The Science of Collagen in Wound Healing

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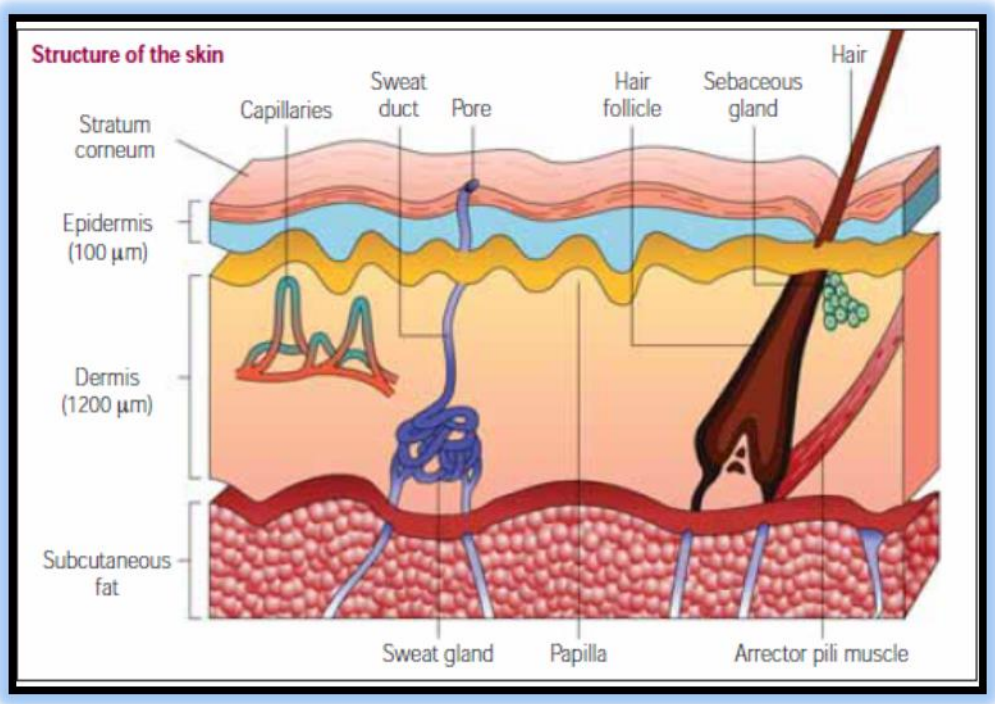
Skin serves as the barrier of the body against injuries. Skin is the largest organ and is in direct contact with the external environment. Our skin protects the body from various physical, chemical, and biological agents. Disruption of the skin structure results in injury or wound formation. Skin is a vital sensory and response organ. The ski is made up of multiple layers of cells with specific structures and functions. Healthy skin is made of the following principal layers: Epidermis, dermis, and subcutaneous tissue.



The layers of the skin differ in thickness in different tissues, but their composition and function are same throughout. Skin is metabolically active and dynamic and continuously undergoes regeneration. ***Epidermis*** is the outermost layer of the skin. It is composed of four layers of stratified squamous epithelial cells. Epidermis lacks blood vessels and hence nutrient uptake, and waste disposal is by diffusion into dermis. Layers of epidermis are as below from outermost to inside:

- *Stratum corneum* is a layer of dead keratinocytes and lipids. It forms physical barrier of skin. These cells are continuously shed and replaced by cells from underlying layer.
- *Stratum granulosum* is a layer of tightly bound keratinocytes in senescence phase.
- *Stratum spinosum* is a layer of differentiated keratinocytes, which secrete keratin. This keratin forms intact in-penetrable layer of cells.
- *Stratum basale* is a layer of actively dividing keratinocytes and is responsible for regeneration of outer layers.

Dermis is usually 3-5 mm in thickness lying below stratum basale of epidermis. Dermis is composed of connective tissue (Collagen fibrils) and elastic tissue embedded in mucopolysaccharide matrix. It consists of blood and lymph vessels, nerve endings, glands

(Sweat gland and sebaceous gland), hair follicles and attached musculature. In addition to these structures dermis also has fibroblasts, macrophages, mast cells and plasma cells. This layer nourishes and supports epidermis. It has distinct role in restricting the invasion of pathogen in case, epidermis is ruptured. Dermis is crucial site in wound healing due to presence of all necessary functional components.

Subcutaneous layer is a bridge connecting dermis to underlying body components. It consists of adipose tissue and responsible for insulation and protection from mechanical damage to the structures below. It is a source of high energy fat molecules. This is the layer through which the blood vessels and nerves reach dermis. Integrity of skin structure is pre-requisite for its function. In case of injury, skin is involved in healing along with its normal functions.

Acute, Chronic, and Hard-to-heal Wounds

As skin is the first defense barrier, it is also most susceptible organ for injury being directly in contact with external elements. In the open wounds, skin is compromised, and underlying tissue may be exposed. In closed wounds though skin is intact, the internal tissue is damaged. Open skin wounds are characterized as acute wounds and chronic wounds depending on their healing progress. Wounds can also be simply differentiated as wounds with tissue loss (Ex. burn wounds, wounds after harvesting of graft) and the ones without tissue loss (Ex. surgical wounds).

Acute wounds are caused by surgical incision or accidental traumas. When the skin and underlying tissues are subjected to intolerable forces, traumatic wounds resulted. Open skin acute wound is categorized as abrasions, avulsions, contusions, crush wounds, cuts, laceration, puncture etc. depending upon characteristics of the injury. Burn wounds are also characterized as acute wounds. Thermal injury causes cellular dysfunction and denaturation of proteins. Burn wounds are not uniform in depth. The area closest to source of injury is affected most and severity reduces radically outwards. These wounds are prone to infection and demand efficient and rapid treatment. A surgical wound is either incision or suture made by the surgeon during surgical operation. Wound breaks the integrity of skin including epidermis and dermis.

Chronic wounds are the ones which take prolonged duration for healing. Such wounds may take months or even years to heal completely. Chronic wounds are characterized by prolonged inflammatory phase, slow forming ECM (Extracellular Matrix) and a decreased rate of epithelialization. These wounds result from prolonged illness or metabolic disorders. Some of the causes of chronic wounds include diabetes, vascular disease, infection, Immobility, trauma, surgery, radiation injury etc. In case of diabetes, high glucose concentration in blood leads to host of metabolic problems. It interferes with blood flow and cause peripheral neuropathy. Absence of some of the important ECM component may be the reason for chronic nature of this kind of wound.

