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Chlorophyllin--A Healer? A Hypothesis for its Activity

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Abstract: Chlorophyllin has a long history of use in medicine. It has found utility as an active ingredient in several topically applied enzymatic debridement agents. The literature describes the biochemical and clinical implications of chlorophyllin. The following review describes chlorophyllin's mode of action and reasons for its initial use in papain-urea-based enzymatic debridement agents. In addition, a critical review investigates whether it is biochemically feasible to describe chlorophyllin as a wound-healing agent.

Sibbald et al.[1] and Falanga[2] first defined wound bed preparation (WBP) in 2000. Schultz et al.[3] expanded the definition in 2003. Wound bed preparation can be described as the management of the wound to accelerate endogenous healing or to facilitate the effectiveness of other therapeutic measures. There are 5 basic modes of debridement: sharp, mechanical, enzymatic, autolytic, and biological (maggot therapy). A given mode is generally chosen based upon the need of the patient, the care setting, and the licensure of the practitioner. There have been many options for enzymatic debridement over the years. Several sources from which these enzymes are obtained include the following:

1. **Bacterial sources:** *Clostridium histolyticum* (produces bacterial collagenase), *Hemolytic streptococci* (produces streptokinase and streptodornase), and *Bacillus subtilis* (produces subtilisin)
2. **Plant sources:** papaya (produces papain), pineapple (produces bromelain), and fig (produces ficin)
3. **Mammalian sources:** pancreatic tissue (produces trypsin and chymotrypsin) and serum (contains fibrinolysin/plasmin)
4. **Others:** *Bacillus thermoproteolyticus* (produces thermolysin) and Antarctic krill (produce krillase).

Thermolysin (TLN) belongs to the zinc-metalloproteinase super family. This extracellular endopeptidase catalyzes the hydrolysis of peptides at specific amino acid sequences.[4] The reaction mechanism is fundamentally similar to that of *C. histolyticum* collagenase. Although TLN directly attacks type IV collagen, TLN is able to convert certain pro-matrix metalloproteases (proMMPs) into active MMPs. For example, TLN can activate proMMP-8 and proMMP-1.[5] This activation aids in the debridement of the wound. Krillase, a protease extract from Antarctic krill (*E. superba*), has been investigated as a new type of enzymatic debriding agent for necrotic ulcers. The predominant enzymes in the preparation represent trypsin-like activity associated with 3 serine proteinases.[6]

Over the years, various proteolytic enzymes have been employed (eg, papain, ficin, streptokinase, streptodornase, trypsin-chymotrypsin, collagenase) for the debridement of wounds. The activity of various enzymes has been investigated via *in-vitro ex-vivo*, and *in-vivo* models and clinical studies. For example, 1 study compared the activity of 2 topically applied enzymatic debridement agents (bacterial collagenase and papain-urea) in a clinical setting.[7] Another study examined the activity of several debriding agents via *in-vitro* and *ex-vivo* models.[8] A comprehensive review of WBP including an algorithm designed to guide the clinician in decision making for debridement modalities provided keen insight into specific aspects of various enzymatic debriding agents.[9] Another review focused solely on debridement modalities, including enzymatic debridement agents.[10] Klasen provided a review of collagenase and subtilisin use in burns.[11] Literature reviews have also compared and contrasted various enzymatic debridement agents.[12,13] Articles describing the appropriate use of certain topically applied enzymes, as well as uses in conjunction with other wound care modalities, can also be found in the literature.[14,15]

The old concept of using proteolytic enzymes to digest dead tissue as an adjunct to the management of "dirty," infected wounds is most likely related to observations from natives of tropical countries where the papain-rich latex obtained by scratching the skin of the green fruit of the papaw tree (*Carica papaya*) has long been used for the treatment of eczema, warts, ulcers, and other types of sores.[16] In addition to applying papain-rich latex to wounds, occasionally natives would

expose the wounds to urine, then wrap them in green leaves from the same plant. These 3 naturally occurring materials contain the chemical compounds papain, urea, and chlorophyll, respectively. Urea is a component of mammalian urine, and chlorophyllin is a derivative of chlorophyll.

