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Review Article

Toxicological Outcomes and Pharmacological Needs in Chronic Wound Healing

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Abstract

Chronic wound healing is an intricate process where tissue, often the epidermis, repairs itself after injury. Wound care technologies that accelerate the healing process are an emerging area of translational research for patients in the health care system as well as war fighters in field services. Accelerated wound healing refers to the efforts made, intrinsically or anthropogenically, to reduce the time required for the normal time course of the healing process to occur. Example research thrusts and prototypical products that have shown an increase to the healing process include endogenous (e.g. stem cell therapy), pharmaceutical (e.g. antiseptics), surgical (i.e. skin homograft), and topical (e.g. wound dressings). Controlling the continuum of wound injury-to-recovery is critical to minimizing inflammatory response, infection, fibrosis and scarring. There is a need to further develop analytical methods that implement safe and effective products and protocols that is cost-effective, easy to use, and is measureable by both patient and health care professionals. This perspective highlights recent technological advances in the field of wound healing with an emphasis on chronic wounds. The barriers that hinder go-to-market strategies are discussed along with recommendations to the wound management community.

Keywords: *Wound healing; Biomedical technology; Biological markers; Safety; Cost analysis*

Abbreviations: USD: United States Dollars;

Introduction

Wound care has many different treatment options and modalities. However, this care is generally regarded as supplemental therapy to the intrinsic process of wound healing - the natural process of injured tissues repairing themselves after sustaining injury [1]. Characterizing the wound healing process is critical to point-of-care practice and has utility in the developing fields of regenerative medicine and tissue engineering [2-5]. A more interdisciplinary approach is needed to progress the fields further and have a positive impact to this serious condition that inflicts millions of people each year.

In the recent past, the prevalence of those suffering from chronic wounds has dramatically increased. Various factors can contribute to insufficient wound healing such as age, smoking, obesity and chronic diseases, such as diabetes, and venous/arterial insufficiency. These factors delay wound healing and increase the risk of developing chronic wounds. Chronic wounds currently pose a significant burden worldwide estimated to be as much as 4% of healthcare budgets [6,7]. In addition, costs associated with wound care in the United States healthcare system is approximately $50 billion annually, including an average cost to heal per wound between $3.00-$10,000.00 USD [8]. The field of wound care has many different treatment options and modalities. Current treatment strategies do not guarantee complete healing and the risk or reoccurrence can be relatively high. Improving wound treatments is a necessity and the scientific community in conjunction with clinicians should focus not only on finding new treatments for wound healing, but also on improving the effectiveness of current therapies. Wound care, however, is generally regarded as a supplemental therapy to the intrinsic process of wound healing - the

natural process of injured tissues repairing themselves after sustaining injury [1]. The importance of characterizing the wound healing process is critical to point-of-care practice as well as having much utility in the developing fields of regenerative medicine and tissue engineering [2-5].

Literature Review

Acute Wounds

Acute wounds typically heal in a very orderly and efficient manner characterized by four distinct, but overlapping, phases: hemostasis, inflammation, proliferation, and remodeling (Figure 1). Acute wounds progress through these normal stages of wound healing and show definite signs of healing within four weeks. Specific biological markers are often used to characterize the healing process of acute wounds; likewise, unique biologic markers can also characterize pathologic responses resulting in fibrosis and chronic (i.e. non-healing) wounds. Treatment is therefore determined based on the wound phase and levels of specific biomarkers. If treatment is tailored to a distinct healing phase, then the probably of normal, timely healing increases [9].

Figure 1 outlines the stages, timing, and biomarkers involved in the wound healing process. After injury, hemostasis begins the healing process with blood coagulation and cellular production of cytokines and chemokines. The process lasts minutes for normal healing and hours for abnormal healing. Inflammation follows this initial stage could take as little as a few hours to weeks, depending on the health of the individual. Immune cell recruitment, phagocytosis, and cell clearance from the site accelerates the sterilization and allows for cell proliferation over the next few days. Cells go though angiogenesis and tissues begin to strengthen and eventual remodel into proper form to resume normal tissue function.

Growth factors continue to be produced to complete the wound healing process over a few weeks to months. It is usually in cell proliferation stage when non-healing wounds tend to stop the wound healing process. At this stage, the healing process does not support tissue strengthening nor produce the needed growth factors to individual cells. This normal month long process enters into an iterative loop between inflammation and angiogenesis with little evidence of new tissue formation.

