pressure by a powered device. The therapy improves rate of granulation and better remodelling of wounded tissues. **Ultraviolet C radiation** ranging from 200-280nm and erythemal effectivity is accomplished at wavelengths of 250nm where nucleic acid absorption happens leading to accelerated DNA synthesis in fibroblasts, increased oxygenation and capillary blood flow for granulation tissue formation and anti-bacterial and anti-viral effects on wound surfaces. UV radiations can contribute to wound healing by upsurging epithelial cell turnover and hyperplasia to release prostaglandins and initiate cell proliferation for re-epithelization. A dose dependent application of this radiations may also cause shedding of peri-ulcer epidermal cells and sloughing of necrotic tissues and eschar [88]. **Vacuum-assisted wound closure** is applied in the form of dressings in order to seal the wound area and to place a negative pressure onto the wound surface that produces an adhesive friction to the tissues and contracts wound depth for efficient closure [88]. This therapy can significantly observably reduce water loss of the split thickness graft area, curtails post-wounding duration and restricts the relapse of infection during wound repair [88].The accomplishment of vacuum assisted closure therapy in treating chronic injuries has now led to its use in specialized clinical situations such as transient abdominal closure, skin avulsion, poststernotomy mediastinitis, acute and subacute wounds, wound with bony prominence, osteomyelitis and as a graft reinforcement [88-90] and in reconstructive surgeries. **Hyperbaric oxygen therapy** confines the use of hundred percent oxygen @ one atmospheric pressure to enhance oxygen inundation in the blood by forming oxyhaemoglobin. Hyperoxic environments indorses wound repair through an increase in growth factors and formation of iNOS that regulates collagen formation, wound contraction and endothelial progenitor cell proliferation [88]. This therapy has been utilized in chronic and poorly healing wounds, acute wounds, and diabetic foot ulcers. A systematic assessment on the healing capacity of hyperbaric oxygen therapy in diabetic foot ulcer patients was found to be much superior in comparison to other surgical procedures. **Electrical simulation** gathers both positive and negative charged cells *viz.*, neutrophils, phagocytes, epidermal cells, fibroblasts onto the wounded area so that each of the cell perform their specific cellular activities pertaining to wound

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healing. Endogenic electric field plays an imperative role in wound-healing largely by triggering protein synthesis and cell migration. Several clinical investigations have confirmed that electrical stimulation with steady direct currents is advantageous in wound acceleration. Human fibroblasts cells subjected to high voltage pulsed current stimulation (HVPCS) did intensify the healing rate of soft tissue wounds as per reports [88]. Both protein and DNA syntheses rates became higher by applying specific blends of HVPCS voltage and pulse rate. Besides, cell migration was prominent near the wound area in response to endogenic electrical field (electrotaxis: the directional migration of cells toward the anodic or cathodic electrode of an applied electrical field). Researcher Yung Shin Sun experimentally aimed at optimizing the direct current stimulation therapy for enhancing the progression of wound repair. He standardized the parameters in exogenous electrotherapy and developed a three-dimensional wound model consisting of different tissue types in the skin layers and using the finite element method the distribution of electrical field near the wound area was evaluated [91].

**Engineered metal composites implemented for healing**

The lucrative physicochemical characteristics of nanomaterials makes them of particular interest in various biomedical applications. Nano-sized materials comprise of nanoparticles, nano-scaffolds, nanocomposites and biomaterials that offers an unmatched approach to accelerate wound repair and tissue remodeling process. Their dimensions and shape govern their specificity, biological efficacy, cellular response, penetrability and targeted delivery to the site of injury. Nanomaterials are comparatively non-toxic and exhibit high antibacterial properties. Also, nanoparticles, nanospheres, nano-capsules, nano-emulsions, nanocarriers and nano-colloids could serve as materials for tissue regeneration. **Nanoparticles** both metallic and non-metallic, principally aid in wound repair and management by either possessing inbuilt inherent features that assist wound contraction or as delivery vehicles/carriers for assisted therapy [92]. The most extensively studied ones are silver, gold and zinc nanoparticles owing to their unique dimensional, functional, chemical, biochemical properties [93,94].