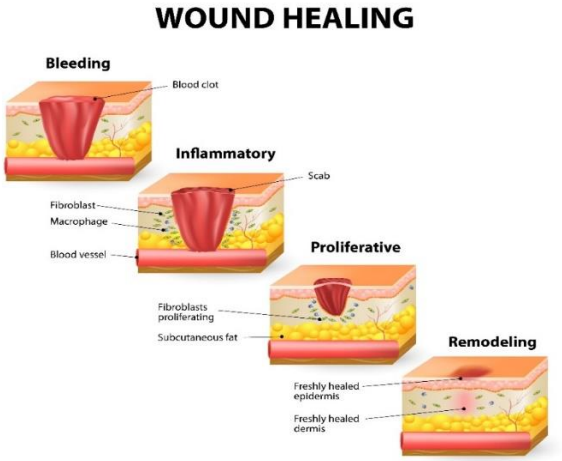
Hard-to-heal wounds are acute or chronic wounds that have failed to heal in a timely and orderly fashion despite standard wound care. Utilizing a systematic approach to wound hygiene has been shown to promote and support these wounds towards wound healing trajectory.

Collagen is one of the body's key natural resources and a component of skin tissue that can benefit all stages of the wound healing process. Collagen is the most prevalent protein in the human body. There are at least 28 identified types of collagen, each with its own unique molecular form and type specific purposes. Collagen, in its proper molecular form specific to skin tissue with self-assembling capabilities, has benefited human skin tissue healing.

The living body is like a complicated computer software program. Whenever there is injury to the skin tissue, the body is programmed to respond to such a wound with a series of complex sequential cellular and vascular activities. Regardless of size or type, all wounds should follow the same cascade of healing. Scientific research typically refers to wound healing as a series of overlapping phases in a timely and orderly fashion. While the body's complex healing mechanisms occur simultaneously, individual areas of a wound may respond at different rates. This can be due to underlying disease, comorbidities, and complications.

The sequential wound healing activity stages are ***hemostasis*** *bleeding/injury*, ***inflammatory***, when the wound bed must maintain bacterial balance; followed ***proliferative***, when tissue granulation and angiogenesis occur to fill wound bed; and finally, the ***remodeling*** stage, where new tissue is strengthened.

A thorough understanding of the factors affecting wound healing is essential in achieving wound closure. This article will examine some of key elements involved in wound healing, and how hard-to-heal wounds must be redirected to the normal healing path.



Collagen Properties

For many years, the scientific community has identified collagen as the common element in wound healing. Research indicates that collagen plays an important role in the body's natural healing response. Collagen is a protein with unique chemistry and specific function. In the proper form, collagen has many responsibilities in the body including cellular activity and providing an organized matrix in skin. Practical knowledge in understanding the

collagen's chemical, physical, and biological properties will help clinicians recognize the advantages in utilizing collagen-based products in wound care.

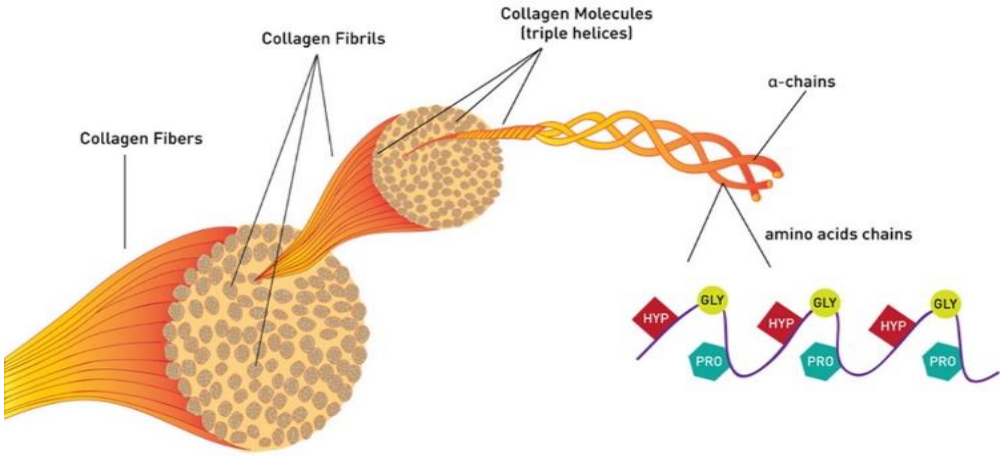
Therapeutic Facts about Collagen

- ✓ Most abundant protein in human body - 25% of total protein content is collagen.
- ✓ Collagen constitutes 60% of body's dry weight.
- ✓ Collagen provides strength and structure to tissues.
- ✓ Collagen is a key component of connective tissue.
- ✓ Collagen is highly versatile, absorbable, supports cell and tissue regeneration, and remodeling.
- ✓ Type I Collagen from bovine skin is most well studied and characterized.
- ✓ Collagen can be obtained from many sources including bovine (cattle), equine (horse), porcine (swine), avian (bird), ovine (sheep), and Piscean (fish).

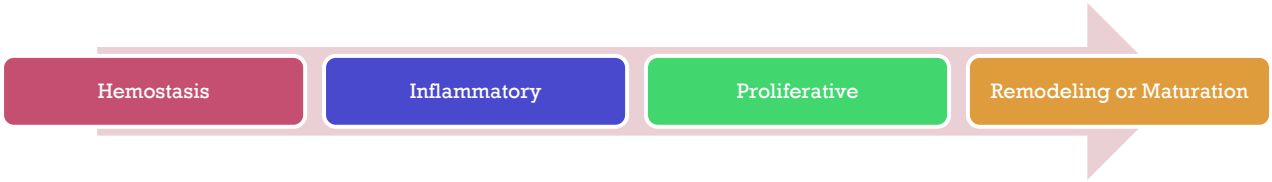
Collagen in the Proper Formulation

→Molecules self-assemble into fibers

- ✓ Chemistry consists of helical and nonhelical domains.
- ✓ Molecules must be complete to provide appropriate biological response.
- ✓ Self-assembled molecules form fibers and aggregates.
- ✓ Intact fibers cause cell growth, activity, and migration.
- ✓ Aggregates have specific binding sites for cells and cell binding proteins in the body.
- ✓ Functional aggregates result in strong, healthy tissues.
- ✓ Distribution and orientation reflect tissue function.