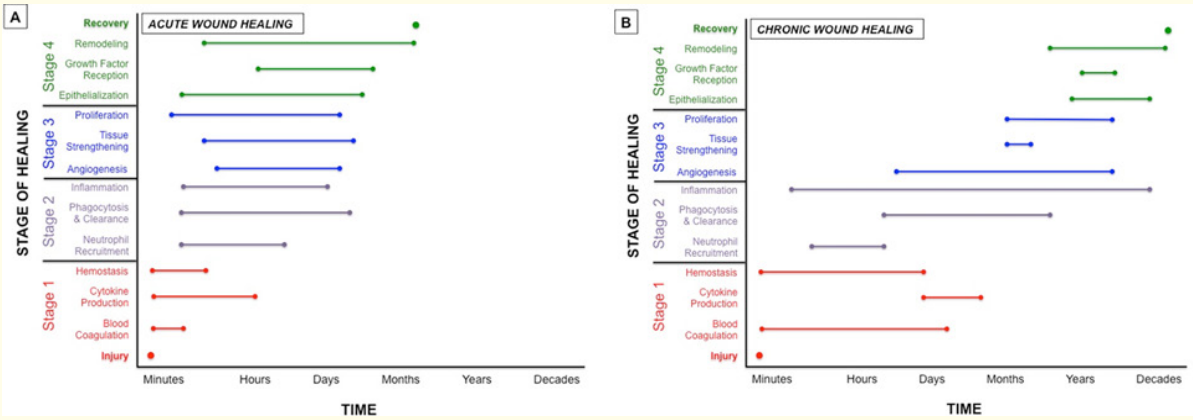

Figure 1: *Stages involved in the normal wound healing process with adverse events inhibiting recovery. Wounds can be described as injuries that break the skin or other body tissues. They include cuts, scrapes, scratches, and punctured skin and often occur because of an accident or intent to injure. Surgery, sutures, and stitches also cause wounds. From the left, injury marks the onset of the wound. Immediately following injury, the four stages of healing begin, initiated with hemostasis and ending with tissue remodeling. At the end of the process, the wound is generally regarded as recovered from injury. The boxes at the bottom of the figure list the most common adverse events that can occur in the wound healing processes. Any one of these – or combinations thereof – result in a chronic wound.*

The normal healing response begins the moment the tissue is injured. As the blood components spill into the site of injury, the platelets come into contact with exposed collagen and other elements of the extracellular matrix triggering the platelets to release clotting factors, essential endogenous growth factors, and cytokines such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-ß) [10]. Following hemostasis, the neutrophils then enter the wound site and begin the critical task of phagocytosis to remove foreign materials, bacteria, and damaged tissue. As part of this inflammatory phase, the macrophages continue the process of phagocytosis and the release of more PDGF and TGF-ß. Once the wound site is cleaned out, fibroblasts migrate in to begin the proliferative phase and deposit new extracellular matrix. The new collagen matrix then becomes cross-linked and organized during the final remodeling phase. In order for this efficient and highly controlled repair process to take place, there are numerous cell-signaling events that are required [11-18].

Chronic Wounds

A chronic wound is a wound that does not heal in the orderly set of stages previously mentioned. In addition, chronic wounds do not progress normally through the stages of healing rather, healing "stalls" in certain wound healing stages (i.e., inflammation) longer than acute wounds. Chronic wounds do not progress and do not show evidence of healing within four weeks [6]. In pathologic conditions, such as chronic wounds (e.g., non-healing chronic ulcers), this efficient and orderly process is absent and the wound is trapped in a state of chronic inflammation. Abundant neutrophils infiltration, associated reactive oxygen species, and destructive enzymes characterize chronic inflammation. Healing can only proceed after the inflammation is controlled. In contrast, excessive matrix deposition, reduced remodeling, and increased densities of mast cells characterize fibrosis. By understanding the functional relationships of these biological processes of normal compared to abnormal wound healing,

New strategies can be designed to treat injury as it relates to the specific pathological condition of the individual. This paper reviews the important processes involved in wound healing as well as the current research that is geared to accelerate chronic wound healing.

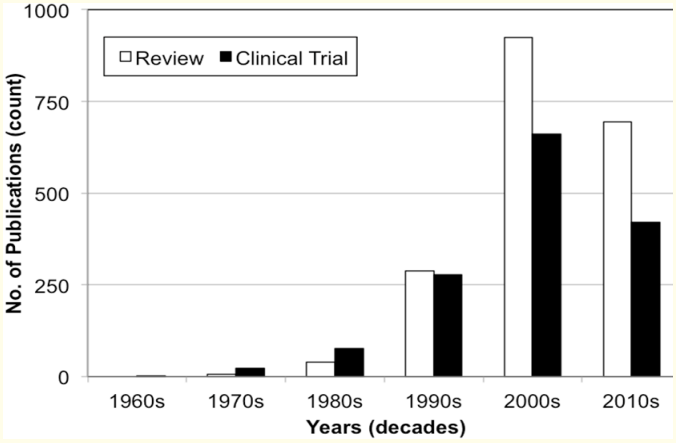

Figure 2: *Number of publications in the field of chronic wound over time. The publications are divided between the numbers of review articles versus clinical trial studies for chronic wound healing.*

The previously mentioned Figure 2 illustrates the number of publications versus the number of clinical trials across various decades from the 1960's through 2010's. As illustrated in the figure, the number of manuscripts and clinical trials has decreased since the early 2000's thus confirming the need to advance the science in this field and discover need therapeutics to mitigate chronic wounds.

This review discusses the cellular processes involved in effective wound healing, the adverse events that most commonly prohibit wound healing and promote chronic wound states, the current methods of treatment, and the research gaps needed that should be addressed to accelerate the recovery time of patients. This paper reviews the important processes involved in wound healing and current research geared at accelerating chronic wound healing process.

The current state of the science and future pharmacological needs is discussed. Finally, an outlook for the future trends and challenges of promising new products and protocols are discussed. The paper does not address some of the less common therapies such as biodebridement, electrical stimulation, or laser stimulation; nor does it address specific complications arising from diabetes, cancer, surgery, cigarette smoke, oral wounds, burns, or leg ulcers. For a list of relevant articles on literature addressing less common therapies and complications associated with wound healing, see Tables 1 and 2, respectively.