**Silver nanoparticles (AgNPs)**, the potential candidate of choice for wound repair, have high surface: volume ratio and show excellent activities at low concentrations and are superior to the traditional silver compounds formerly used. Neat AgNPs can regulate the release of anti-inflammatory cytokines that facilitate rapid non-hypertrophic scar devoid wound contraction [95,96]. AgNPs have the capacity to

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initiate proliferation and differentiation of keratinocytes to augment epidermal closure and re-epithelialization. Myofibroblast differentiation from normal fibroblasts to promote speedy tissue renewal is also facilitated by AgNPs [96]. Reports, however, on their toxicity at increased concentrations by Szmyd *et al.* has shown that keratinocyte feasibility, absorption, migration and differentiation is affected *via* specific cell death initiating stimulus: caspase 3 and 7, ultimately leading to DNA mutilation [95,96]. Therefore, it is recommended to use lower safe doses, together with antimicrobic preparations to attain improved effectiveness. AgNPs in conglomeration with the polyketide antibiotic can significantly reduce bacterial load both in epidermal and deep dermal layers in a mice model and quicken healing [94,95]. Hence the combination of nanoparticles with conventional antibacterial mediators or dressings, can more competently be used for repair of infected wounds. Like, microcellulose reinforced with AgNPs behave as antimicrobial coatings for open wounds and has shown high antibacterial performance against Gram negative pathogen [94]. Experimental evidences by Holban *et al.* also depicted that coated polyester-nylon dressings with AgNPs can prevent biofilm formation and bacterial colonization while upholding a low toxicity profile [94]. AgNPs form sulphur bonds with the bacterial plasmalemma proteins or bind to enzymatic thiol moieties which participate in respiratory chain reactions and cell death [95]. Furthermore, these nanoparticles can hinder with DNA synthesis and curb bacterial multiplication in the wounds. Experimental observations by Lu *et al.* showed that the incorporation of silica into AgNPs can give rise to non-toxic mesoporous disulphide structures (Ag-MSNs) which can very efficiently adhere to open wounds and have outstanding bacteriostatic activity [94]. Ag infused veneers warrants quicker wound healing and evade microbial colonization on wound site, as observed in an *in vivo* canine model [96]. Commercially available silver impregnated dressings, Acticoat©- nano-sized AgNPs (< 15 nm size) are being currently explored for its reparative, anti-infective and pain lessening facets and this is under clinical trial for burn wounds but Anticoat© may be complaint in evading burn wound infections upon its amalgamation with silver sulphadiazine and chlorhexidine digluconate formulation [97]. **Gold nanoparticles (AuNPs)** represent their potency in tissue rejuvenation, targeted drug delivery and wound repair due to their extraordinary biocompatibility. AuNPs nanomaterials because of its stabilizing properties can be used as reinforcements with many other nanomaterials. Since neat gold particles do not exhibit a substantial activity, they need to be incorporated or combined with some matrix or therapeutic agent, a carrier, biomolecules, etc. for efficient antimicrobial activity. The cross-linking of AuNPs with collagen, chitosan, gelatin, alginate and their incorporation with various polysaccharides, growth factors,

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peptides, and cell adhesion proteins enables their attachment onto the gold nanoparticle surface without any alteration in the structural conformation of the biomolecule. This conglomerated moiety modified AuNPs displays excellent biocompatibility and biodegradability, and are suited for healing. Similar to collagen, gelatin and chitosan can also easily be incorporated with AuNPs, showing safe and positive effects in enhancing wound healing [98,99]. Additionally, by modifying the surface plasmon resonance of AuNP, these exhibit thermo-responsive behaviour, which is supported by *in vitro* and *in vivo* experimental data [98]. The mechanism of action of AuNPs follows either targeting the cell wall or binding to DNA to stall the double-helical structure from unwinding during replication or transcription, therefore contributing to bactericidal and bacteriostatic activities. They can thus show multidrug-resistance to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. AuNPs are also potent antioxidants [99]. Low concentrations of AuNPs are associated with keratinocyte growth and differentiation [99, 100]. Observations made by Marza *et al*. on basic fibroblast growth factor-AuNPs impregnated petroleum jelly mixtures showed enhanced angiogenesis and fibroblast proliferation, which aided speedy wound recovery [101]. The effect of colloidal AuNP coupled with quercetin on fibroblast cell migration assisted-wound healing mechanism was depicted by Madhyatha et al. $Au^{Qur}$NPs displayed enhanced cell proliferation and migration of fibroblasts, which was directed through the TGFβ1 dependent SMAD signaling pathway. This initial study on nanocuetical engineered gold particles brings forth molecular and cellular evidence-based data to elevate the promising healing applications of $Au^{Qur}$NPs in upcoming nanomedicine for skin etiology[102] (Figure 2).

