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Advancement in biomaterials for chronic wounds therapy

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Abstract
Wound healing, as a complex biological process in the body, comprising four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. For a wound to heal successful and fast, all four phases must occur in the proper sequence and in time. Many factors like oxygenation, infection, age, stress, diabetes, etc may cause improper or impaired wound healing. A better understanding of the influence of these factors on repair may lead to therapeutics that improves wound healing. This paper reviews the latest applications in advanced dressings like skin substitutes, biologic wound products including growth factor applications, silver, platelet rich plasma, by inhibition of matrix metalloproteinase activity in chronic wounds by a polyacrylate super absorber in microbial prophylaxis as an adjunct in wound healing. A number of recent advance techniques are discussed, with the aim of developing strategies to improve the rate of tissue repair in wound healing.

Keywords: Wound, homeostatsis, inflammation, therapeutics, infection, skin substitutes, silver

Introduction
The wound-healing process consists of four phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution [1]. For a successful wound healing, all four phases must occur in the proper sequence and within particular time frame. In future, gene therapy may allow genes or gene-derived messengers in healing to be delivered directly into a wound at directed time [2]. Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure.
Chronic wounds can be defined as, wounds which have failed to progress timely and orderly reparative process to restore anatomic as well as functional integrity in 3 months [3]. Chronic wounds are often identified by the presence of a raised and hyper proliferative non advancing wound margin. Fibroblasts derived from the wound bed of chronic wounds of various etiologies represent a senescent, premature, or differentiated phenotype, which respond inefficiently to normal stimulatory messages.
By supporting the wound with growth factors and biologic substances, we can help augment or modulate the wound healing process itself. Similarly besides biomaterials, regardless of the nature of the many silver-containing products currently available, elemental silver requires ionization for antimicrobial efficacy [4]. This paper will review several new technologies in biomaterials for chronic wounds therapy.

Wound Healing
The process of wound healing as a complex dynamic process consists of four highly integrated & overlapping phases [1]:
1. Hamostasis
2. Inflammation
3. Proliferation, and
4. Tissue Remodelling Or Resolution

Factors affecting the wound healing
Multiple factors can lead to impaired wound healing. In general terms, the factors that

influence repair can be categorized into local and systemic. Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state of the individual that affect his or her ability to heal. Many of these factors are related, and the systemic factors act through the local effects affecting wound healing.

Local factors that influence healing

1. Oxygenation

Oxygen is most important for cellular metabolism, especially energy production in terms of ATP, and also critically essential for several wound healing processes. Oxygen prevents wounds from induces angiogenesis, infection, stimulates keratinocyte differentiation, migration, and re-epithelialization, thus, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction, these all steps results in healing of wound [5, 6].

Massive vascular disruption and tremendously increased oxygen consumption by metabolically active cells, the microenvironment of the early wound is devoid of oxygen (hypoxic). In addition, several systemic conditions, including advancing age and diabetes, can create impaired vascular flow, thus setting the stage for poor tissue oxygenation. Transcutaneously oxygen tensions in chronic wounds ranges from 5 to 20 mm Hg, in comparison to the control tissue values of 30 to 50 mm Hg. Temporary hypoxia triggers wound healing, but prolonged or chronic hypoxia delays wound healing [7]. In the process of wound healing, reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide (O_2) are acting as a cellular messengers for stimulation of various key processes associated with wound healing, which includes cell motility, cytokine action (including PDGF signal transduction), and angiogenesis [6].

In summary, the proper oxygen level is very crucial factor in wound healing. Hypoxia stimulates wound healing such as the release of growth factors and angiogenesis, on the other hand oxygen is needed to sustain the healing process.

2. Infection

Inflammation is a integral part of the wound-healing, and is important for removing contaminating micro-organisms. Once skin is injured, micro-organisms that are normally sequestered at the skin surface entered the underlying tissues. In the absence of effective immunity, inflammation may be prolonged causing incomplete and delayed microbial clearance is. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and elongate the inflammatory phase. If this continues, the wound may enter a chronic state and fail to heal. This prolonged inflammation also leads to an increased level of matrix metalloproteases (MMPs), a proteases that can degrade the ECM, with increased protease content the naturally occurring protease inhibitors decreased. This kind of shift in the protease balance may cause growth factors that appears in chronic wounds to be rapidly degraded [8, 9]. Similar to other infective processes, the bacteria in infected wounds occur in the form of biofilmsproponded that *Staphylococcus aureus, Pseudomonas aeruginosa,* and *β-hemolytic streptococci* are the most common bacteria in infected and clinically non-infected wounds [10]. Mature biofilms develop around the protected microenvironments and are more resistant to conventional antibiotic treatment, if biofilm contain *P. aeruginosa*, protects the bacteria from the phagocytic activity of invading polymorphonuclear neutrophils, causing the failure of antibiotics as a remedy for chronic wounds [11].