Prior to the turn of the 20th century, literature on the use of papaya latex preparations for treating sloughing ulcers, removing impacted cerumen, and dissolving diphtheritic membranes became available.[17-19] More recently, it has been found that the major insoluble constituents of inflammatory exudates, fibrin and desoxyribonucleoprotein derived from the nuclei of dead degenerating cells, could be rapidly lysed by the local application of a mixture of enzymes obtained from the secretory products of certain strains of hemolytic streptococci. The major constituents of this enzyme mixture, streptokinase (an activator of plasminogen, the naturally occurring precursor of a proteolytic and fibrinolytic enzyme of human plasma) and streptodornase (streptococcal desoxyribonuclease), provided the basis for enzymatic debridement.[20-26] The history of enzymatic debridement agents has been turbulent, with only 3 enzymatic systems currently used widely (ie, bacterial collagenase, papain-urea, and papain-urea-chlorophyllin).

Chlorophyllin has an interesting history. Approximately 800 chlorophyllin derivatives have been prepared as a result of efforts to identify the structure of chlorophyll following the discovery of the similarity in structure of chlorophyll and hemoglobin.[27] Chlorophyllin plays a dual role in the wound—healing and controlling odor. Of the 800 chlorophyllin derivatives, the sodium salt of the copper compound has displayed the highest degree of antibacterial and cell growth-stimulating activity. This derivative is commonly referred to as chlorophyllin copper complex sodium and can be found in conjunction with papain and urea in topically applied enzymatic debriding ointments. Chlorophyllin provides wound healing activity and odor control to these commonly used enzymatic debriders.

Sack and Barnard[28] found capillary blockage to be characteristic of chronic lesions but showed the cause of blockage to be agglutinated erythrocytes. The agglutinins in these cases were tissue breakdown products (ie, depolymerized mucoproteins, mucopolysaccharides). While acknowledging the value of proteolytic enzymes to remove necrotic tissue and debris, the authors noted that depolymerized mucoproteins were the products of enzyme digestion of necrotic tissue and that ideally a proteolytic enzyme should be applied in conjunction with an antiagglutinin material (ie, sodium copper chlorophyllin). Miller[29] confirmed this concept and noted that when enzymes are used without an accompanying antiagglutinin, removal of the products of digestion from the wound must be frequent and effective. Both groups of investigators incriminated tissue breakdown products not only as agglutinins but also promoters of inflammation and consequently necrosis of tissue cells. In separate clinical studies by Morrison and Casali[30] and Miller,[31] ointments containing papain and urea but without chlorophyllin proved to be potent debriding agents. However, the ointments produced inflammatory reactions that necessitated their discontinuation. Additionally, in order to assure optimal availability of nutrients (eg, to a decubitus ulcer), the integrity of the capillary and lymphatic systems must be assured.[31]

Chlorophyllin controls the inflammatory effects ordinarily associated with the products of enzymatic digestion. Sodium copper chlorophyllin is a historically established wound-healing agent. It appears that the primary value of chlorophyllin is as an antiagglutinin and anti-inflammatory substance, because it allows continuous and prolonged use of the proteolytic ingredients, papain-urea, which may otherwise cause inflammation and hemagglutination of capillaries. The favorable clinical results seem to be due to the fact that the proteolytic ointment (containing papain, urea, and chlorophyllin) thoroughly cleanses the lesions of all necrotic tissue and debris and then maintains optimal circulation and drainage so as to allow for full availability to the tissues of hematologic and nutritive elements.[31]

