HELICOLL INNOVATIVE TECHNOLOGY, DESIGN, FEATURES, & BENEFITS

What is Innovation?
Innovation refers to something new or a novelty incorporated into an existing product, idea, or field for an improvement.

Accordingly, all the existing advanced wound healing products are falling behind our HELICOLL product in several ways.

Advantages of HELICOLL vs. the competitors are as follows:

- Highly biocompatible, non-immunogenic and highly bioactive.
- US patents prove the purity of type-I collagen as well as the surface chemistry modification through phosphorylation.
- PHOSPHORYLATION of HELICOLL's type-I collagen is DEFINITELY an INNOVATION to the product vs. the competitors.
- It enhances the cell signaling and practically reduces the healing time and would have better patient care and safety by default compared to other products.

Unfortunately, there seems to be major misconceptions about collagen protein as listed below:

- Structural configuration
- Surface modifications like crosslinking
- Inclusion of contaminants of other immunogenic molecules like elastin and immunogenic collagen types etc.

All products that are made of "Intact Tissue-based Membrane" matrices (such as OASIS™, EPIFIX™, AMNIOFIX™, CYTAL™) naturally contain at least 15% of high immunogenic molecules primarily **elastin**, and other allergenic biological materials like glycosaminoglycans and other types of collagen besides type-I.

It is very likely that these products and even the allograft skin grafts may be losing their usage as an ideal skin regenerative matrix over time. Hence, these tissue regenerative products would lose their ground for clinical applications when an advanced Innovative Helicoll is approved by the GPOs.

HELICOLL is derived as a NANO-TECHNOLOGY based biomatrix engineered to provide significant superiority over existing products in the market. HELICOLL, AS WE KNOW OF, IS THE ONLY PRODUCT CLINICALLY PROVEN TO HAVE NEW BLOOD CAPILLARIES FORMED INTO THE MATRIX WITHIN 4 TO 5 DAYS UPON APPLICATION.

We appeal to consider, based on the given evidence, HELICOLL as the truly INNOVATIVE tissue regenerative wound healing collagen matrix product. We wish to defend our technology any

time among all the experts like lead Scientists, Clinicians, Nurse practitioners and the viewer. Hope fully every reviewer would assert their endorsement in favor of HELICOLL.

INNOVATIVE TECHNOLOGY

Wound Healing and Collagen:

The wound healing process is a complex series of events that begins at the moment of injury and can continue for months to years. This process has four phases: the blood clotting phase, inflammatory phase, the proliferative phase, and the maturational phase.[1]

Collagen, the most abundant protein found in the body, is the main supportive protein of cartilage, connective tissue, tendon, skin, and bone. There are at least 13 different types of collagen. Types 1, 3, 4, 5, and 7 are specific for skin.[2]

Collagen plays an integral part during each phase of wound healing and is an excellent hemostatic agent as it absorbs 40 - 60 times its weight in fluid. Collagen exposed during wound formation activates the clotting phase, when the collagen is native and bioactive, and is responsible for cell signalling that influences the migration of inflammatory cells to the wound bed.[1-4]

Collagen dressings have been used in various forms for tissue repair and wound healing[5] as it constitutes more than 80% of the structural proteins of the body. Compared to many other modern non-biological dressings, collagen dressings remain a poorly understood and probably underused material. Biodegradable (bio utilized) collagen dressings are derived from animal tissues. These collagen dressings maintain a physiologically moist microenvironment that promotes healing and the formation of granulation tissue.[6]

The healing of skin tissue requires the development of a vascularized granular tissue bed, filling of large tissue defects by dermal regeneration, and the restoration of a continuous epidermal keratinocyte layer. Several experimental results suggest that collagen is an ideal material for tissue regeneration compared to other non-biological wound healing materials.[6-8]