Systemic factors that influence healing

1. Age

In old age, delayed wound healing is associated with an altered inflammatory response (such as delayed T-cell infiltration into the wound area), altered chemokine production and reduced macrophage phagocytic capacity [12]. It is commonly observed that, in healthy older adults, aging causes a temporal delay in wound healing, but not an actual impairment in terms of the quality of healing [13]. In clinical study on mice delayed collagen synthesis, angiogenesis, and re-epithelialization have also been observed in aged mice as compared with young mice [14].

2. Stress

Stress has a great impact on health, production and reproduction. The pathophysiology of stress results in the deregulation of the immune system, mediated primarily through the sympathetic nervous system and hypothalamic-pituitary- adrenal axis [15, 16]. Stress also reduces the expression of IL 1α and IL-8 at wound sites—both chemo attractants are necessary for the initial inflammatory phase of wound healing. The hypothalamic-pituitary-adrenal and the sympathetic- adrenal medullary axis regulate the release of pituitary and adrenal hormones. Stress, up-regulates glucocorticoids (GCs) and reduces the levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α at the wound site [16]. The GC functions as an anti-inflammatory agent and modulates the Th1-mediated immune responses that are essential for the initial phase of healing.

Stressors can lead to negative emotional states, such as anxiety and depression, which may in turn have an impact on physiologic processes and/or behavioral patterns that influence health outcomes. Psychological stress impairs normal cell mediated immunity at the wound site, causing a significant delay in the healing process. All of these factors may come into play in negative impact on the wound healing process.

3. Diabetes

Several unregulated cellular functions are involved in diabetic wounds, such as defective T-cell immunity, defects in leukocyte chemotaxis, phagocytosis, and bactericidal capacity, and dysfunctions of fibroblasts and epidermal cells. These defects are responsible for inadequate bacterial clearance and delayed or impaired repair in individuals with diabetes [17, 18].

Hyperglycemia also causes oxidative stress and production of ROS exceeds the anti-oxidant capacity of the cell. The formation of advanced glycation end-products (AGEs) under hyperglycemia and the interaction with their receptors (RAGE) are associated with impaired wound healing in diabetic condition.

In diabetes, a persistent inflammatory phase is occurs associated with a delayed formation of mature granulation tissue as well as reduction in wound tensile strength [19, 20]. While, in health the acute wound healing process is guided and maintained through integration of multiple signals (cytokines and chemokines) released by keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets [22].

In diabetes; eNOS phosphorylation in the bone marrow is impaired, which directly limits EPC mobilization from the bone marrow into the circulation [21]. Lack of healing is also

indicated by a thicker cornified layer with nuclei and epidermis

Advanced wound care therapies for chronic wounds

Biomaterial: ''Any substance or combination of substances, other than drugs, artificial or natural, which can be used for any period of time, which replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual''

A. Collagen- The term collagen includes chemically distinct macromolecular proteins. The roles of collagen wound products in ulcer healing may be [23].

1. To act as a substrate for hemostasis,
2. To provide a template for cellular attachment, migration, and proliferation
3. To provide a scaffold for more rapid transition to mature collagen production and alignment,
4. Chemotaxis to cellular elements of healing such as granulocytes, macrophages, and fibroblasts.

B. Fibracol Collagen-Alginate wound dressing (Johnson and Johnson, New Brunswick, NJ): A therapeutic product for an advanced wound care device composed of collagen and calcium alginate fibers. It received FDA approval in August of 1998 for topical use for burns and pressure, venous, and diabetic ulcers.

C. Promogran: (Johnson and Johnson) therapeutic product consists of 55% collagen and 45% oxidized generated cellulose. It was approved by the FDA in February of 2002. Promogran is an absorbent open pored, sterile, freeze-dried matrix used as a topical treatment for chronic wound ulcers. Promogran is composed of natural materials which physically bind to and inactivate damaging proteases while binding and protecting growth factors [24].