Topic	Reference(s)
Nanosilver	Atiyeh BS., *et al.* "Effect of silver on burn wound infection control and healing: review of the literature". *Burns* 33 (2007): 139-148. Chaloupka K., *et al*. "Nanosilver as a new generation of nanoproduct in biomedical applications". *Trends Biotechnol* 28 (2010): 580-588.
Vacuum Assisted Closure	Morykwas MJ., *et al*. "Vacuum-Assisted Closure: A New Method for Wound Control and Treatment: Animal Studies and Basic Foundation". *Annals of Plastic Surgery* 38 (1997): 553-562. Argenta LC and Morykwas MJ. "Vacuum-Assisted Closure: A New Method for Wound Control and Treatment: Clinical Experience". *Annals of Plastic Surgery* 38 (1997): 563-577.
Biodebridem ent and biosurgery	Wollina U., *et al.* "Biosurgery supports granulation and debridement in chronic wounds– clinical data and remittance spectroscopy measurement". *International Journal of Dermatology* 41 (2002): 635-639. Davydov L. "Maggot therapy in wound management in modern era and a review of published literature". *Journal of Pharmacy Practice* 24 (2011): 89-93.
Electrical stimulation	Kloth Luther C and Joseph M McCulloch. "Promotion of wound healing with electrical stimulation". *Advances in Skin & Wound Care* 9.5 (1996): 42. Baker Lucinda L., *et al*. "Effects of electrical stimulation on wound healing in patients with diabetic ulcers". *Diabetes care* 20.3 (1997): 405. Kloth Luther C and Jeffrey A Feedar. "Acceleration of wound healing with high voltage, monophasic, pulsed current". *Physical Therapy* 68.4 (1988): 503.
Laser stimulation	Conlan Michael J., *et al.* "Biostimulation of wound healing by low-energy laser irradiation: A review". *Journal of clinical periodontology* 23.5 (1996): 492. Posten W Wrone DA., *et al*. "Low-level laser therapy for wound healing: mechanism and efficacy". *Dermatol Surgery* 31 (2005): 334-340.

Table 1: *Literature addressing less common therapies.*

Topic	Reference(s)
Diabetes	Brem Harold and Marjana Tomic-Canic. "Cellular and molecular basis of wound healing in diabetes". The Journal of clinical investigation 117.5 (2007): 1219. Fahey III Thomas J., et al. "Diabetes impairs the late inflammatory response to wound healing". Journal of Surgical Research 50.4 (1991): 308. Falanga Vincent. "Wound healing and its impairment in the diabetic foot". The Lancet 366.9498 (2005): 1736.

Cancer	Zederfeldt B., *et al*. "Wound Healing and Cancer". Surgical Oncology". *Springer Berlin Heidelberg* (1989). 246. Singer Adam J and Richard AF Clark. "Cutaneous wound healing". *New England journal of medicine* 341.10 (1999): 738. Chang Howard Y., et al. "Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival". *Proceedings of the National Academy of Sciences of the United States of America* 102.10 (2005): 3738.
Surgery	Skuta Gregory L and Richard K Parrish II. "Wound healing in glaucoma filtering surgery". *Survey of ophthalmology* 32.3 (1987): 149. Scappaticci Frank A., *et al*. "Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab". *Journal of surgical oncology* 91.3 (2005): 173.
Cigarette smoke	Silverstein Paul. "Smoking and wound healing". *The American journal of medicine* 93.1 (1992): S22. Jones John K and Robert G Triplett. "The relationship of cigarette smoking to impaired intraoral wound healing: a review of evidence and implications for patient care". *Journal of oral and maxillofacial surgery* 50.3 (1992): 237.
Oral wounds	Landesberg Regina., *et al.* "Inhibition of oral mucosal cell wound healing by bisphosphonates". *Journal of Oral & Maxillofacial Surgery* 66.5 (2008): 839. Guo Shujuan and Luisa A DiPietro. "Factors affecting wound healing". *Journal of dental research* 89.3 (2010): 219.
Burns	Subrahmanyam M. "A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine". *Burns* 24.2 (1998): 157. Mester E., *et al*. "Effect of laser rays on wound healing". The American Journal of Surgery 122.4 (1971): 532. Maenthaisong Ratree., *et al*. "The efficacy of aloe vera used for burn wound healing: a systematic review". *Burns* 33.6 (2007): 713.
Leg ulcers	Vuerstaek J., *et al*. "State-of-the-art treatment of chronic leg ulcers: a randomized controlled trial comparing vacuum-assisted closure (VAC) with modern wound dressings". *Journal of vascular surgery* 44.5 (2006): 1029. Paletta Christian E., *et al*. "Major leg wound complications after saphenous vein harvest for coronary revascularization". *The Annals of thoracic surgery* 70.2 (2000): 492.

Table 2: *Literature addressing complications associated with wound healing.*

Discussion

Scale of Healing (Severity Versus Age Of The Wound)

In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exist in steady-state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the normal (physiologic) process of wound healing is immediately set in motion. Acute wound healing is a dynamic stepwise process consisting of partially overlapping phases that are determined by interacting events on a molecular, cellular and extracellular matrix (ECM) level. In contrast, chronic wounds are defined as wounds that do not follow the well-defined stepwise process of pathological healing but are trapped in an uncoordinated

and self-sustaining phase of inflammation. This impairs the constitution of anatomical and functional integrity in a physiologically appropriate length of time. The etiology of chronic wounds is diverse, but more than 80% are associated with venous insufficiency, high blood pressure or diabetes mellitus [19,20]. Despite the different underlying etiology, most chronic wounds show a similar behavior and progress. This uniformity is due to consistent components of the multifactorial pathogenesis of most chronic wounds: local tissue hypoxia, bacterial colonization, repeated ischemia-reperfusion injury and cellular as well as systemic changes of ageing [21-25].