Collagen's Role in Wound Healing



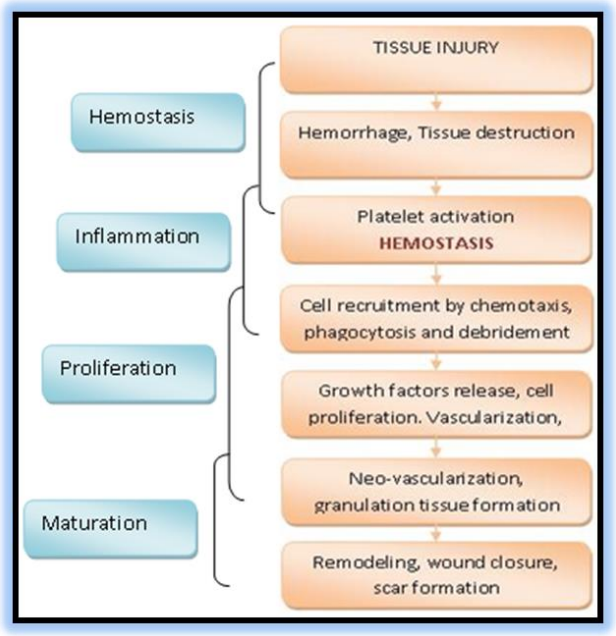


Figure above: The figure above depicts steps involved in wound healing. Wound healing starts with hemostasis in which, platelets along with ECM factors form blood clot and prevent hemorrhage. In the next step inflammation, body mounts defense response against possible invading pathogens. It involves various leukocytes and protein factors. In proliferative phase cells responsible for regeneration of lost tissue (Fibroblasts, endothelial cells, keratinocytes) undergo activation and proliferation and restoration of the tissue. In last step the scar formation takes place and wound closure takes place.

Hemostasis Phase

Hemostasis is particularly important in surgical wounds as copious blood loss affects mortality of patient. Basic measures for hemostasis include direct pressure application, sutures, staples, gauzes, sponges etc. In addition to this, thermal and chemical treatments are also routinely followed for this purpose. Many biological polymers like fibrin, cellulose, gelatin is also used to prepare wound treatment products. These molecules act by both physical and biological mechanisms and help in healing the wound. Use of these materials ensures maintenance of moist environment hence continuous exposure of wound to proteases, chemotactic factors, and plasma proteins. These agents give improved results than traditional methods. One of the most effective agents used for topical application is Collagen. Bovine collagen is formulated in different forms and exclusively used for wound management.

- ✓ Collagen actively participates in wound healing. It also acts as topical hemostatic agent hence reducing blood loss.

- ✓ Collagen binds to specific receptor sites on platelet membranes which swell and release substances to initiate hemostasis.
- ✓ Collagen binds fibronectin, causing platelet activation, adhesion, and aggregation.
- ✓ Activating cofactors for thrombosis.

Inflammatory Phase

Open wounds are exposed to environmental factors and hence are frequently contaminated with bacteria. Many times, they heal without complications but in some cases when balance cannot be maintained between host and pathogens, infection is established, and healing is delayed. Higher bacterial load inflicts higher metabolic products and deplete wound oxygen tension retarding the healing process. Inflammatory response defends the tissue from pathogens and cleans debris. Inflammation is characterized by classical symptoms which are: Pain, rise in temperature), redness, swelling and loss of function. Swelling during inflammation is a result of accumulation of vascular fluid at the site of injury. This occurs because of vasodilation which occurs immediately after initial vasoconstriction. Endothelial products and products of mast cells (leukotrienes, prostaglandins) mediate vasodilation. It also results from appearance of gaps between endothelial cells lining capillaries and hence causes leakage of plasma. Components of complement system also contribute to this process and act as chemo attractants for cellular mediators of inflammation.

- ✓ Collagen is chemotactic to monocytes and leukocytes.
- ✓ Monocytes transform into macrophages which scavenge and phagocytize foreign bodies and debris.
- ✓ Attracting plasma components.
- ✓ Removal of bacteria and debris by phagocytosis and adsorption.

Leukocyte migration to the wound area is directed by complement factors (C3a, C5a), ECM breakdown products, TGF β, TNF α (Tumor necrotic factor), IL1(Interleukin), PDGF etc. Some of these factors are released by degranulation of platelets followed by their activation by collagen. After reaching the tissue site, neutrophils come out of circulation/vessel and enter the wound matrix. Monocytes also traverse capillary wall and arrive at matrix. Together fibronectin, elastin, complement factors, thrombin, TGFβ cause activation of monocytes into macrophages. Neutrophils and macrophages are cells of immune system and can detect foreign cells and particles, attach it, phagocytose them and ultimately digest them. Neutrophils loaded with cell debris are also digested by macrophages.

Inflammation is regulated by many chemical factors. This regulation is of utmost importance to achieve healthy state. Lack of adequate response can cause setting of infection whereas if prolonged it can cause chronic inflammation and massive tissue destruction. Histamine is a chemical secreted by mast cells at the onset of inflammation for vasodilation. Kinins from blood plasma perform similar function. Prostaglandins to some extent increase permeability of blood vessels and attract leukocytes. Components of complement system exist in inactive state in blood plasma. Upon activation, they signal leukocytes for inflammation. As mentioned earlier the cellular response in further stages of inflammation is brought about by growth factors secreted by platelets, macrophages.

Macrophages are important cellular mediators of inflammation. Their function is not limited to immunity. Macrophages release many metalloproteases which digest ECM components and initiate granulation tissue formation. Upon activation and phagocytosis, macrophages release cytokines and chemokines. Macrophage-produced cytokines are involved in angiogenesis, fibroblast migration and proliferation, collagen production, keratinocytes activation and migration and in turn wound contraction. TGF β is important cytokine released by macrophages. Its release is controlled in autocrine manner by these cells. TGF β stimulates release of PDGF, FGF-2, TNF α and IL1 attracting and activating many cell types involved in later stages of wound healing as shown in the figure. Macrophages also secrete nitric oxide which acts as chemical signal as well as antibacterial agent. Presence of activated macrophages marks beginning of proliferation phase of wound healing.