Advanced dressings

The process of autolysis is important in wound care. If an occlusive dressing is provided as a barrier to the outside environment, the body's own phagocytic processes will provide debridement of wound [25].

Tegaderm (an occlusive film), which are permeable to air and water vapor, but impermeable to fluid and microorganisms to hydrocolloids, thus, maintaining a moist environment for autolysis.

For heavily exudative wounds, there are a range of absorptive products including various hydrophilic foam dressings, hydrogels, hydro fibers, and alginates, which can absorb up to 20 times their weight [26]. An advanced dressing enhances healing and thus reduces overall treatment period. Advanced wound dressing encompasses following dressing methods for better wound healing:

1. Skin substitutes

The advances in temporary and permanent coverage of wounds have made significant gains with advancing technology in biomaterials and tissue engineering. Biobrane, an invention, in temporary dressing composed of knitted nylon mesh bonded to a thin silicone membrane and coated with porcine polypeptides [27]. Bioengineered skin substitutes, both biosynthetic skin substitutes and cultured autologous engineered skin, are available to provide temporary or permanent coverage, with the advantages of availability in large quantities and negligible risk of infection or immunologic issues.

- Derma graft: It is a dermal tissue substitute that received FDA approval in 2001 for treating wounds in diabetic condition lasting more than 6 weeks. Dermagraft contains neonatal fibroblasts on a bioabsorbable polyglactin mesh. The fibroblasts produce dermal collagen, glycosaminoglycans, growth factors, and fibronectin to support wound healing [28].
- Apligraf is a similar skin substitute made from cultured skin cells but is a bilayer construct that contains both dermal and epidermal components used alone in chronic wound ulcers, showing increased healing times several time. Apligraf received FDA approval in 1998 for delayed wound healing in diabetes or stress [27, 29]. Apligraf is composed of an epidermal layer of allogeneic neonatal keratinocytes and fibroblasts from neonatal foreskin on bilayered type I bovine collagen. Both Apligraf and Dermagraft are metabolically active products increase the healing process by stimulating fibro vascular in growth and epithelialization in tissues [30, 31].
- Integra is a semibiologic bilayered dressing composed of a matrix of type I bovine collagen, chondroitin-6- sulfate, a glycosaminoglycan from shark cartilage, under a temporary silicone epidermal sheet. The pore size (70–200 μm) is designed to allow only the migration of the body endothelial cells and fibroblasts [32].
- OASIS Wound Matrix a commonly used biologically active dressing is an extracellular matrix product derived from the small intestinal submucosa of pigs. It received FDA approval in 2000 and is indicated for the treatment of diabetic ulcers and chronic vascular ulcers [33].

2. Keratinocytes

Keratinocyte-based therapies for wound healing exist in a various forms. Different keratinocyte sources have been utilized; the patient's own skin cells, donor cells from cadavers or patients undergoing cosmetic procedures, and bioengineered "immortalized" keratinocytes have all been used [34].

- In addition to using different cellular sources, therapies may vary in their use of fresh, cryopreserved, or lyophilized keratinocytes. These products differ in level of metabolic activity and ease of storage and transportation. These products do not act as grafts or serve as permanent skin replacements, as they are rapidly replaced by the host's own keratinocytes.
- Keratinocytes stimulates proliferation and migration of host epithelium from wound [35].
- The most important advantage of cultured keratinocyte allografts is the large surface area obtained from a relatively small biopsy of healthy skin from the patient [36].

Kaloderm is a path-breaking dressing means particularly effective on cutaneous wound healing. It can be applied on the wound that accelerate the wound healing through growth factors and cytokines [29].

3. Growth factors

Growth factors enhances the wound healing process by stimulating fibroblasts and keratinocytes via transmembrane glycoproteins [37, 38].

- Human platelet-derived growth factor is a substance naturally produced by the body to help in wound healing. It works by helping repair and replace dead skin and

other tissues, attracting cells that repair wounds, and helping to close and heal the ulcer [39].

- Regranex Gel (becaplermin 0.01%) was approved by the FDA in 1997 for the treatment of diabetic foot ulcers. Regranex is a genetically engineered product that mimics PDGF in the body. It is used as an alternate to traditional ulcer care strategies, such as daily dressing changes, initial sharp debridement, treatment of infection (if present) and pressure relief [40, 41].

4. Platelet rich plasma (PRP)

PRP consists of plasma that has a platelet concentration above baseline i.e., five times more than normal platelet counts [42].