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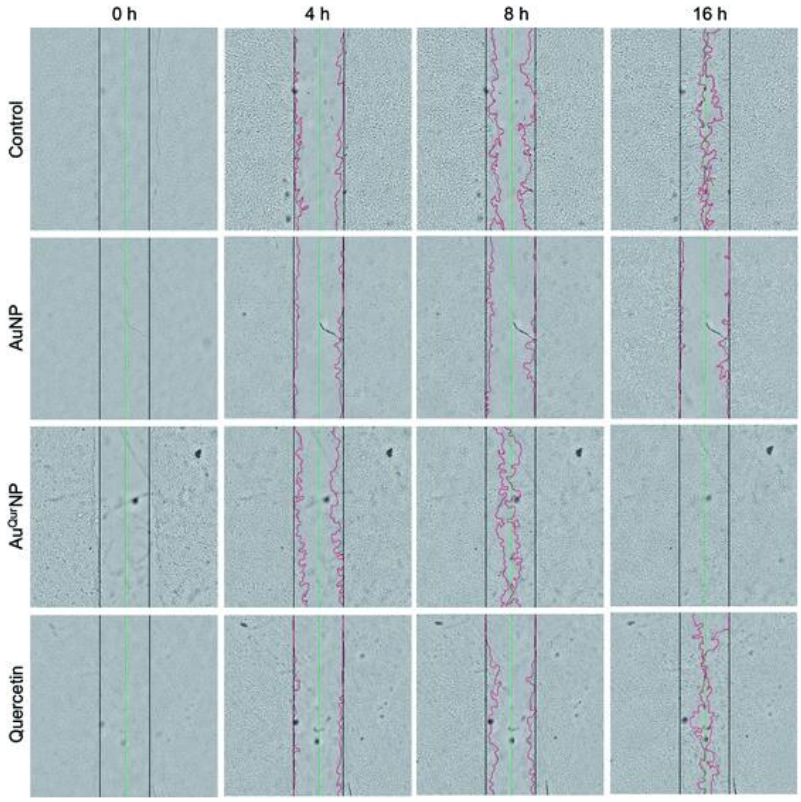


**Figure 2:** *In vitro* wound assay of human keratinocyte cells treated with AuNP (5 µg l−1) or AuQurNP (5 µg l−1) or pure quercetin (15 ng ml−1) for different time period (0, 4, 8, 16 h). Non treated cells were used as control. Black, green and red lines depict the start, end point of cell migration and migratory cell edge respectively (10X). [original image adapted from reference 102]

**Zinc oxide nanoparticles (ZnONPs)** exhibit potent antibacterial activity and it in combination with hydrogel-based wound dressings [25], can activate keratinocyte migration and improve re-epithelization [25]. A recent study on the assessment of ZnONPs based chitosan hydrogel formulations presented high absorbency of wound exudates and aided hemostatic blood clotting and antibacterial effectivity simultaneously [26]. ZnONPs and collagen based bioresorbable matrix with orange essential oil has been seen to substantially heal burn wounds while also decreasing the chances of sepsis. This wound dressing was seen to augment angiogenesis, form new tissue and exhibit biocompatibility and no cytotoxicity when evaluated *in vitro* and *in vivo* [103]. Yet, their inherent toxicity makes them less used in wound healing therapies [25]. ZnONP toxicity is dose dependent, which with higher doses acts as a mitochondrial dysfunctioning agent to release reactive oxygen species and block gene expression of superoxide dismutase and glutathione peroxidase in human keratinocytes, ultimately giving rise to membrane oxidative stress and cell death. ZnONPs are also carcinogenic in nature, according to some experimental examinations [105]. Creating core-shell nanocomposites by combining two metals such as biogenic AuNPs with a thin coat of ZnO to form AuZnO core-shell

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nanocomposites, were assessed to find out that the antibacterial and anti-biofilm efficacy against *Staphylococcus aureus* and methicillin resistant *Staphylococcus haemolyticus* [106]. ZnONPs have good tissue adhesive properties as exhibited in mice skin models [107].

