4576 Enterprise Street, Fremont, CA 94538. Tel. (510) 396-8581

PRE-READING INFORMATIONAL MATERIAL PACKAGE ON HELICOLL® SKIN SUBSTITUTE / ADVANCED BIOLOGICAL SKIN GRAFT PRODUCT

Product Name: HELICOLL®

Product Category: Bioengineered skin Substitute / Regenerative biological skin graft category Advanced Wound Care product used surgically for the treatment of all wounds especially for the non-healing wounds.

Submitted to
WHOM IT MAY CONCERN

Our Contact:

Manufacturer & Technology Holder	
S. Gunasekaran, PhD (for Technology) guna@encoll.com +1 (510) 396-8581	Murugan G. (for Business & Sales) murugan@encoll.com +1 (510) 709-8663

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PRELEUDE / INTRODUCTION

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 & potentially carcinogenic 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, a carcinogen**, 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. Hopefully every reviewer would assert their endorsement in favor of HELICOLL.

Thank you and please go through the details in the following pages of this document.

Product Specific Information

Product Name & Description:

HELICOLL®:

Helicoll is a non-immunogenic, bio-compatible and highly bioactive type-I collagen membrane construct.

Helicoll has been clinically documented for faster wound healing. (see the recent Stanford University Dermatology clinical study publication). It encourages the formation of new blood capillaries within 4 to 5 days upon the application of the product over the wound (see attached USP Monograph with references). No other skin substitute has shown such a clinical result.

EnColl ENHANCING LIFE THROUGH COLLAGEN

- California based corporation; founded in 1994.
- Core expertise to develop, manufacture and globally market FDA approved collagen-based medical products.
- Patent protected manufacturing of high purity, non cross-linked, sterile medical grade type-I collagen
- Proven faster tissue in-growth potential.

OUR MISSION: To become a Leading Biotech Company committed to improve quality of life through innovative type-I Collagen products that stimulate tissue regeneration

LEADERSHIP EXPERIENCE

- Over 25 years of experience in healthcare
- 40 years experience in Tissue Regenerative Products
- 7 years experience in medical supply distributions

Contra-Indications: Helicoll is derived from a bovine or ovine source and should not be used in patients with known sensitivity to such material. This device is not indicated for third-degree burns.

Precautions: Do not resterilize. Helicoll is sterile if the package is dry, unopened and undamaged. Do not use if the package seal is broken. The device must be used prior to the expiration date. Discard all open Helicoll and any unused portions. Helicoll is available by medical prescription only.

Storage: Helicoll should be stored in a clean, dry location at room temperature.

Sterilization: Helicoll has been sterilized with ethylene oxide.

Shelf Life: Helicoll shelf life is 3 years.

Available Sizes (in inches & in cm):

0.5 in dia disc (1.27 cm dia disc) **1 sq cm**	**1.0 in dia disc** (2.54 cm dia disc) **5 sq cm**	**0.8 in x 1.6 in** (2 cm x 4 cm) **8 sq cm**
1.2 in x 1.6 in (3 cm x 4 cm) **12 sq cm**	**1.6 in x 1.6 in** (4 cm x 4 cm) **16 sq cm**	**2 in x 2 in** (5 cm x 5 cm) **25 sq cm**
2 in x 4 in (5 cm x 10 cm) **50 sq cm**	and other custom sizes. (Each individually sterile packaged)	

(U.S. Patents 5,814,328; 6,127,143 & 6,548,077)

Helicoll®

COLLAGEN BASED STERILE
BIOENGINEERED SKIN SUBSTITUTE

Website: www.helicoll.com

US FDA K # 040314, issued Aug. 2004

Manufactured & Marketed by:

ENCOLL

Enhancing life through collagen

Address: 4576 Enterprise St. Fremont, CA 94538 USA
Tel: (510) 659-1466, Email: info@helicoll.com

CP0005.F

Helicoll®

Collagen Based Sterile Bioengineered Skin Substitute

For Partial and Full Thickness Wounds, Second-degree Burns, Trauma, Skin Ulcers, and Skin Donor Sites.

ENCOLL

Enhancing life through collagen

Advanced Patented High Purity Type-I Collagen Technology for Wound Care Professional

Helicoll®

Helicoll is a bioengineered high purity Type-I collagen (>97% pure) forming an acellular skin substitute construct that is highly bioactive, cell conducive, and supportive towards enhancing tissue generation for wound management. **Helicoll** is an acellular dermal replacement product and is within the definition of a bioengineered skin substitute. It provides a framework that promotes the regeneration of blood vessels and supports biologic cell migration due to the resorbable properties of Helicoll. Treatment course typically involves 1 to 4 applications.