- Platelet-rich plasma (PRP) is derived from newly drawn whole blood prepared by specialized centrifugation to create plasma having a platelet concentration above baseline. Thus, PRP contains a high level of platelets and a full complement of clotting and growth factors which aid in healing by attracting undifferentiated cells and activating cell division [43]. Autologous platelet rich plasma (PRP) is a safe, simple, affordable and less expensive procedure in the treatment of chronic wound with reportedly good results as it is biocompatible and safe [44].
- PRP enhances wound healing by promoting the healing process by seven growth factors present in it. They are platelet derived growth factor (αα, αβ, αβ), fibroblast growth factor, vascular endothelial growth factor, epidermal growth factor, transforming growth factor [45].

5. Silver

The use of silver to prevent and treat infection is both one of the earliest forms of wound care, documented as early as 69 BC, and one of the latest technologies for antimicrobial prophylaxis. Silver has a very broad spectrum of microbial coverage, including yeast, fungi, mold, and even antibiotic-resistant bacteria. Silver is a bactericidal material that kills on contact by:

1. Inhibiting the respiratory chain at the cytochrome level
2. Interfering with electron transport
3. Denaturing nucleic acids, inhibiting DNA replication
4. Altering cell membrane permeability

Elemental silver requires ionization for antimicrobial efficacy. The highly reactive charged silver ion (Ag+) reacts by binding to negatively charged particles such as proteins, DNA, RNA, and chloride ions. While this is responsible for its antimicrobial properties, it also complicates delivery as the silver ions are readily bound to proteins and chloride in the wound bed fluid. Many delivery systems exist, with the key to the most effective product being one that can maintain an adequate concentration of silver with long enough residual activity [4, 46].

Nanocrystalline silver dressings were developed and introduced in the late 1990s and are the latest forms of silver wound dressings. At present, products contain two layers of high-density polyethylene net sandwiching a layer of rayon/polyester gauze. The outer layer is coated with a nanocrystalline (<20 nm) and noncharged form of silver and the inner layer helps maintain a moist environment for wound healing. Since, noncharged silver is less reactive with negatively charged particles in the wound; it is deactivated much more slowly and provides an initial large bolus of silver followed by a sustained release into the wound [47]. Unlike routine dressing procedures, nanocrystalline dressings require less frequent dressing changes. Thus, decreases patient discomfort as well as provides less disruption to the healing wound bed.

6. The inhibition of matrix metalloproteinase activity in chronic wounds by a polyacrylate superabsorber

Excessive matrix metalloproteinase (MMP) levels have been observed in wound fluid of impaired healing wounds. This is thought to interfere with granulation tissue formation as newly formed extracellular matrix and cytokines are degraded and the wound becomes deadlocked, unable to progress to the next healing stages [48]. Polyacrylate superabsorber particles effectively inhibited MMP. It is now well acclaimed that polyacrylate superabsorber particles can rescue the highly proteolytic microenvironment of non-healing wounds from MMP activity so that more conductive conditions allow healing to proceed [49, 50].

7. Fibroblast growth factor 2 dimer with superagonist *in vitro* activity improves granulation tissue formation during wound healing

Fibroblast growth factor 2 (FGF2), expression is impaired in diabetic and pressure ulcers as well as in chronic wounds. FGF2 moderates cell proliferation, differentiation and migration of multiple cell types. Thus, FGF2 is plays critical role in wound healing, angiogenesis, bone regeneration, neuroregeneration, and can even result in scarless healing. FGF2 activity is dependent on the formation of a tetrameric complex, consisting of two FGF2 proteins and two FGF receptors (FGFR1) [20].

Conclusion

This article was an attempt to review the recent advances in biomaterials for chronic wound therapy. Emerging technologies present novel approaches to future wound care. Basics of good wound care should not be neglected, to derive maximum benefits from these evolving technologies and therapies. Clear guidelines focussing on the principles of effective wound bed preparation have to be followed to ensure effective outcome with the use of newer wound care products. In addition, to make the best use of advanced products clinical trials will have to include more complex wound types. Purely neuropathic ulcers are relatively straightforward and many clinicians believe they can be effectively treated with sound surgical debridement and off-loading. While it might be argued that accelerating the healing of these relatively simple wounds may prevent complications arising from infection, more needs to be done to show cost-effectiveness to our society as a whole.

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