Proliferative Phase

Successful wound healing is indicated by initiation of proliferation phase after inflammation. It is characterized by formation of granulation tissue. Granulation tissue is composed of many mesenchymal and non-mesenchymal cells with distinct phenotypes and an extensive neo-vasculature embedded within the loosely assembled matrix composed of collagens, fibronectin, and proteoglycans. In the wounds with loss of tissue (Burn wound, skin graft harvest) it is very important to restore the lost tissue mass as well as lost ECM. This function is achieved in proliferation phase wherein the cellular multiplication and secretion of ECM components occur. At this phase though macrophages are present, their role shifts more towards secretion of signaling molecules. These molecules mediate fibroplasia, epithelialization, and angiogenesis during this phase.

PDGF, TGFβ, EGF and fibronectin secreted by platelets and macrophages and low oxygen tension in wound stimulate fibroblast migration from adjacent tissue to injury site. These factors along with IGF (Insulin like growth factor) stimulate proliferation of fibroblasts. These cells secrete many metalloproteases (MMP1, MMP2 and MMP3) for reorganization of provisional matrix. TGFβ is important growth factor affecting fibroblasts in this phase. It controls secretion of proteases by fibroblasts. It boosts transcription of matrix proteins (Collagen, fibronectin, proteoglycans) by fibroblasts, thus new matrix is established. This structure is a pool of growth factors needed by subsequent stages. During this phase, collagen provides bridge for epithelial cells to cross the wound surface. Also stimulates cellular differentiation and establishment of dermal-epidermal junctions. To regain the original structure of skin *epithelialization* is important. It also sets up physical barrier. Main effecter cells for this function are keratinocytes and epidermal stem cells from neighboring area. These cells migrate to the wound site due to many factors like extracellular matrix, integrin receptors, collagen I, matrix metalloproteases (MMPs), and growth factors: EGF, TGFα, KGF (Keratinocytes growth factor). They arrange over the wound surface until monolayer is formed by contact inhibition; following this they terminally differentiate into stratified epithelial cells. These cells attach to underlying stroma and establish new basement membrane. Collagen I stimulate production of MMP1 (Collagenase 1) by keratinocytes. Cleavage of dermal collagen provides keratinocytes with mechanism to maintain their directionality during re-epithelialization. This establishes stroma for further epithelialization.

At this stage, the cells in wound site are highly active and consume high oxygen; in addition, it has high lactate content, low pH, and low oxygen tension. This stimulates formation of blood vessels *i.e., angiogenesis* in granulation tissue. Endothelial cells are principal effecter cells for this. Endothelial sprouts from capillaries at the margin of wound extend inside. These cells form capillary structure through cell migration and proliferation. As the sprouts from different direction converge, they fuse with each other and form capillary network. Endothelial cells secrete MMPs for degrading matrix collagen for easy movement. This is regulated by two important cytokines - bFGF(basic fibroblast growth factor) and VEGF (Vascular endothelial growth factor). At this stage, the necessary structures of skin are present although they need to undergo maturation to achieve complete healing.

- ✓ Establishes new tissue and angiogenesis (new blood vessels).
- ✓ Collagen attracts monocytes which transform into macrophages. Macrophages release substances that result in fibroplasia and angiogenesis.
- ✓ Collagen provides support for the growth of new capillaries. The presence of new capillaries is essential for the deposition of new fibers.
- ✓ Creating extracellular matrix (ECM) structure for new tissue growth.
- ✓ Collagen binds with fibronectin, which promotes cell binding and fibrillogenic influences, fibril dimensions, and stimulates fibroblast proliferation and migration.
- ✓ Collagen is chemotactic to fibroblasts, which govern the restoration of new tissue by depositing oriented and organized fibers.

Remodeling or Maturation Phase

As healing progresses, dermis and epidermis start establishing and dermo-epidermal junctions are formed. Along with it, keratinocytes continue epithelialization and wound contraction begins. Many of the granulation tissue cells undergo apoptosis and provisional ECM is dissolved. Macrophages continue phagocytosis and debridement necessary for the inward migration of the vasculature and mesenchymal cells deeper into the wound. Fibroblasts secrete collagen under chemotactic regulation of TGFβ, PDGF, and EGF. Pro collagen undergoes post-translational modification in the extracellular environment to form tropocollagen. This in turn forms fibrils in tendons by self-association and cross linking. Collagen makes up of 50 % of the scar tissue. It is present in combination of type I and type III collagen. Fibroblasts transform into myofibroblasts due to mechano-tension which corresponds to the commencement of connective-tissue compaction and the contraction of the wound. The contraction is thought to be stimulated by TGFβ1 orβ2 and PDGF, attachment of fibroblasts to the collagen matrix through integrin receptors, and cross-links between individual bundles of collagen. As the scar matures, collagen fibers are arranged along the skin stresses. Though contraction of wound is important for its closure, sometimes it can compromise the mobility and function of affected area. Remodeling of the wound can continue for several months; although it cannot achieve quality of undamaged tissue, it gradually strengthens and restores its functions.

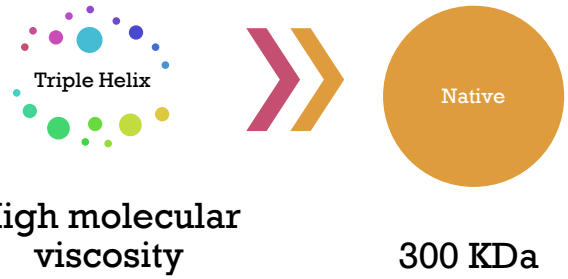
- ✓ Collagen directly supports the growth, attachment, differentiation, and migration of keratinocytes.

- ✓ By binding with fibronectin, collagen provides a provisional matrix for keratinocyte migration.
- ✓ Collagen reduces scarring by depositing oriented and organized fibers and by regulating the amount of collagenase expressed by keratinocytes.
- ✓ Acts as a matrix for epithelial cells to spread across the wound surface called epithelialization.