Acute wounds generally proceed through an orderly and timely reparative process that results in a durable restoration of anatomic and functional integrity. However, various physiologic and mechanical factors may impair the healing response, resulting in a chronic wound that fails to proceed through the usual stepwise progression. For example, local infection, hypoxia, trauma, foreign bodies, or systemic problems such as diabetes mellitus, malnutrition, immunodeficiency, or medications are most frequently responsible [26-30].

All wounds are contaminated, but most successfully resist invasive infection. When the concentration exceeds 100,000 organisms per gram of tissue or the immune system becomes compromised, infection frequently ensues [31]. Debridement (surgical, enzymatic, and/or by dressing changes) and antibiotics are the mainstays of antibiotic treatment. Cellular hypoxia retards wound healing through various means. A state of oxygen deficiency, hypoxia, may cause an impairment of function. When the cell is unable to extract adequate oxygen, the partial pressure of oxygen within the cell declines, which leads to a reduction in mitochondrial respiration and oxidative metabolism [32]. Systemic disease can dramatically prolong or interrupt wound healing. Glycosylation in diabetes mellitus impairs neutrophils and macrophage phagocytosis of bacteria, prolonging the inflammatory phase [27].

Wounds exert heightened metabolic demands, particularly within granulation tissue. Amino acids such as methionine, proline, glycine, and lysine, are essential for normal cell function and the repair of cutaneous wounds. Fatty acids are critical constituents of cell membranes and are the substrate for the eicosanoids that mediate the inflammatory process [33].

In recovering tissues after sustaining injury, the use of medications is thought of as therapy or, some clinicians believe, a "killing therapy" [34,35]. Corticosteroids, any of a group of steroid hormones produced within tissue or made synthetically, can blunt the processes of the entire inflammatory phase. There are studies that suggest that short-term non-steroidal anti-inflammatory drug (NSAIDS) have a negative impact on healing. However, the question of whether long-term NSAIDs interfere with wound healing remains unanswered. NSAIDs can interfere with inflammation modulation and inhibit platelet function and, by extension, have the potential to create a chronic wound [36,37] Animal studies have shown that systemic use of ibuprofen has demonstrated an anti-proliferative effect on wound healing. This anti-proliferative effect results in decreased numbers of fibroblasts, weakened breaking strength, reduced wound contraction, delayed epithelialization [38-41] and impaired angiogenesis [41]. Clinical recommendations have long suggested that, to avoid anti-platelet effects, patients should discontinue NSAIDs before surgery. The exception to this rule is cardiac patients who are on a low-dose aspirin regime to mitigate the risk of a deleterious cardiovascular event [42,43]. NSAIDs have also been used topically on the surfaces of chronic wounds with somewhat positive results. For example, the local use of ibuprofen- foam provides moist wound healing, reduces persistent and temporary wound pain, and benefits chronic venous leg ulcer healing [43].

Barriers that Inhibit Successful Wound Healing

During the time period when injured tissues recover, complications tend to occur that inhibit the healing process. Coagulation and inflammation is the body's immediate natural response to injury. Coagulation, also known as disseminated intravascular coagulopathy, is a pathological process characterized by the widespread activation of the clotting cascade that results in the formation of blood clots in the small blood vessels throughout the body. The process of blood coagulation protects the wound and prevents further blood loss. The clots that form, via platelet aggregation, are held together and in place by fibrin. Complications in blood clot formation, thrombosis (excess clots) or hemophilia (inability to form clots), disrupt the fibrin networking process and subsequently keep the wound "open" and diagnosed as chronic [44].

Once coagulation has been achieved, blood vessels dilate to allow antibodies, white blood cells, growth factors, enzymes, and nutrients to reach the wounded tissue [3,35 and 45]. It is at this stage that the characteristic signs of inflammation can be seen: erythema, heat, edema, pain and functional disturbance [2, 12]. While inflammation is generally recognized as a normal physiological event and provides ample evidence that the recovery process has begun, excessive inflammatory response can induce additional pain, discomfort, and hinder other key measures the wound needs to heal.

Cytokine/chemokines production accompanies inflammation. Biomarker production of compromised tissue normally serves as the method to communicate repair mechanisms to the rest of the body [46]. In the last 10 years, there has been an increase in the research efforts that explore the early biomarkers along the path to healing. These areas of research include inflammatory cytokine response and oxidative stress [47,48]. For example, the forkhead transcription factor, FOXO1, is a critical regulator of wound healing in that it is responsible for regulating transforming growth factor- beta (TGF-β) expression as well as protecting damaged (and healing) cells from oxidative stress. In the absence of FOXO1, tissue healing is mitigated due to the onset of oxidative damage and reduced growth factor induction. On the other hand, an overproduction of FOXO1 can contribute to excess inflammation and trigger cellular apoptosis. These phenomena are not unique to FOXO1; other critical regulators include other growth factors (GFs), interleukins (ILs), and tumor necrosis factors (TNFs). Almost all of the cytokines involved the wound healing process require accurate production concentrations in order to be helpful and not exacerbate the condition.