To summarize, as necrotic tissue is degraded via the action of papain-urea, various breakdown products are produced. These breakdown products are known to promote the blockage of capillaries and lymphatic vessels, thus preventing an adequate supply of nutrients to the wound site as well as removal of toxins from the wound site (resulting in inflammation). These products of the digestive action of papain-urea are referred to as agglutinins (ie, depolymerized mucoproteins, mucopolysaccharides). These compounds cause the agglutination (clumping) of red blood cells (hemagglutination) in capillaries, leading to local necrosis, and attack viable cells, resulting in inflammation and further necrosis.[28] Two key properties of sodium copper chlorophyllin—1) prevention/inhibition of hemagglutination and fibrin formation and 2) protection of viable cells from the necrotizing action of toxic substances in the inflammatory exudate—explain the action of chlorophyllin in promoting wound repair (Figure 1). As a result of the action of chlorophyllin, the inflammatory response is diminished, resulting in a decrease in pain, swelling, and erythema and allowing the wound to granulate in many cases. Slow healing wounds appear, in part, to be the result of an inability of the host's tissues to overcome by natural processes the local effects of partially depolymerized mucoproteins.[28] Tissue culture studies on the growth of fibroblasts indicate that the introduction of chlorophyllin abolishes the "lag phase" ordinarily preceding the proliferation phase.[32] By inhibiting fibrin formation and hemagglutination in capillaries, macrophages are able to gain access to the wound site more easily (Figure 2). Macrophages (via the expression of TNF-α and IL-1β) promote the proliferation of endothelial cells and fibroblasts in the wound site (Figure 3),[33] resulting in the development of healthy granulation tissue (Figure 4). Chlorophyllin has been described as a wound-healing agent because of its various activities in the wound environment. Functionally, chlorophyllin allows continuous and prolonged use of the proteolytic ingredients, papain-urea, which may otherwise cause hemagglutination of capillaries and lymphatic vessels, inflammation, and necrosis.