Triple Helical Collagen: The Native Active Component

Collagen is one of the most critical components of the body and found in connective tissue derived from the basement membrane of organs including hard and bony structures, collagen serves different functions. Nearly 28 types of collagen have been identified, but collagen type I is the most common in skin, bone, teeth, tendon, ligaments, vascular ligature, and organs. The main source of Type I native collagen extraction is in bovine due to the availability, as well as biocompatibility. The extractions can take place in various tissues including bones, tendons, lung tissue, or connective tissue.

From wound healing to lending structural support to skin and bone and expansion of arterial network, collagen plays an important role in the sustenance of life. In skin, collagen offers continuity while in bone, the reinforcement for the mineral deposited bone building units is provided by collagen. In blood vessels, collagen forms a stiff layer offering maximum expansion while providing protection from injuries. A comparison of this process in the physical world will be that of the steel radials wrapped by rubber sheets as in the case of automobile tires.



Usually, collagen-based products are made from 100% pure bovine collagen that is non-hydrolyzed and non-denatured. It maintains the chemical and structural integrity of original bovine collagen which is a triple helical structure. Collagen has efficacy rate of approximately 95% through wound closure and about 50% decrease in healing time than traditional methods. These materials mainly consist of type I collagen which is structural protein of ECM.

The Collagen Scaffolding

The collagen scaffolds used in wound treatment bind to fibroblasts via integrins ligands and hence blocks generation of microscopic contractile forces deployed to contract wound in injured region. Blocking of contraction is necessary for regeneration of adult tissue at the site of injury. Three-dimensional microenvironment of the collagen scaffold was found to up regulate several genes related to matrix remodeling and angiogenesis as compared with standard dressing methods. The adult wound contains large, parallel collagen bundles that are oriented perpendicular to the wound surface. Collagen scaffolds reduce scarring by depositing oriented and organized fibers and by regulating the amount of collagenase expressed by fibroblasts. If external collagen contains more fibronectin sites; increased (in vitro) fibronectin binding stimulates fibroblast migration and hence less time for healing. This may be due to chemotactic nature of collagen degradation products. Both the 3D scaffold and chemotactic factors likely assist more rapid migration of epithelial cells.

Thus, collagen absorbent pads provide lucrative means for preventing operative and/or post-operative hemorrhage. Microfibrillar collagen products are made by purifying bovine collagen and processing it into micro-crystals which can then be manipulated into hemostatic agents in a wide variety of formats. These collagen products are available in various formats and hence can be chosen according to the intended use.

Collagen has density lower than water, is superabsorbent and tough due to its unique molecular structure. It is active hemostatic agent and helps the body to heal itself. Use of collagen pads stops bleeding, removes fluids which are a source of infection by absorption and forms better quality tissue. As collagen absorbs fluid, it swells to fill the spaces in wound which would otherwise store pus and become infected. It has low antigenic potential and is poor media for microbial propagation, hence remains in contact with wounds for prolonged periods causing hemostatic, spatial, mechanical, nutritional, and chemotactic effects. Other properties of collagen making it ideal material for preparation of wound healing scaffold are mentioned below.

- ✓ Collagen constitutes more than 30% of dry weight vertebrates, thus available in abundance and easily purified from living organisms.
- ✓ Bovine collagen invokes immunologic reaction in minority of the population. For majority of the individuals, it is non-immunogenic, compatible with the body's immune system.
- ✓ Collagen being non-toxic and biocompatible can be formulated in several different forms. This is also supported by its synergistic action with other bioactive components.
- ✓ It is biodegradable and bioabsorbable. Biodegradability of collagen can be controlled by the cross linking in the higher molecular structures. It is also considered as source for bio-plastic due to high tensile strength and minimum expressibility. Structure of collagen is such that it has many functional groups which can be easily modified to produce desirable materials.

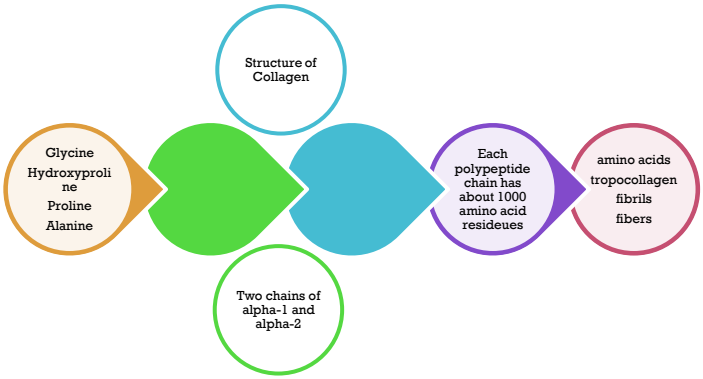
Native Collagen Properties

Native collagen properties are very different than hydrolyzed collagen. Native collagen properties of molecular weight (~100-300 KDa, isoelectric point (pI)(7.0-8.3), viscosity (high)and film formation is most commonly used in various industries due to excellent biocompatibility and biodegradability, low immunogenicity, and high versatility to fabricate films.

Molecular Genetics of Human Collagen: Cellular Expression of Collagen Protein

The turnover of biological collagen is a dynamic process. As mentioned earlier in this monograph, collagen is essential for structural integrity, proliferation of cells, controlling vessel bleeding, formation of new blood vessels, healing, and remodeling of injured areas etc. All types of collagen are translated by the RER (Rough Endoplasmic Reticulum) compartment and further processed inside the Golgi network in the cells to produce the procollagen inside the intracellular space. However, subtle differences occur at the downstream propeptide processing and subsequent intermolecular bonding patterns to achieve the differences in functional specificities of large number of collagen types.

In the case of collagen Type I, for example, because of translation, two separate peptide chains are formed corresponding to alpha-1 and alpha-2 designations. In the subsequent steps, these chains are translocated to the Golgi compartment for further processing where posttranslational modifications occur to derive distinct features along with processing of signal peptides. These polypeptides undergo further processing and with eventual elimination of signal peptide portions for mature type I collagen fibrils. The defining downstream events in creating a triple helical identity occur within the ER luminal space where hydroxylation of proline and lysine occur with the help of a set of enzymes and cofactors where prolyl hydroxylase and ascorbic acids play a major role.