During proliferation, the wound is reconstructed with new granulation tissue comprised of collagen and extracellular matrix. Within the granulation tissue, a new network of blood vessels will develop. This process is referred to as angiogenesis [15,49]. The development of healthy granulation tissue is dependent upon the fibroblast receiving sufficient levels of oxygen and nutrients supplied by the blood vessels [50,51]. Healthy granulation tissue is rough and uneven in texture; it does not bleed easily and is pink or red in color. If oxygen and other essential nutrients are not supplied to the injured tissue, the granulated tissue then becomes dark and tender to touch indicating ischemia and/or infection [2,5].

Debridement to remove dead tissue is typically used to accelerate wound healing, however, it can also be considered a risk factor for a non-healing chronic wound. There are five main methods of debridement: surgical or sharp, autolytic, enzymatic, and mechanical [52,53].

The type of debridement method employed depends upon many factors, including the size, position, and type of wound, efficiency of debridement method, pain management, exudate levels, risk of infection, and the cost of the procedure [54]. Surgical or sharp debridement, which is thought to be selective, may induce damage to viable tissue, and bleeding is likely. Some level of autolytic debridement, which is the natural and highly selective process by which endogenous proteolytic enzymes break down necrotic tissue, occurs in all wounds. Autolytic debridement may not take place fast enough to encourage rapid wound healing and closure and is therefore used in combination with another method of debridement. Enzymatic debridement, another highly selective method, uses naturally occurring proteolytic enzymes that are manufactured by the pharmaceutical and healthcare industry specifically for wound debridement. These exogenously applied enzymes work together with the endogenous enzymes in the wound. Additionally, mechanical debridement is a nonselective, physical method of removing necrotic tissue and debris from a wound using mechanical force. This debridement method is generally easy to perform and is more rapid than autolytic and enzymatic debridement. However, this nonselective method can damage healthy granulation tissue both in the wound bed and at the margins of the wound thus causing significant discomfort to the patient and compromising complete wound healing and closure [55-57].

Wound colonization is the presence of replicating microorganisms adherent to the wound in the absence of injury to the host. This is very common is wound scenarios and most of these organisms are normal skin flora. *Staphylococcus epidermidis*, other coagulase negative Staph., *Corynebacterium* sp., *Brevibacterium* sp., *Proprionibacterium acnes, Pityrosporum* sp. are all examples of normal skin flora. Wound Infection, on the other hand, is referring to the presence of replicating microorganisms within a wound that cause host injury. There are some pathogens that are common in these infections that include *Staphylococcus aureus*, Beta-hemolytic *Streptococcus (S. pyogenes, S. agalactiae), E. coli, Proteus, Klebsiella*, anaerobes, *Pseudomonas, Acinetobacter, and Stenotrophomonas (Xanthomonas).*

As is the case with infective processes, the bacteria in a wound occur in the form of biofilms, which are aggregated bacteria in complex communities embedded in a self-secreted extracellular polysaccharide matrix [9]. Biofilms play a significant role in a large number of infections in humans, and due to the intrinsic resistance of these structures to an array of antimicrobial agents and host defense mechanisms, such infections can be difficult to treat effectively. Some of the common bacterial strains observed in infected and clinically non-infected wounds are *Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa)*, and β-hemolytic *streptococci* [28,54,58,59]. Of these bacterial strains, P. aeruginosa and *Staphylococcus* appear to play an important role in bacterial infection in wounds. It has been estimated that biofilms are associated with 65 percent of nosocomial infections and that treatment of biofilm-associated infections costs > $1 billion annually in the United States [30,60]. Dermal wounds, both chronic and acute, are susceptible to infection due to the development of microbial communities within the wound environment. It is likely that these communities exist within biofilms, although to date their characterization and role has not been elucidated.

The microbial flora in wounds appears to change over the course of healing. In a recently formed wound, different microorganisms from endogenous and exogenous sources will contaminate the wound surface. The properties of the wound surface will predetermine which microorganisms will attach, grow and remain components of an early biofilm. Immediately after injury, normal skin flora predominates however, after about 4 weeks facultative anaerobic gram negative rods will colonize the wound [61]. In addition, as long-term chronic wounds are developed the bacterial colonization contains more anaerobes than aerobes. Aerobic gram-negative rods also infect wounds late in the course of chronic wound degeneration usually acquired from exogenous sources like bath or foot water [54,61-64].

Colonizing bacteria will modify the habitat and create a microenvironment that encourages the attachment and growth of secondary colonizing microorganisms. If unchallenged, and with favorable conditions, a complex community of microorganisms is likely to develop. It has been proposed that as a microbial biofilm develops the community will ultimately form a more stable polymicrobial "climax community" [65-69]. Antibiotics are used to treat bacterial infections. However, biofilm-related infections do not yield so easily to this form of treatment, because they provide a protective mechanism that renders bacterial cells less susceptible to both antibiotics and biocides [28,60,66 and 70]. Therefore there is a tremendous need to develop technologies that provide a wound environment that is favorable for healing while reducing excessive bacterial invasion.

Current Technologies, Modalities, and Therapies

As previously mentioned, research and development in the area of wound healing has significantly increased in the last decade. Modern technologies contribute to the success of natural recovery while intervention modalities accelerate the healing process. Some of the most successful current therapies can be categorized into one of the following four categories: stimulated endogenous, pharmaceutical, surgical, and topical.