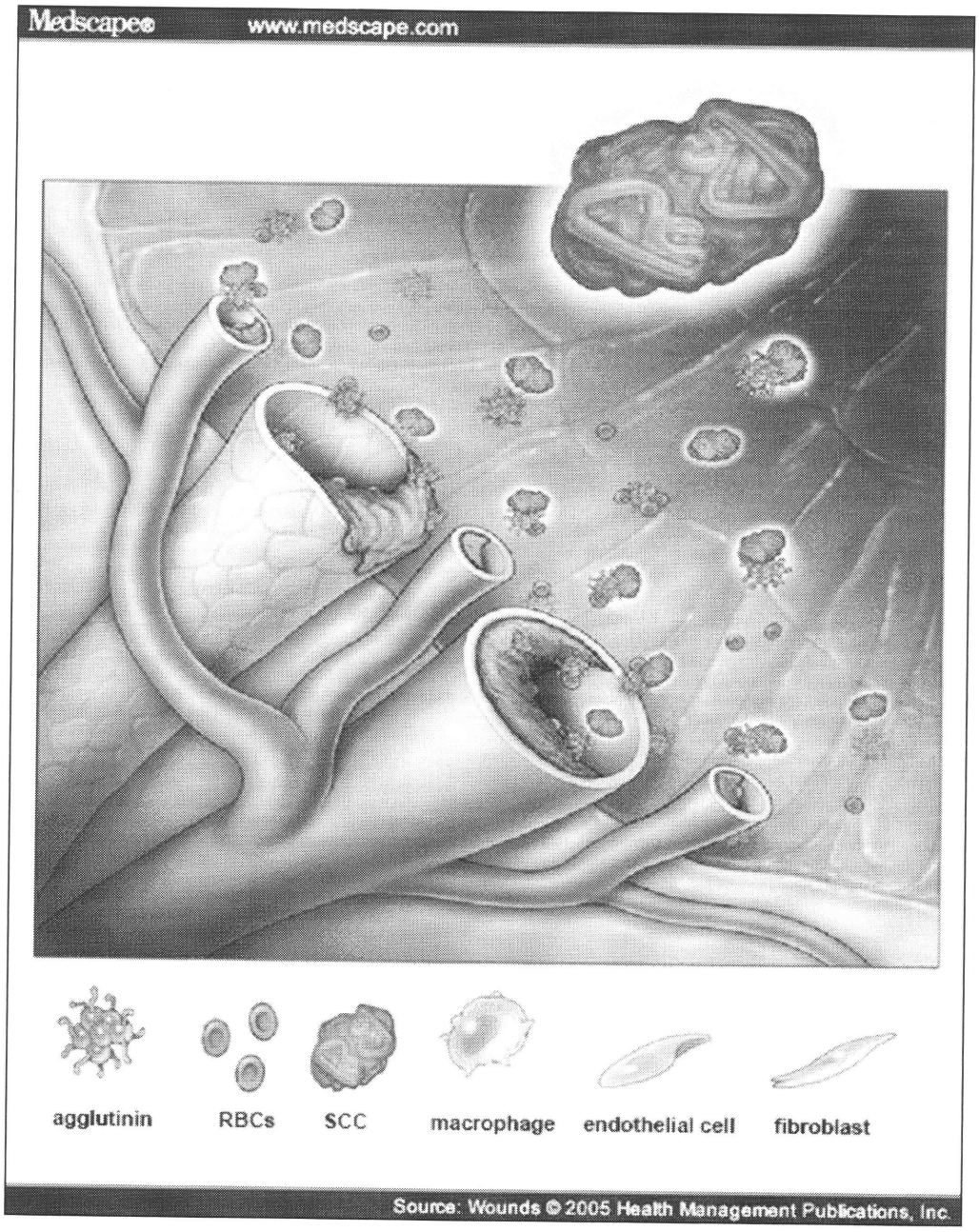

Figure 1.

As necrotic tissue is degraded via the action of papain-urea, agglutinins (depolymerized mucoproteins, mucopolysaccharides) are produced. Agglutinins cause the clumping (hemagglutination) of red blood cells (RBCs) in capillaries and attack viable cells, resulting in inflammation and necrosis. Lymphatic vessels are also blocked. Sodium copper chlorophyllin (SCC) inhibits hemagglutination and fibrin formation and protects viable cells from necrosis.