Structure of Collagen

More than 30 types of collagen types are found in vertebrates which vary according to their location and structure. The type I collagen is most abundant and is exclusively used for bio-scaffold preparation. It makes up of 96% of dermal collagen. Structure of collagen enables it to act as scaffolding molecule in the ECM. The structure of the molecule also gives it vital characteristics such as thermal stability, mechanical strength, and the ability to engage in specific interactions with other biomolecules.

Collagen is made up of three amino acid chains. These polypeptides called pro-collagen are synthesized within cell and then secreted outside. These polypeptides are then cleaved extracellularly and self-associate into primary collagen molecule called 'tropo-collagen'. Tropo-collagen is rod shaped triple helix over its entire length. It is formed by self-association of 2 identical α1 (I) chains and one α2 (I) chain. These chains contain approximately 1040 amino acid residues with Glycine at every third place. Apart from glycine, proline and hydroxy proline are the second abundant amino acids in collagen. Thus, primary structure of this protein is repeating tripeptide Gly-Xxx-Yyy. Presence of glycine at every third place allows close packaging of chains in the helix and rigid proline molecules prohibit rotation about peptide bond. Overall collagen tertiary structure is stabilized by water bridges, hydrogen bonds and electrostatic and hydrophobic interaction among its amino acid side chains. Collagen type I is a glycoprotein with less than 1% carbohydrate content. The sugar components are either a single galactose unit or a disaccharide of galactose and glucose O-glycosidically attached via hydroxylysine residues. The tropocollagen molecules align along the helix axis and form bundle called ‘collagen fibrils. Collagen fibers in the ligaments are formed by bundling of collagen fibrils in hierarchical structures.

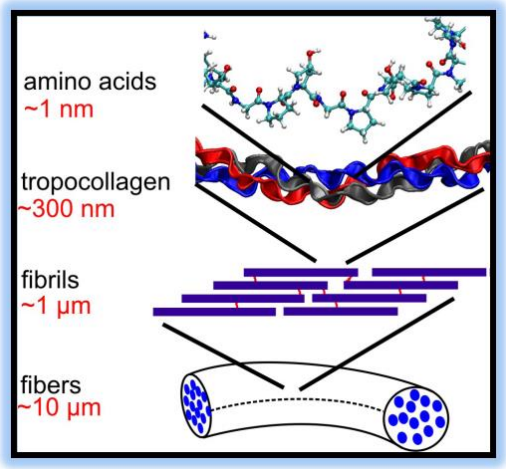


Figure: Primary structure of collagen is made up of two α1 polypeptides and one α2 chain. This These three peptides self-associate and form 'tropo-collagen' which is a rod-shaped molecule. Tropo-collagen is then arranged lengthwise to form fibrils. The fibrils in turn associate longitudinally and make up collagen fibers in the extracellular matrix.

Wound Management Product Algorithm
Medifil® II Collagen Particles & Skin Temp® II Collagen Sheets

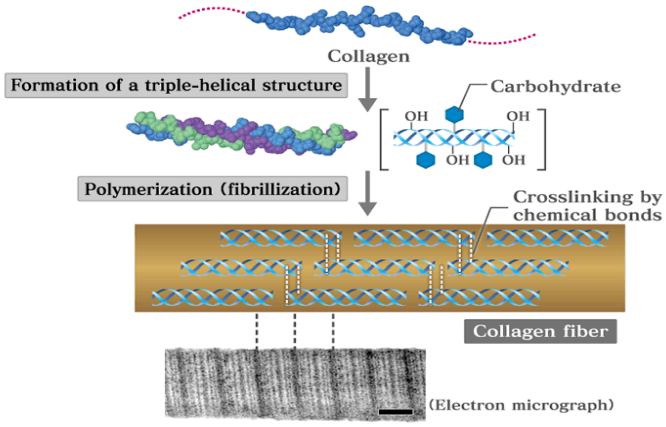
**Hard-to-heal wounds are acute or chronic wounds that have failed to heal in a timely and orderly fashion using standard of care.*

Wound Classifications by Depth	Kollagen™ Product	Secondary Dressing	Frequency of Dressing Change
Minimal exudate Superficial thickness Partial thickness Full thickness	Medifil II particles Skin Temp II sheet	Non-adherent gauze, hydrocolloid, transparent film, and or composite.	Daily up to 3 days
Moderate exudate Partial thickness Full thickness	Medifil II particles Skin Temp II sheet	Non-bordered or bordered foam dressing, and or specialty absorptive dressings.	Daily up to every other day
Heavy exudate Partial thickness Full thickness	Medifil II particles Skin Temp II sheet	Non-bordered or bordered foam dressing, and or specialty absorptive dressings.	Daily up to every other day
Tunneling, Undermining Epibole	Medifil II particles Skin Temp II sheet	Non-bordered or bordered foam dressing, and or specialty absorptive dressings.	Daily up to every other day

Human BioScience's Kollagen™ Technology Advantage

Kollagen technology by HBS is one of the most efficient modalities for complex wound management. It mimics the body's own collagen and actively participates in healing. Bovine collagen products from HBS India Ltd play an active role in all stages of wound healing. Kollagen technology preserves Type-1 collagen's native structure during manufacturing processes. As stated earlier, the collagen being triple helical, begins blood coagulation cascade. Reduce the duration of inflammatory phase, helps in proliferation of cells to establish the new tissue and result in minimum to no scar.

- Collagen molecule consists of three amino acid chains with characteristic Gly-X-Y repeat units and arranged in triple helical form. These helical chains entwine to form super helix.
- Non-helical end regions of the molecule form cross links with other collagen molecules.
- Collagen fibrils arrange in bundles to form collagen fibers. Arrangement of these fibers is specific to tissue function, Ex. In skin the collagen fibers provide support and structural integrity.
- HBS' Kollagen technology conserve all the properties of human type 1 collagen and provides most effective biomaterial for efficient and quick wound healing.



Collagen has a specific chemistry and structure. When creating a collagen product, the most important task is to maintain that chemistry and structure. Many products in the market claim to be collagen, and while they may start with Type I collagen, their processing alters the molecular structure to the point where, under a microscope, it is no longer identifiable as true collagen. The collagen supplied in just fiber form cannot provide desired environment for various essential cell types to bind and proliferate. Collagen supplied in fiber form may attract various important cells essential for effective wound healing; but fiber form cannot provide optimum "wound bed" required for these cells to migrate and grow. These products do not have essential pore structure to facilitate cell adhesion and proliferation. When a product lists "denatured" or "hydrolyzed" collagen as one of its ingredients, it means the structure has been changed to the point where it fails to meet the scientifically accepted definition of collagen.