Stimulated Endogenous Therapies

Stimulated endogenous therapies include the use of additional growth substances (e.g. growth factors, collagen, and cytokines) to stimulate wound healing [71-74]. For example, mesenchymal stem cells (MSCs) are responsible for the repair response of tissue wounds. They function by recruiting cells to and secreting growth factors at the site of injury. Like other types of stem cells, MSCs differentiate into various lineages. The critical cell-types in wound repair are bone, cartilage, tendon, and fat. Boosting the production of these natural occurring helper cells or modifying their localized concentration at the injury site via injection has improved wound healing [75].

Growth substance stimulation can also improve wound healing. Growth factors are naturally occurring proteins that influence the stages of wound healing during tissue repair process. These macromolecules have the ability to capture growth factors within the growing fibrin network at the wound site [76]. If these growth factors are trapped and not available to bind to cell surface receptors, the cells lose their ability to multiply in an expedient manner, thus slowing the healing process. By supplementing the amount of growth factors in the localized area, cells are able to receive the nutrients needed for maturation and proliferation [77]. Some studies have

shown the positive effects of adding recombinant human platelet derived growth factor (rhPDGF), fibroblast growth factor (FGF), or epidermal growth factor (EGF) to wounds [78]. Topical delivery of growth factors to chronic wounds must consider resistance to rapid degradation from the wound's proteolytic environment and in addition, they must have sustained release. Efforts in chronic wound healing have readily accomplished this using gene therapy [79-82]. Multiple novel delivery systems, including adenovirus and slow-releasing polymers are being investigated as growth factor delivery systems with varying success. Some of the most promising growth factors that require further clinical testing are VEGF, bFGF, and GM-CSF [83-85]. PDGF-BB has already been approved by the U.S. Food and Drug Administration (FDA) and is currently used in the treatment of ulcers [86-88]. Sustained, simultaneous growth factor therapy, or living cell therapy, which is also FDA approved, may be considered as multiple growth factor therapy as both healthy keratinocytes and fibroblasts produce at least 17 different growth factors. These healthy cells will secrete these factors simultaneously thus stimulating a patients' cells to participate in healing process [89].

Another stimulated endogenous therapy that influences the wound healing process is collagen. Collagen is produced by fibroblasts and is an important component of new tissue. There are a few examples of products currently on the market that utilize topical collagen; these products have shown wound healing improvement by providing a scaffold for newly recruited cells and growth factors to adhere onto while cell populations grow and tissues thicken. An important consideration for using collagen products is moisture control. The wound and collagen need a specific amount of moisture at the wound site for adequate healing: too much moisture promotes infection, while too little moisture collapses the fragile fibrin network [90].

Pharmaceutical Therapies

The pharmaceutical therapies associated with wound healing revolve around pain and infection mitigation. Pain medication is used to aid in the patients' comfort throughout the scale of injury to recovery and have been shown to slow the healing process. Antiseptics are used preventively to disinfect the area immediately after injury as well as prohibit conditions where bacteria and other microorganisms breed uncontrollably. In recent years, curcumin (a.k.a. turmeric) has been investigated as a potentially novel therapeutic for wounds. It is believed to function as an inflammatory mediator and an antimicrobial agent. Chemically, it is a polyphenol that the FDA has granted GRAS (generally recognized as safe) status [91,92].

Surgical Therapies

The two most commonly used surgical procedures that assist in the wound healing process are skin grafting and resurfacing. A skin graft is a patch of skin that is removed by surgery from one area of the body and transplanted to another area [93]. Skin resurfacing and body -contouring surgery utilizes a laser to modify scar tissue, discoloration, and other skin formation to that of healthy and aesthetically pleasing skin tone and contour. This procedure is usually performed when the healing process is near completion. There are a few complications associated with these procedures, most notably the fact that patients require anesthetics. Skin grafting is an invasive procedure requiring a surgeon, where body contouring is an quicker procedure requiring a dermatologist only. In addition, post-operation bleeding or thrombosis increase the risks of subsequent injuries to an already strained immune system response [94].

Topical Therapies

A fourth therapeutic area, and arguable the most used administration, is topical, such as bandages, dressings, or sealants [95,96]. The three major characteristics that all deployed bandages should possess are antimicrobial, biocompatible, and stable. These characteristics are most important for the use of negative pressure wound therapy (NPWT) [97-99]. NPWT is a recently developed therapy that has become mainstream modality for the most severe wounds, such as those occurring in combat, car accidents, or debilitating workplace injury [64,100 and 101]. Today, the therapy is conveniently packaged as a small portable vacuum unit for home use. The technology has the ability to remove contaminated fluid or instill nutritional fluid. The system requires the use of a bandage or "sponge" that is flexible and porous.

In addition to vacuum therapies, impregnated bandages are used as a topical agent in wound healing. The most common structures of these bandages are either natural fiber or polymeric mesh doped with salt complexes, easily dissolved into cationic and anionic species. This chemical system has been shown to exhibit bactericidal and bacteriostatic properties [102,103] Leached cations and anions

are released from the fiber or mesh, penetrate the bacterial cell, and degrade the integrity of the cytoskeleton structure. Mammalian cells are also vulnerable to this physicochemical process, but to a lesser exist. Excess ionic content inside cells is known to produce metallothionein (MT), which in turn increases the uptake of any other foreign entities from outside to inside the cell membrane. Thus, this topical therapy often produces mixed results.