**Nano based composites**

Cost effective and renewable sources of nanoparticle synthesis is gaining much importance due to the high costs, energy consumption and requirement of additional resources to dispose of toxic by-products of artificial nanoparticle synthesis process. Phytochemicals resent in plants *viz.*, alkaloids, phenols, amino acids, proteins and many more have been used to stabilize several nanoparticles like Ag ions in AgNPs. *Ocimum sanctum* merged AgNPs embedded into a Carbopol gel base attained ≈96% of wound contraction by the 14th post wounding day as revealed by Sood and co-workers [108] and also possessed activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [108]. Gelatin has *in situ* reductive properties along with its non-polar amino acids that stabilizes AgNPs. Development of gelatin-chitosan-Ag porous composites with sizes 100–250μm are biocompatible, bioderadable and non-immunogenic, which upon cross-linking with tannic acid does exhibits therapeutic and anti-bacterial characteristics with low cytotoxicity [109]. Shao et al. used *Barleria gibsoni* leaf extracts for synthesis of ZnONPs gel formulation for both Gram-positive and Gram-negative infected burn wounds [104]. Polymers such as chitosan can also serve as nanomaterial dressings or drug carriers due to their biocompatible polymeric networks to hold optimum moisture for a balanced wound environment [104]. Chitosan being cationic, attract most metals, proteins and dyes to form complexes [110] and its degradation products can activate ECM synthesis [110]. Chitosan assisted wound-healing therapies, include hydrogels, membranes, films, sponges and scaffolds [110]. Chitosan nanoparticles have immunomodulatory and nontoxic effects on human dermal cells as revealed by Chen et al. in his assembled acellular porcine dermal matrix using a naturally-derived chitosan oligosaccharide [111]. Also, the presence of the functional group aldehyde in AgNPs, formed during in situ reaction, confers the chitosan-AgNPs scaffolds with broad spectrum antimicrobial properties wound associated *Escherichia coli* and *Staphylococcus aureus* [111]. A combination of chitosan-PVA complex improved the antioxidant and antimicrobial efficacy when compared to polymer alone. Besides, it also conferred strong Gram-negative activity against *Klebsiella* species and further enhanced *in vivo* wound repair by forming granulation tissue and re-epithelialization, while demonstrating no cytotoxicity [109]. Holban *et al.* experimentally displayed the biofilm inhibition capacity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* by polylactic acid-chitosan-magnetite-eugenol amalgamated nanospheres [110]. An infrared-irradiation triggered thermo-sensitive hydrogel-based drug delivery system loaded with ciprofloxacin was developed by Gao *et al.* triggered by near-infrared light stimulation. The mixture of polydopamine nanoparticles/glycol chitosan, being photothermally active, generated hyperthermia

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leading to bacterial cell leaching. Besides, polydopamine nanoparticles in combination with the drug ciprofloxacin exhibits a controlled released when stimulated with near-infrared light and showed minimal leakage under physiological conditions [112]. Calreticulin (calcium-binding protein) based AuNPs, chitosan/AuNP nanocomposite have been used for diabetic lesions. Calreticulins regulates the proper folding of proteins and the nanocomposite indorses fibroblast-keratinocyte-endothelial cell growth, migration and division and collagen formation without hindering the cell proliferation [113]. The biopolymer cellulose triggers repair *via* the implementation of multiple local growth factors such as epidermal growth factor and basic fibroblast growth factor [113]. Nanocellulose dressings due to its anti-infective properties and amplified tensile properties has been explored as scaffolds [25]. Bacterial cellulose mimics the skin structure with a high surface area per unit, increased biocompatibility, hydrophilicity and no cytotoxicity. 3D porous networks of nanocellulose have high water retention capacity, ensuring a moist environment appropriate for healing [25]. The wound healing potential of cellulose-ZnONPs composites was displayed by Khalid *et al.* [114]. Bacterial nanocellulose derived from Gram-negative *Gluconacetobacter xylinus*, in combination with silver nanoparticles showed enhanced healing and reduced colonization of wound associated *Staphylococcus aureus in vitro* [25].