These products exhibit limited biological responses that aid in wound healing. The term "biological response" is an important distinction. Kollagen products maintain the chemistry and structure of collagen, and most importantly, Kollagen elicit a biological response necessary to promote wound healing.

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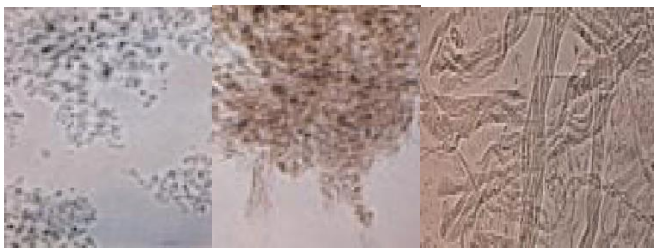
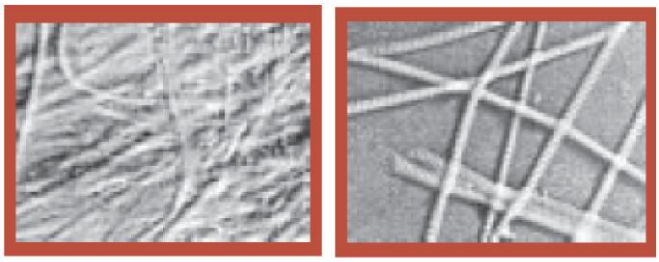


Image 1&2: Denatured or hydrolyzed collagen fibers lacking non-helical domains and forma of disorganized fibrils. It shows absence of ordered structure.

Image 3: Ordered structure of collagen triple helix. In this structure fibers self-assemble into aggregates which resemble rope-like structures.

The pore structure of these scaffolds significantly affects cellular activity. Very small pore size itself act as physical barrier and does not allow cell penetration. This reduces the efficacy and desired activity of the dressing. If the pore size is too big, the cell density in the scaffold remains low and molecular mechanisms for the healing are slowed down. HBS' Kollagen technology maintains optimum pore size inside the Type-1 collagen matrix which not only helps in attracting several cell types essential for wound healing but also provides ideal three-dimensional scaffolds for these cells to attach and grow. The scanning electron micrographs below show that in HBS Skin Temp® II, the fibril structure of collagen is preserved which is identical to human skin. The images below show that even after going through manufacturing process, the fibril structure is preserved in Skin Temp® II.



Kollagen™ (Skin Temp^{II}): Magnification 10.000x

Collagen: Magnification 33,000x

Conclusion

Collagen dressings are not all the same. When selecting an advanced wound care collagen dressing consider the type and source of collagen, manufacturing process, cost-effectiveness, user friendliness and bioactive technology of the dressing. A complete wound assessment is imperative in the clinical rationale and justification of appropriate dressing usage. Healthcare clinician should be familiar with facility formulary, dressing categories, indications, and contraindications. Dressing change frequency is based on the wound assessment, physician order, and dressing manufacturer guidelines. Most collagen dressings require a secondary dressing.

Glossary of Terms

Angiogenesis: The process by which new blood vessels are formed.

Hard-to-heal Wounds: Acute or chronic wounds that take longer than normal to heal despite standard of care in a timely and orderly fashion.

Edema: The presence of an abnormally large amount of fluid in the, intercellular tissue spaces of the body.

Fibroblasts: A connective tissue cell. They differentiate into chondroblasts, collagen oblasts, and osteoblasts, form the fibrous tissues in the body, ten dons, aponeuroses, supporting and binding tissues of all sorts.

Full thickness wound: A full thickness wound indicates that damage extends below the epidermis and dermis (all layers of the skin) into the subcutaneous tissue or beyond (into muscle, bone, tendons, etc.).

Granulation Tissue: Small, beadlike masses of red/pink tissue formed in wounds.

Infection: Invasion and multiplication of microorganisms in body tissues.

Inflammation: A localized protective response elicited by injury or destruction of tissues which serves to destroy, dilute, or wall off both the injurious agent and the injured tissue.

Keratinocyte: The epidermal cell which synthesizes keratin; constitutes 95 percent of the epidermal cells and with melanocyte, forms the binary cell system of the epidermis.

Leukocyte: White blood corpuscles which act as scavengers, helping to combat infection.

Macrophages: Any of the many mononuclear phagocytes found in tissues.

Monocyte: A mononuclear phagocytic leukocyte.

Moist Wound Healing: To maintain a moist environment at the wound bed to enhance epidermal cell migration and encourage epithelialization.

Necrosis: Slough or eschar, devitalized tissue, or also referred to non-viable tissue.

Neovascularization: New blood vessel formation in abnormal tis sue or in abnormal positions.

Neutrophils: Granular leukocytes that have a nucleus with three to five lobes. They have the properties of chemotaxis, adherence to immune complex es, and phagocytosis.

Partial thickness wound: Partial thickness wounds are wounds that extend only into the first two layers of skin, which are the dermis and epidermis.

Platelets: A disk-shaped structure, found in the blood of all mammals and chiefly known for its role in blood coagulation.

Superficial thickness wound: Superficial thickness wounds involve loss of the epidermis layer only.