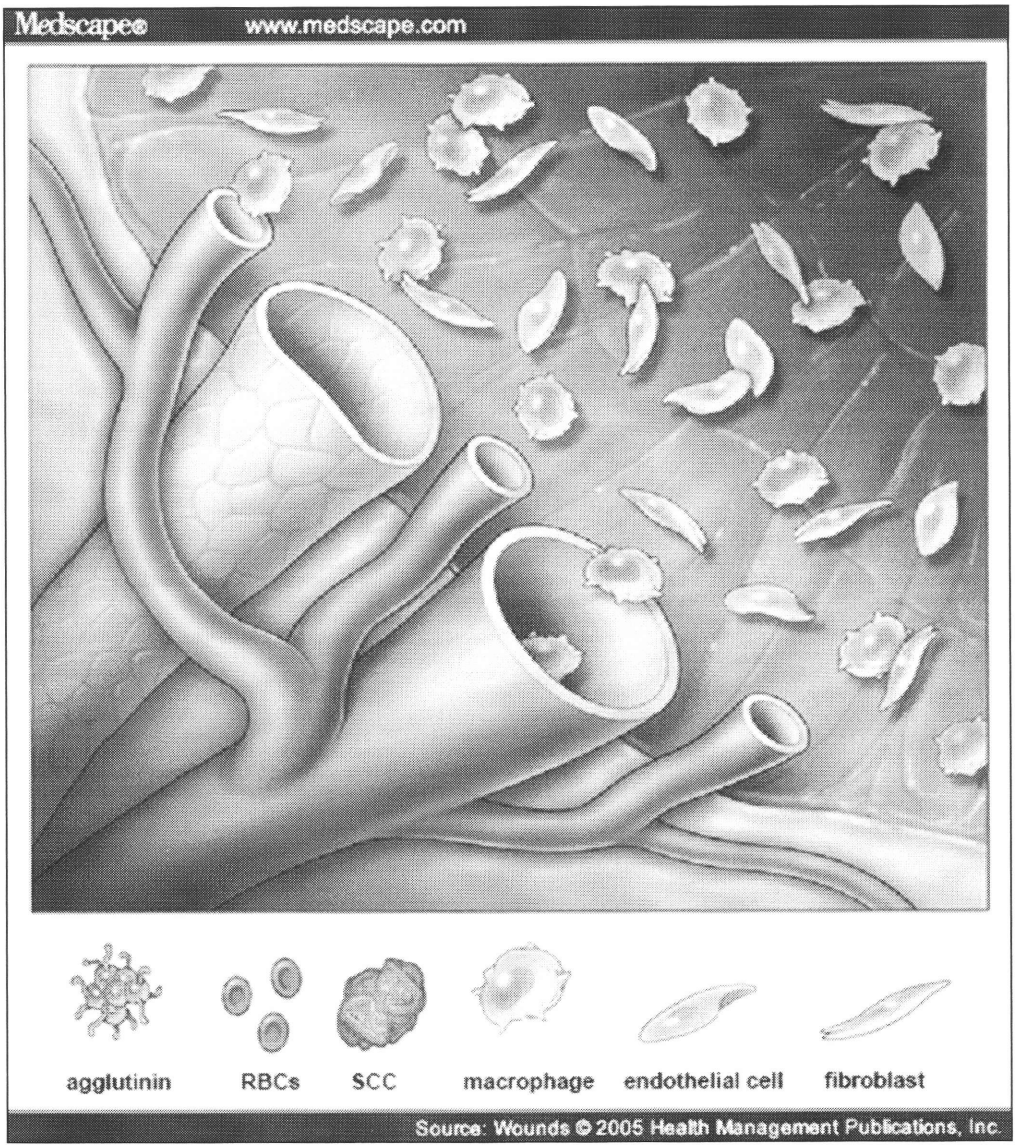

Figure 2.

Macrophages (via the expression of TNF-α and IL-1β) promote the proliferation of endothelial cells and fibroblasts at the wound site, resulting in the development of healthy granulation tissue. As fibroblasts produce various matrix proteins, cytokines, growth factors, GAGs, and endothelial cells produce capillaries, and granulation is achieved. In addition, the copper component of SCC is felt to play a role in collagen deposition/stability.