In a wound where the basement membrane has been destroyed, similar to a second or third degree burn, the wound is re-epithelialized from the normal cells in the periphery and from the skin appendages provided the basement is intact (e.g., hair follicles, sweat glands). The granulation phase and tissue deposition require nutrients supplied by the capillaries, and failure for this to occur results in a chronically unhealed wound. Fibroblasts differentiate and produce ground substance and then collagen. Many different cytokines are involved in the proliferative phase of wound repair. The steps and the exact mechanism of control have not been elucidated. Some of the cytokines include PDGF, insulin-like growth factor (IGF), and EGF. All are necessary for collagen formation. Epithelialization, angiogenesis, granulation tissue formation, and collagen deposition are the principal steps in this anabolic portion of wound healing.[9,10]

Helicoll Manufacturing and Chemistry:

Helicoll is an acellular collagen matrix free of contaminants (the final production, processing and packaging of Helicoll occurs in an FDA approved clean room). Contaminants not eliminated during processing or packaging could cause an immunological response when applied to the host wound which interferes with the healing process.[7] Contamination from other types of collagen such as Type-II and Type-III are potentially immunogenic and such types of collagen are completely removed in preparing Helicoll.

Our method was developed in order to address the problems presented by other commonly used collagen preparations. Our EnColl process is predicated in part on the discovery that collagen may be prepared in a manner in which all non-collagenous materials are removed, while retaining the native molecular quaternary structure and other characteristic features of collagen (e.g., length, diameter, and periodicity of collagen Type-I fibrils; see **Figure 1**).

Figure 1: Microphotographs of Helicoll collagen fibrils[11]

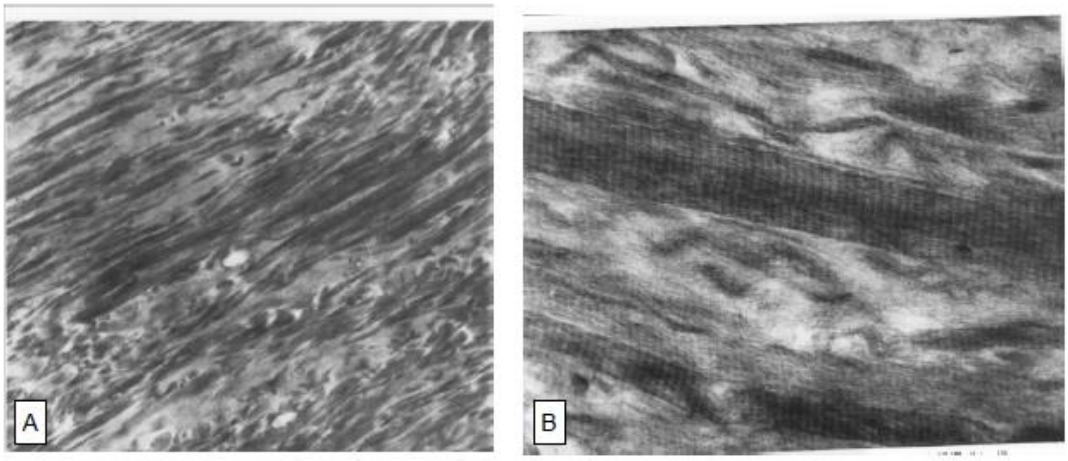


Image of meshwork of collagen fibrils. A: 100X; B: 1000X, showing periodic banding.

Helicoll is tested and manufactured in the FDA-certified clean room which is a controlled environment that filters all incoming air to remove all dust particles and possible contaminants that may interfere with the healing process. To be FDA certified, the clean room must meet the standards for controlled environments set forth in ISO 14644-1.

Helicoll collagen dressing does not require refrigeration and can be stored at normal room temperature for three years as stated in the FDA approval (See the FDA Approval information above). NASA scientists in 2010, upon reviewing the product information and in consideration possible use of the product on the 21-month Mars missions in 2035 and beyond, determined that the product is ideal for their missions. They stated that they believe the product has an incredible nine-year shelf life at standard temperature and pressure. Important for their missions is ease of application, storage requirements, size and healing rate.