**Nanoscaffolds**

The use of nanomaterials in scaffoldings offer several advantages. Scaffolds mimic the extracellular matrix and have been developed using a variety of techniques; electrospinning being the most common. Electrospinning can give rise to highly porous polymeric nanofibers that can also be used in wound healing applications [26]. Further, nanopolymers like the dendrimers, also show anti-inflammatory and antibacterial characteristics. Studies on a porcine model of superficial partial thickness wounds displayed enhance healing potency of an electrospun polymer nanofiber dressing with least risk of infection. Chitosan-poly-vinyl alcohol nanofibrous scaffolds upon application to rat diabetic wounds models had improved healing rates in comparison to controls [115]. *In vivo* study in Wistar rats of a silver nanoparticle spun nanofiber membrane exhibited numerous favourable effects of reduced cytotoxicity, broad spectrum antibacterial action, abridged inflammation and higher healing rates [116]. Recombinant human epidermal growth factor, another nanocarrier has been revealed to stimulate healing of full-thickness diabetic wounds. Nevertheless, their restricted use is due to the highly proteolytic environment they possess and the down-regulation of associated growth factor receptors and signalling molecules in case of chronic wounds [117]. However, the results, do vary between experiments. Zhang *et al.* defined a hydrogel with Ca2+ cross-linker was capable of releasing preloaded bFGF. Observations that both calcium and bFGF led to the growth and division of

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fibroblasts in the early re-epithelialization phases, persuading wound shrinkage on both *in vitro* and *in vivo* models [118]. Nano-fibrous mesh networks developed by electrospinning have been used for gene encapsulation in wound-dressings. Gene-activated matrix therapy can simultaneously alter the expression of a target gene involved I regeneration and bridges the gap between tissue engineering and gene therapy. Wang et al. optimized gene delivery system based on the antimicrobial peptide LL-37 imbedded on ultra-small AuNPs, increased the complete antibacterial action in topical treatment of diabetic lesions. Furthermore, LL37-AuNPs composite boosted cellular and nucleus diffusion, thus accomplishing high gene delivery efficacy. This system possessed biocompatibility, endorsed angiogenesis through expression of VEGF expression, and improved re-epithelialization and granulation tissue formation [119]. Conjugated microRNA-146a (miR-146a) with cerium oxide nanoparticles was used for diabetic wound healing process where the conjugate precisely regulated gene transcription and pro-inflammatory cytokine synthesis [120] by suppressing interleukin-1 receptor-associated kinase 1 (IRAK-1) and tumor necrosis factor receptor-associated factor 6 (TRAF-6) production which are key controllers of NFκB pathway. This in turn shoots up the activity of NFκB thus overexpressing IL-6 and IL-8 [65]. Nanoceria have scavenging activity due to the coexistence of two oxidation states (3+ and 4+) in valence cerium atom. Hence, these nanoparticles may diminish oxidative stress and reinstate the balance between oxidants and antioxidant enzymes in diabetic lesions. A 100 μg dose of cerium oxide nanoparticles-miR-146a combination enhanced diabetic wound healing without altering the wound tensile [64]. Stem cell therapy another feather in the cap of tissue engineering and regenerative medicine represents another possible beneficiary of the nanoscaffold technology, due to their substantial stem cell migration and differentiation. These multifaceted nanomaterials with numerous enhancing properties thus, represent advantages in compared to standard treatment procedures adapted in clinical practice.

**4. Conclusion:**

Dermal wound healing has been most explored in recent times and has encouraged scientists to reconnoiter the fundamental underlying cellular and molecular mechanisms for developing innumerable therapeutic products for wound repair applications. Though the rudimentary portrayal of wound healing is well explored, there still persists certain mechanisms that are unclear. Various approaches have been employed and improved in part driven by up-to-date developments in science and technology. No single therapeutic approach has efficaciously unravelled the quandary

of treating obstinate, sluggishly healing, or non-healing wounds. Nevertheless, better awareness of features segregating chronic wounds from the non-chronic ones is essential for understanding the complete wound treatments. A better picture of the physiological variations in chronic wounds would help in choosing specific wound management stratagems. A complex cascade of events such as Bacterial inequities, increased incidence of inflammatory cells and proinflammatory growth factors, augmented protease synthesis in wound cells, drops in TIMP, etc. are some of the implications of chronic wound healing. Improved understanding of these chronic wound features and their association with other factors must be well-thought-out when devising a novel therapy for increased treatment efficacy. Forthcoming clinical therapies probably would be focused at employing local environ and triggering cellular responses in chronic wounds. Evaluation of a number of growth factors, their most effective amalgamations, their methods to control excessive inflammation and proteolytic forces, genetic therapy, development of skin counterparts, and the effectiveness of adjunctive treatments are essential key point to initiate proper healing. Additionally, translation of information and optimal delivery of chronic wound care are crucial. In addition, interdisciplinary research on wound care is required to evaluate patient outcomes. The efforts of basic and clinical effort with expert interdisciplinary research offer a great potential for balancing the burden of chronic wounds.

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