References

1. Fu Y., Young J.F., Løkke M.M., Lametsch R., Aluko R.E., Therkildsen M. Revalorisation of bovine collagen as a potential precursor of angiotensin I-converting enzyme (ACE) inhibitory peptides based on. in silico and in vitro protein digestions. J. Funct. Foods. 2016;24:196–206. doi: 10.1016/j.jff.2016.03.026
2. Mass spectrometric analysis of enzymatic digestion of denatured collagen for identification of collagen type.*Zhang G, Sun A, Li W, Liu T, Su Z Chromatogr A. 2006 May 12; 1114(2):274-7.*
3. León-López A, Morales-Peñaloza A, Martínez-Juárez VM, Vargas-Torres A, Zeugolis DI, Aguirre-Álvarez G. Hydrolyzed Collagen-Sources and Applications. *Molecules*. 2019;24(22):4031. Published 2019 Nov 7. doi:10.3390/molecules24224031
4. Adzick NS, Lorenz HP Cell matrix, growth factors and the surgeon. The biology of fetal wound repair *Annls of surg.* 1994; **220(1)**:10-18
5. Atiyeh BS, Ioannovich J, Al-Amm CA, El-Musa KA Management of Acute and Chronic Open Wounds: The Importance of Moist Environment in Optimal Wound Healing *Current Pharmaceutical Biotechnology. 2002;* **3(3):***179-195*
6. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M Growth factors and cytokines in wound healing. *Wound Rep. Reg.* 2008; **16**:585-601
7. Bello YM, Falabella AF, Eaglstein WH. Tissue engineered skin: Current status in wound healing. *Am. J Clin. Dermatol.* 2001; **2(5)**:305-313.
8. Birke JA, Novick A, Hawkins ES, Patout C A review of causes of foot ulceration in patients with Diabetes Mellitus *JPO* 1991; **4(1)**:13-22.
9. Bradley M, Cullum N, Nelson EA, Petticrew M, Sheldon T, Torgerson D. Systematic reviews of wound care management: (2) Dressings and topical agents used in the healing of chronic wounds. *Health Technol Assess* 1999;**3**(17 Pt 2).
10. Diegelmann RF, Evans MC Wound healing: An overview of acute, fibrotic and delayed healing. *Front. Biosci.* 2004; **9**:283-289.
11. Diegelmann RF, Evans MC. Wound healing: An overview of acute , fibrotic and delayed healing. *Front. in Biosci*. 2004; **9**:283-289.
12. Edo AE, Eregie E, Ezeani IU Diabetic foot ulcers following rat bites. *Afri J. Diabe.Medi* 2010; **18(2):** 19.
13. Friess W. Collagen – biomaterial for drug delivery *EJPB* 1998; **45**: 113–136
14. Ghalbzouri AE, Hensbergen P, Gibbs S, Kempenaar J, Schors R van Der, Ponec M Fibroblast facilitates re-epithelialization in wounded skin equivalents. *Lab. Invest.* 2004; **84**, 102–112
15. Grinnell F, Bennett MH Fibroblast adhesion on collagen substrate in the presence and absence of plasma fibronectin. *J. Cell Set. 1981;* **48**, 19-34
16. Harding KG, Morris HL and Patel GK. Healing chronic wounds. *BMJ* 2002; **324**:160-163
17. Haroon ZA, Raleigh JA, Greenberg CS, Dewhirst MW. Early wound healing exhibits cytokine surge without evidence of hypoxia. *Ann.Surg.* 2002 **231(1)**:137-147.
18. Kolenik S, Mc Groven TW, Leffel D, Surg D Use of lyophilized bovine collagen matrix in post operative wound healing. *Dermatol Surg* 1999; **25**:303-307
19. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. *Clin. in Dermatol*. 2007; 25:9-18.
20. Martin P .Wound healing- Aiming for perfect skin regeneration. *Science* 1997; **276**:75-81
21. McDougall S, Dallon J, Sherratt J, Maini P Fibroblast migration and collagen deposition during dermal wound healing: mathematical modeling and clinical implications. *Phil. Trans. R. Soc.*2006; **364**:1385-1405
22. McIntosh C, Kelly L Importance of wound debridement in management of diabetic foot ulcers. *Wound Essentials* 2009; **4**: 122-125.
23. Moon CH, Crabtree TG. New Wound Dressing Techniques to Accelerate Healing. *Curr. Treat. Options in Infe. Diseases* 2003, **5:**251–260.
24. Peng T. Biomaterials for hemorrhage control. *Trends Biomater. Artif. Organs* 2010; **24(1)**:27-68.
25. Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC Activity of Collagenase-1 is required for keratinocyte migration on Type I collagen matrix. *J. cell. Biol.* 1997; **137**:1445-1457
26. Raghow R. Role of extracellular matrix in post-inflammatory wound healing and fibrosis. *FSEB J* 1994; **8**:823-831
27. Samudrala S Topical hemostatic agents in surgery: A surgen's perspective *AORN J*.2008; 88(3):S2-S11
28. Schalkwijk CG, Stehouwer Coen DA Vascular complications in diabetes Mellitus: the role of endothelial dysfunction. *Clin. Sci.* 2005; **109**:143-159.
29. Singer AJ. Cutaneous wound healing. *NEJM* 1999; **341**:738-746
30. Smyth SS, Mcever RP, Weyrich AS, Morrel CN, Hoffman MR, Arepally GM, French PA, Dauerman HL, Becker RC. Platelet functions beyond hemostasis. *J Thromb Haemost* 2009; **7**: 1759–66.

31. Still J, Glat P, Silverstein P, Griswold J, Mozingo D. The use of collagen sponge/living skin composite material to treat donor sites in burn patients. *Burns* 2003; **29**:837-841.
32. Sundaram CP, Keenan AC Evolution of hemostatic agents in surgical practice *Indian J. Urol* 2010; **26(3)**:374-378
33. Tonnesen MG, Feng X, Clark RAF Angiogenesis in wound healing. *J. Invest. Dermatol. Sympo. Proc.* 2000; **5**:40-46
34. Turjman F, Massoud TF, Vinters HV, C Ji, Tardy M, Guglielmi G, and . Vin˜uela F Collagen Microbeads: Experimental Evaluation of an Embolic Agent in the Rete Mirabile of the Swine. *AJNR Am J Neuroradiol* 1995; **16:**1031–1036
35. Werner S And Grose R. Regulation of Wound Healing by Growth Factors and Cytokines *Physiol Rev2003;* **83**: 835–870
36. Widgerow A Cellular/extracellular matrix cross talk in scar evolution and control. *Wound Rep. Reg.* 2011; **19**:117-133
37. Woo KY, Sibbald RG, Fogh K, Glynn C, Krasner D, Leaper D, O¨ sterbrink J, Price P, Teot L. Assessment and management of persistent (chronic) and total wound pain. *Int Wound J* 2008;**5**:205–215.
38. Yannas IV, Tzeranis DS, Harley BA Biologically active collagen scaffolds: Advances in processing and characterization. *Phil. Trans. R. Soc. A.* 2010; **368**:2123-2139Monaco JL, Lawrence WT. Acute wound healing: An overview. *Clin. Plastc Surg.* 2003; **30**:1-12.