The EnColl process may be used to prepare highly purified collagen from various animal sources (including humans) as most, if not all, contaminating conjugated proteolipids and phospholipids are removed through use of a specific mixture of organic solvents. Unlike previously reported enzymatic methods and patents filed for collagen preparation,[12] the EnColl method utilizes a two-step enzyme treatment process. This two-step treatment processes ("Twice Treatment Process" or "TTP") renders collagen polymers non-inflammatory when implanted.[13]

INNOVATIVE TECHNOLOGY BACKGROUND

The use of papain, an enzyme extracted from papaya, is known to break the disulfide bonds of cysteine[14]. As many immunogenic molecules contain cysteine disulfide bonds[15], papain may be used to degrade these molecules and render them non-immunogenic. In comparison with other collagen preparations for biomedical applications, better results in terms of reduced immunogenicity are obtained with EnColl's collagen.[13]

In addition, papain has been reported to have a lytic effect on elastin, one of the contaminants that is difficult to remove from purified collagen.[12,13] Initial experiments involving a one-step papain treatment to remove immunogenic sites from collagen were largely unsuccessful in altering the *in vivo* performance of purified collagen. These observations led to the development of the EnColl processes, which result in the breaking and loosening of the natural crosslinks of collagen fibers (e.g., aldol condensation). In this manner, the papain used in the second treatment step of EnColl's patented process (i.e., papain is used in two treatment steps) is provided access to most, if not all of the collagen molecules' surfaces, and facilitates the release of trapped immunogenic sites from the collagen preparation. These developments resulted in one embodiment of the two-stage EnColl process, in which papain is used at two specific stages of the process (i.e., before and after the treatment of the collagen with a reducing and/or an unfolding agent). These methods therefore, provide means to produce highly purified collagen that is non-immunogenic.[16]

The collagen is further bioactivated by varied degrees of controlled modification of phosphorylation. Purified collagen can be chemically modified by covalently binding phosphates to hydroxyl groups of hydroxylated amino acids. This reaction (an example for serine is given below in Figure 2) likely involves covalent bonding of phosphate to hydroxyl group of serine, tyrosine and/or threonine, hydroxylysine and hydroxyproline.[17] The reaction is controlled, in order to limit the degree of reaction. EnColl's phosphorylated collagen renders unique abilities in the growth of soft or hard tissue as needed by the physiological system.

Phosphorylation exposes multiple free binding sites which allow the collagen-connective tissue framework to develop quickly. This proper alignment and binding of collagen fibres causes the maturation process to accelerate wound healing. This allows epithelial regeneration to occur leaving no scar formation.

Using patented technology, Helicoll collagen is phosphorylated to provide better healing. Protein phosphorylation is reversible through protein phosphatases, enzymes that hydrolytically remove

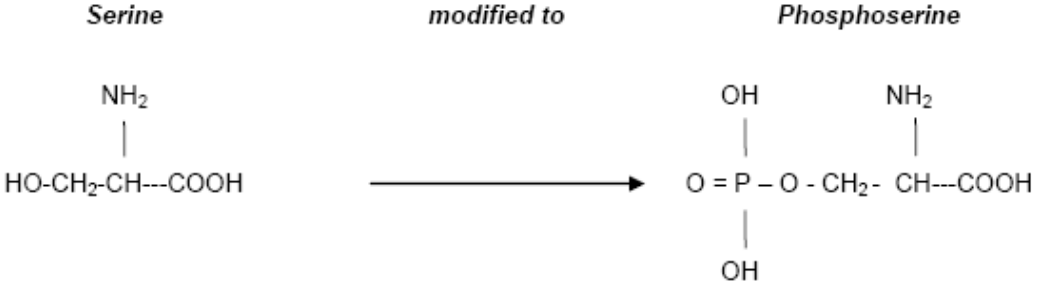
specific phosphoryl groups from modified proteins. These protein phosphatases are one mechanism for the termination of a signaling process.