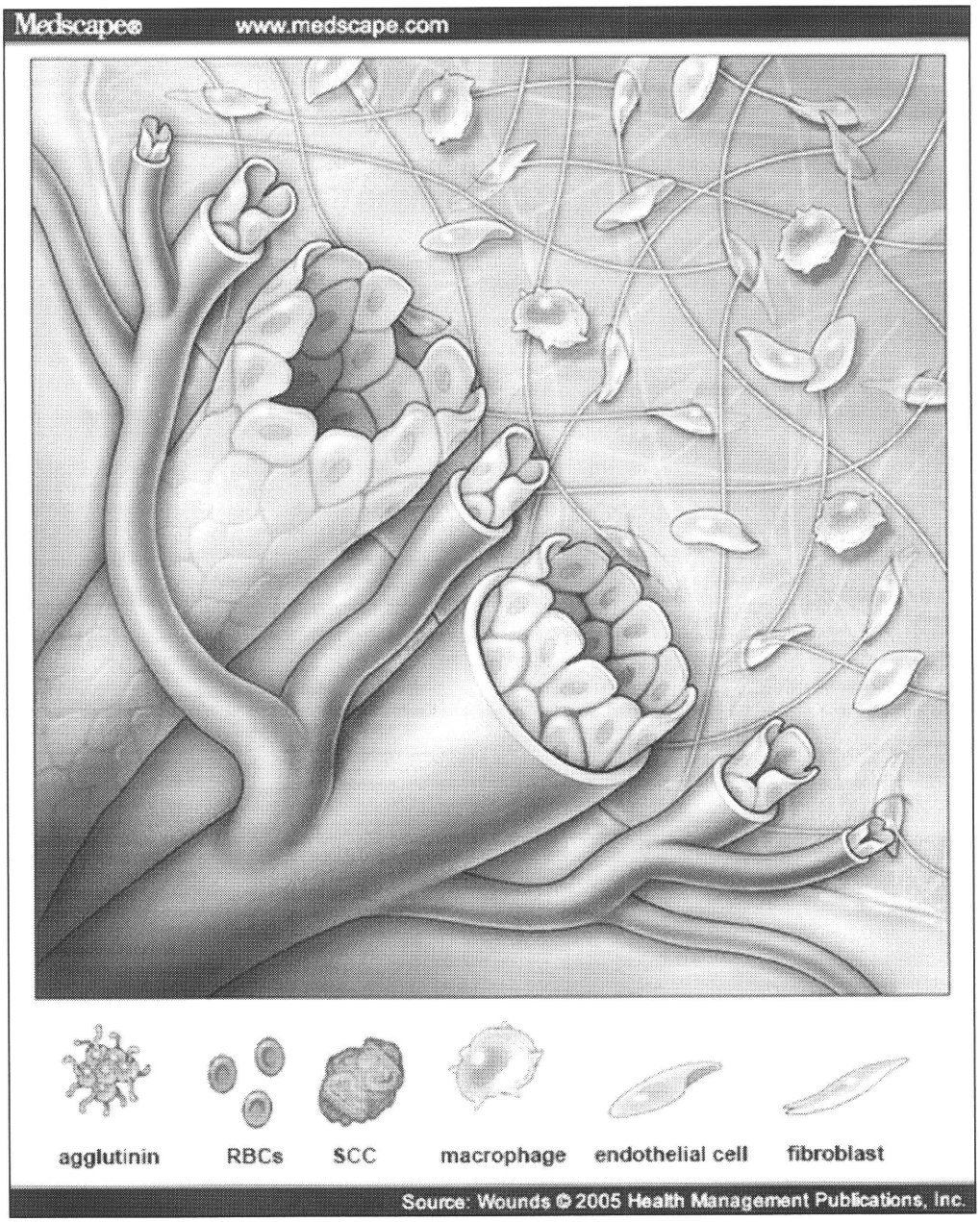

Figure 3.

As a result, the inflammatory response is diminished, resulting in a decrease in pain, swelling, and erythema. In many cases, the wound progresses onto granulation followed by closure. By inhibiting fibrin formation and hemagglutination in capillaries, macrophages are able to gain access to the wound site more easily.