No matter which treatment – stimulated endogenous, pharmaceutical, surgical, or topical – is used to promote wound healing, the use of oxygen (a.k.a oxygenation) can have a dramatic effect on the healing process [104]. Hyperbaric oxygen involves pressurizing the wound in a sealed chamber where an atmosphere of 100% oxygen is created. Its most sensationalized use is decompression after deep-sea dives, but it is also effective at mitigating carbon monoxide poisoning, crush injuries, radiation injury, unhealed skin grafts, bacterial infections, and chronic wounds. Wound healing repair processes such as collagen deposition and infection control are dependent upon oxygen. Increased oxygenation promotes these complex events.

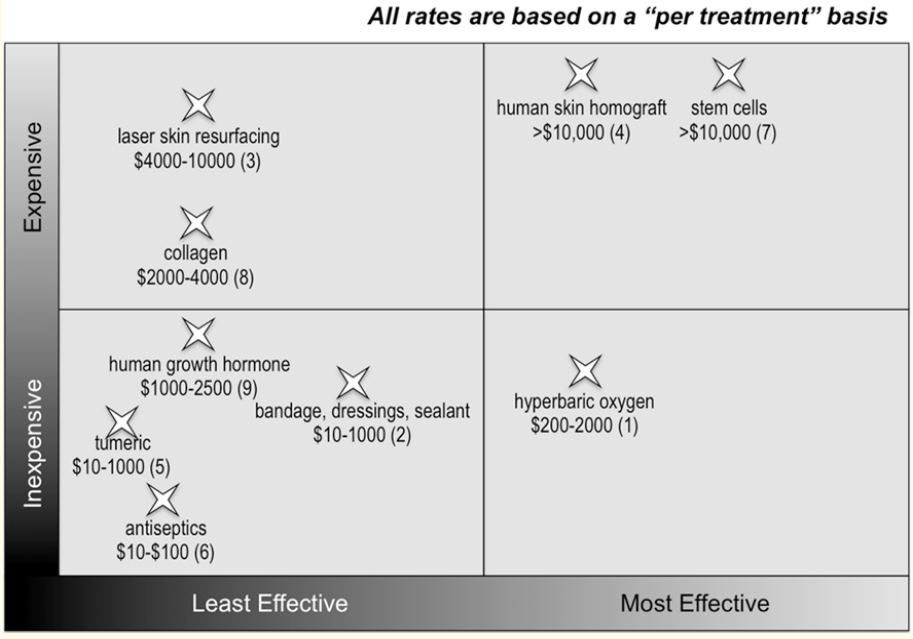

Figure 3: *Market analysis in terms of cost and efficacy of the treatment modalities discussed in this paper. Each modality cites a reference for efficacy and cost information: (1) The Cochrane Library 4(4):1002; (2) Health Technology Assessment 3(17):1; (3) American Journal Of Clinical Dermatology 4(1):1; (4) Clinical Laboratory Medicine 25: 587;(5) Journal of Trauma-Injury, Infection, and Critical Care 51(5):927; (6) Wounds 15(5):149;(7) Stem Cell Research & Therapy 5(1):28; (8) Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 6:17; (9) Proceedings of the National Academy of Sciences USA 107(43):18611.*

Future Research Considerations

Nationally, chronic wounds affect about 6.5 million people and over US $25 billion are spent annually on treatment of chronic wounds [105]. Treating those wounds is one of medicine's most challenging problems. The field of wound care contains varying treatment options and modalities as the number and types of practitioners caring for wounds. There is success obtain when using the classical wound care treatment option, however, there continues to be new products and technologies developed to add to the wound care arsenal. Continuing research in these areas of new development and in modifying or improving historical methods is critical to reducing the burden of chronic wounds so many patients face.

Research Models and Model Needs

The selection of an appropriate model is critical in collecting meaningful data. Each study has to make decisions on how accurately the selected model reflects the biological processes that occur in normal human wound healing [106,107]. The motivation of every study is to have a perfect equivalent of the human clinical situation and to be able to collect data in a timely matter at low cost. When considering impaired wound healing, there are significant complexities found in the healing process of human wounds. The pathological conditions that result in chronic problems in human beings must be judiciously simulated in an animal model for several reasons. First and foremost, that animal's survival for the length of the study is critical in evaluating the progress of wound healing [3,108-110]. The researcher is tasked with deciding which model would be most applicable to the human condition that is being simulated.

In vitro wound healing scratch assays have been used for quite some time for evaluating wound healing [111-113]. Researchers now are moving to more complex co-cultured and 3D-tissue models to evaluate wound healing scenarios [114]. These in vitro models serve as a starting point to pre-clinical data collection and further are in line with the 3Rs movement. Several factors can be evaluated in biological testing models such as rodent models of wound healing. Tensile strength of the wounded area, histological examination for new cell growth, presence of growth factors, and rate of healing have all been evaluated using rodent wound models. Each of these endpoints is affected by characteristics such as the selection of the strain of rodent, sex of rodent used, weight/age range, and choice of anesthetic agent. In addition there are several porcine models available evaluating various types of wound scenarios like full thickness wounds, burns, and surgical wound healing [115-118]. While there are several biological models available, there is not a completely accurate wound healing model available that mimics chronic wounds in humans. Developing future therapeutics via toxicological mechanistic approaches may not be translational if the models don't accurately mimic chronic wounds in humans. This is a significant need in the area of biological models for wound healing. Each biological test model has utility and has limitations. The goal for researchers to optimize the compromises made for each study to allow for meaningful data to be collected and further for that data to be translational in nature. Development of scenario-appropriate models for evaluating wound healing is an ongoing need and from the point of view of therapeutic development, to understand how well models reflect the ultimate clinical target and how they are limited by species and age differences is key.