Proteins undergo a huge number of post translational modifications. Only certain covalent modifications such as acetylation, fatty acid acylation, glycosylation and phosphorylation are reversible. Among these modifications, phosphorylation is an important and ubiquitous one. The majority of the proteins involved in cell activation are subjected to reversible phosphorylation. The sites of phosphorylation are serine, threonine and tyrosine hydroxyl groups. Aspartic acid, histidine and lysine can also be phosphorylated. Phosphorylation of tissue proteins is involved in natural cell differentiation of stem cells and in preventing pathogenic bacterial invasion.

In nature, the phosphorylation of extra-cellular matrix protein is evidenced by the accumulation of alkaline phosphatase in the regions of tissue formation or repair. The significance of protein phosphorylation is to induce cell signal transduction through a cascade of enzymatic reactions which are all documented in the literature. Collagen is the largest native structural protein present at the sites of tissue repair remodeling or growth. Phosphorylation of collagen makes the molecule biologically more active and becomes essential for the cell signal transduction to happen.

Collagen has specific binding regions for all active components such as cell membrane receptors, ligands, platelets, growth factors and other cytokines for proper interaction that can result in repair, remodeling and regeneration of tissues. Phosphorylated collagen plays an important role due to its ability to bring all necessary factors together and to activate them for the desired result. Additionally, the phosphorylated collagen tends to attract divalent cations such as Ca and Mg. Such divalent cations are essential for activating platelets and other physiological events for faster wound repair or tissue growth. EnColl's patented and FDA approved technology focuses on "collagen - phosphorylation" - and exploit the same for extra-ordinary biomedical applications.

Figure 2: Serine phosphorylation



Biological Characteristics of Manufactured Collagen

EnColl's modified collagen has been shown to possess improved biological characteristics. The modified collagen was found to have increased solubility features under neutral conditions, which helps in the formulation of bioactive coatings on inactive surfaces.[13]

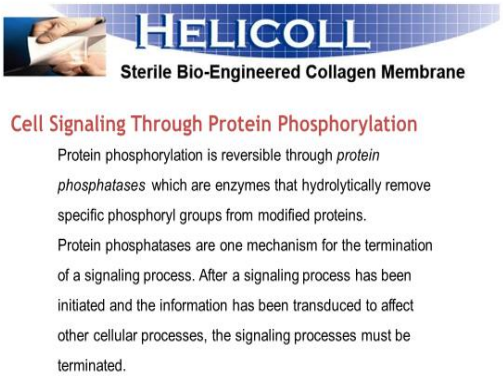
In one of the implant experiments, the modified collagen implants were analyzed for their alkaline phosphatase (an enzyme involved in new tissue formation) activity. The assay used[15,18] was a calorimetric method using the measurement of o-carboxy-phenyl phosphate (OCCP) following the hydrolysis by alkaline phosphatase enzyme. Briefly, samples of approximately 10 mg from each of the harvested collagen implants were dispersed at a rate of 1 mg in 1 mL of Tris buffer (0.1M Tris, pH 8.5) for five minutes. A small amount of detergent (to a final concentration of 0.1M sodium deoxycholate) was added to the dispersed samples to release of membrane-bound enzymes. The optical density was determined at 300 nm, at room temperature. The activity is expressed in units per mg of tissue, as based on the number of micromoles of OCCP hydrolyzed per minute at 25°C, under the conditions described above. The results showed significantly elevated amounts of alkaline phosphatase (34% increase; $p<0.0005$) activity in the modified collagen implants as compared to the unmodified implants.[13]