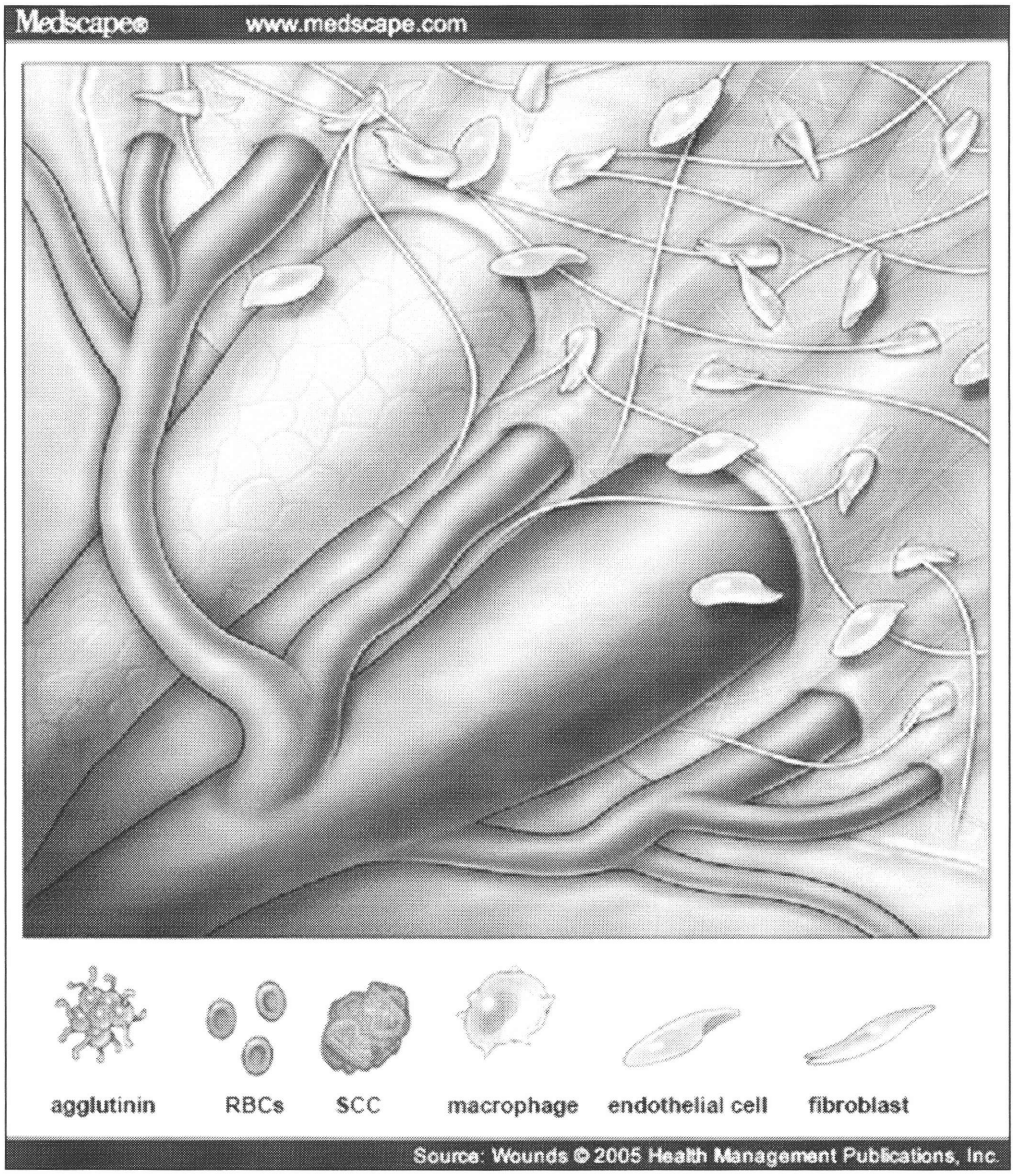

Figure 4.

Wound closure progresses with growth factors produced by fibroblasts, endothelial cells, and keratinocytes present in the wound.

Copper chlorophyllin also has the ability to control odor. Various modes of action with respect to odor control have been described in the literature. Smith demonstrated that sodium and potassium salts of chelated, water-soluble chlorophyllin possess bacteriostatic activity in varying degrees.[34] The deodorizing effects in infected wounds are largely due to chlorophyllin's effect on bacterial metabolism, as it interferes with oxidation-reduction mechanisms of anaerobic organisms. Hainer[35] described copper chlorophyllin's ability to selectively adsorb mercaptans and sulfides. However, the principle mechanism of odor control is due to the interference of bacterial metabolism and to a much lesser degree adsorption of odoriferous compounds. The latest theory on odor control involves nutritional antagonism or competition in which the antimetabolic agent tends to inhibit the action of a specific enzyme critical to the survival of the bacterium but not to the survival of the host's tissue.[27]

Conclusion

Based upon the available literature, chlorophyllin seems to play a positive role in wound repair. Smith[27] suggests that the key to chlorophyllin's benefit is the metabolic antagonism, whereby the growth pattern and activity of infecting bacteria are modified. The modification results in the lessening of the toxicity of certain bacterial metabolic products. Simultaneously, chlorophyllin promotes or stimulates normal cell proliferation, which results in accelerated wound healing. In addition, bacteriostatic (and, on a few occasions, cidal) properties are responsible for chlorophyllin's odor control properties. Adsorption of odoriferous compounds plays a relatively minor role in its odor control activity. Chlorophyllin's ability to mitigate the side effects of papain-urea is well described in the literature. Because of the potential lack of a "lag phase" just prior to the

proliferation phase of healing and the ability of macrophages to gain access to the wound site more easily (via the activity of chlorophyllin), chlorophyllin could promote and/or accelerate wound healing.

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Disclosure: The company by which the author is employed, Smith & Nephew (Largo, Fla), sells a papain-urea-chlorophyllin debridement agent.