The Use of Nanosilver

Silver dressings (including nanosilver dressings) are a comparatively new category of advanced wound care dressings for the treatment of bacteria-infected wounds. The mechanism of action in this topical system involves the dissociation of silver ions from silver particles or salts and subsequent ion intake into the bacteria cell where metabolism dysfunction leads to cell death. The smaller size of the silver particle, then the faster the rate of ion dissociation. Furthermore, if the silver particle is small enough (~50 nm) and maintains a positively charged surface within the wound -dressing matrix, the cell tends to engulf the particle where the "Trojan Horse" mechanism of targeted, concentrated ion release occurs immediately upon decreased cytoplasm pH.

Typically, the structure of the silver dressing is a wound dressing impregnated with ionic silver. It is important to note that this impregnation not only occurs within solid state materials (such as hydrogels and other rigid polymers), but also within ointment creams. The difference between these two product-types is that while the ointment cream usually contains other antibacterial drugs (like neomycin), the wound dressing usually does not.

There are many companies who have developed silver or nanosilver particle based infected wound treatment options. Table 3 lists the companies and their products currently on the market.

Company	Product	Use
Smith & Nephew	Allevyn	Adhesive
Smith & Nephew	Acticoat	Ointment cream
Johnson & Johnson	Actisorb Silver 220	Ointment cream
Coloplast	InterDry Ag Textile	Bandage
3M	Tegaderm Alginate	Dressing
Milliken & Company	Select Silver	Ointment cream
Ferris	PolyMem Silver	Dressing

Table 3: *List of companies and their products using silver or nanosilver particles in their wound healing treatments.*

Silver is believed to have beneficial effects in healing in general; thus the use of drinking or consuming silver as a food supplement. Because of this generally accepted dogma, much research – and by extension commercialization – has bridged silver oral supplements to silver impregnation. Whether it is used as a topical ingredient in an ointment or impregnated into a wound dressing, the use of silver and nanosilver particles in treating wounds has produced contradictory results [119]. Clinically, silver films have been used to coat sutures/catheters and colloidal silver has been used in eye/tongue drops to mitigate or eliminate bacteria infection [120] [121]. Mechanistically, silver has shown antimicrobial properties against gram-positive and gram -negative bacteria [122]. These ions compete for nutrients and produce of toxic metabolites in bacteria. In addition, silver ions block the respiratory cycle of bacterial cell wall [123]. Beneficially, silver ions have demonstrated regulation of inflammatory response without shutting off essential pro-healing functions [124] Contrary, many studies have shown that the same toxicity against bacteria cells can also be observed against mammalian cells [125]. Silver deposits around nerves and in deeper skin layers and may cause permanent skin damage [126]. It contributes to antibiotic resistance and is intimately associated with environmental contamination of other toxic heavy metals such as mercury and lead [127]. Once the wound is washed, silver disturbs the healthy bacterial activity used to keep sewage systems functioning properly [128].

Tissue Engineering Technologies

Bioengineering techniques for achieving wound closure (e.g. stem cells and gene therapy) are currently emerging as alternative wound healing models. Stem cells offer extensive possibilities for migrate to the site of injury or inflammation, participation in regeneration of damaged tissue, stimulation of proliferation and differentiation of resident progenitor cells, secretion of growth factors, remodeling matrix, increasing angiogenesis, inhibiting scar formation and improving tensile strength of wounds [129-135]. Stem cell-based skin engineering represents a promising alternative tool for regenerative strategy for wound therapy.

Conclusion

Wound healing and care are both dynamic and co-dependent practices [136]. In order to optimize the healing process, a collaborative and interdisciplinary approach to wound management is needed. There are six general principles that have the potential to ensure this optimization: safety, environmental surroundings, clinician and patient decision-making, documentation, education, and basic research.

The safety of the wounded individual is safeguarded by clinical practices that are in compliance with regulations, codes of practice, and policies. By extension, the optimal outcome for the individual's health is facilitated when a thorough understanding of the environmental surroundings where the patient resides during the stages of healing. Reasonable and cooperative care management among the clinician, patient, and caregiver along with detailed documentation of the healing process enables the patient to educate themselves in prevention of chronic wounds. Lastly, research and development of novel wound healing tools and modalities strengthen the wound management community's use of effective therapy [137].

Integrated strategies like increased oxygenation, the use of silver particles or stem cells, medicated bandages, and wound irrigation are needed to advance the development of wound care therapeutics. For instance, the addition of oxygen to the inflammation phase of wound healing, could increase in T-cell recruitment to the site of injury thus corresponding to a faster healing process. Wound irrigation offers a simple and effective way to increase the probability of a normal progression of wound healing. Arguably, the greatest challenge of wound care and management is control over microorganisms in the wound bed. Medical practitioners at various levels (i.e., clinicians, nurses, aids, first-responders) should be trained to use the newest wound healing techniques as soon as the technologies are available. These advanced technologies increase the probability to accelerate the wound healing process.

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Conflict of interest

The authors have no conflicts of interests to declare.

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