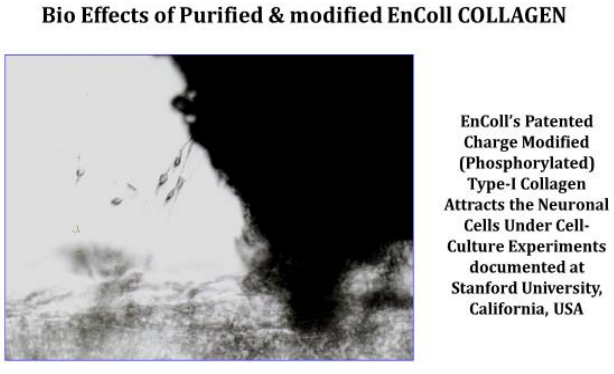
EnColl's patented technology includes chemical modifications in solution or solid form of collagen which can be used for a variety of purposes https://www.helicoll.com/pdf/Evaluation_of_Bovine_Derived_Collagen_of_ENCOLL_Technology.pdf, including, but not limited to, biological implants[13,19-21], grafts[13,22], transplants[11,13], and drug delivery[13,23].

Pre-Clinical Experience

Numerous *in vitro* pre-clinical studies were conducted and are included in the Helicoll patent 5814328.[13]

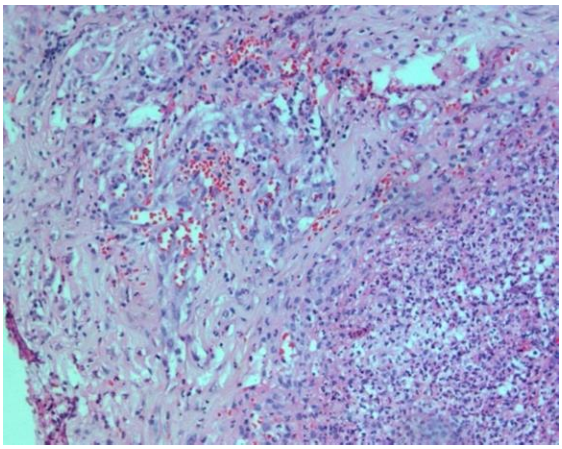
The *in vivo* and *in vitro* tissue culture experiments using mice and rabbits demonstrated that the delivery of growth factors was more effective when delivered through EnColl prepared collagen as compared to native Type-I collagen.[13]





A bioactive collagen dressing, such as Helicoll, induces platelet aggregation. Inflammatory cells, neutrophils and macrophages invade the clotting area. After 4 days (refer to Figure 1) of wound healing, there is a complete connective tissue bridge covering the wound. The site fills with neutrophils and macrophages. At seven days, the inflammatory process recedes and the repair process (proliferative phase) begins with the fibroblastic synthesis and deposition of the extracellular matrix and collagen. Matured skin tissue develops consisting of bricks of fibroblast cells that are mortared by the collagen produced by fibroblasts (see Figure 3). A combination of cells and collagen provides a secure bridge over the interrupted skin tissue.24

Figure 3: H&E staining after Helicoll application



After Helicoll application the acute inflammatory cells, fibroblasts and blood vessels proliferate into the collagen matrix. (50x). Absence of Lymphocytes indicates the non-immunogenic property of the collagen in Helicoll.[11]

The role of pure bioactive Type I, non-immunogenic collagen, such as Helicoll, is to provide binding and bridging sites for multiple chemokines (epidermal growth factor (EGF), fibronectin, fibrinogen, histamine, platelet-derived growth factor (PDGF), serotonin, and von Willebrand factor), necessary for building a connective tissue framework for epithelial regeneration to occur.[25-28]

In Vivo Evaluation of Chemically Modified Collagen in adult New Zealand white rabbit experiments showed more vascularization and fibroblastic in-growth in both of the experimental groups (Example 6 and Example 2, see the Patent reference for further details). Six of the rabbits (24 sites) were operated on bilaterally, with implants placed on both sides of the dorsal mid-line. Each implant comprised 50 mg of dried collagen sample was rolled into an approximately round ball and placed subcutaneously at each site. The animals were observed for three weeks for gross indications of inflammation (e.g., redness, swelling, etc.). No adverse responses were observed for any of the animals. After 3 weeks, the animals were sacrificed and the implants were surgically removed and subjected to histology evaluations. The control

samples had relatively poor vascularization, as well as a prevalence of multi-nucleated giant cells, reflecting the lesser biocompatibility of these samples.

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