Applications

- Partial and full-thickness wounds
- Pressure ulcers
- Venous ulcers
- Chronic vascular ulcers
- Diabetic ulcers
- Trauma wounds: Abrasions, Lacerations, Skin tears, Second-degree burns
- Surgical wounds: Donor sites/grafts, Post- Mohs' surgery, Post Laser surgery, Podiatric, Wound dehiscence.

Advantages of Helicoll Biological Skin Substitute:

High purity Type-I Collagen: Helicoll is a patented reconstituted bioactive collagen sheet, free of immunogenic proteins, lipids, and elastin. The native structure of collagen is not altered or cross-linked which maintains its high bioactivity.

Faster Healing: Collagen phosphorylation attracts cells, regenerates tissue, and stimulates blood capillaries/granulation within 4 to 5 days.

Innovative Technology: Better than intact tissue-based membranes like an amnion, intestinal wall, urinary bladder etc. which contain >15% elastin that is recently discovered to be carcinogenic.

Pain Control: Effectively reduces pain.

Easy Application: No washing needed prior to use. The overall clinical usage of Helicoll is simple and easy as it can be cut, sutured or stapled.

Cost-Effective: Accelerated wound healing and tissue remodeling with minimal applications reduce the treatment cost by over 40%.

Directions for Use:

Note: Helicoll comes in a sterile double packaging as a transparent pliable sheet with a back and a top protection cover sheet of medical grade synthetic polymer.

- Upon opening the sterile package, carefully remove the top sheet of polymer and soak the Helicoll membrane in sterile saline solution for 5 to 10 minutes to easily remove the other backing sheet, (soaking time is not critical for the efficacy of the product).
- Prepare wound area using standard methods to ensure wound is free of debris and necrotic tissue. An initial surgical debridement of the wound may be necessary to ensure the wound edges contain viable tissue.
- Do not apply ointment or any greasy cream on site prior to Helicoll membrane.
- Helicoll membrane can be applied on either surface and it adheres to the wound. In case of dry wounds, sprinkle with sterile saline solution before applying Helicoll.
- Do not try to over-stretch the Helicoll membrane.
- Place carefully over the wound, press out any air pockets under to make sure Helicoll membrane contacts well to the surface of the wound. Any excess Helicoll can be cut and placed as a second layer. Excessive exudate underneath Helicoll can be drained by making slits through the skin substitute.
- If there is a need to secure Helicoll membrane in place, the edges can be taped, sutured or stapled if preferred by the doctor. If a secondary dressing is required, use any non-adherent dressing to prevent unnecessary adherence of Helicoll membrane to the secondary dressing. Change secondary dressing as required.
- Additional application of Helicoll membrane is not generally required unless patient is hyperglycemic.
- Repeated application on every 2nd or 3rd day like a typical wound dressing is not required, unless the wound is infected or accumulates excessive exudate underneath which can be drained by making slit openings in the Helicoll dressing.
- Removal of a Helicoll membrane is not required except when wound is infected; or, if excessive exudate is under the Helicoll membrane; or, for slowly healing chronic ulcers 5 to 7 days after an application of Helicoll membrane. Moisten the Helicoll membrane with saline and gently remove.
- Depending on the treatment modality, sometimes Helicoll may remain intact and gets peeled off as the wound heals which may carefully be removed by moistening with saline soaked gauze for a few minutes. However in some cases, Helicoll may get incorporated into the wound bed in about 4 to 5 days resulting in complete absorption of Helicoll.
- For donor site application, after surgical removal of donor tissue, arrest bleeding by conventional methods, clean the site and apply Helicoll.
- Oral or systemic antibiotics may be given as prescribed in infected cases and in non-infected cases as a preventive measure for better and faster results.

Caution:

Always handle Helicoll using aseptic techniques. Helicoll should not be applied until excessive exudate, bleeding, acute swelling, and infection is controlled. If air pockets appear beneath the applied Helicoll, it can be gently pressed and removed using sterile methods. In case of localized bulging due to fluid accumulation beneath Helicoll, a small incision can be made to exude fluid. This incision can be patched with a small piece of Helicoll adhering to the original applied Helicoll sheet. After application, use an appropriate, non-adherent, secondary dressing to maintain a moist wound environment. Frequency of secondary dressing change will depend on the volume of exudate produced and the type of dressing used. Do not forcibly remove sections of Helicoll that may adhere to the wound. Helicoll may form a caramel-colored gel, which can be rinsed away with gentle irrigation.

HELICOLL REGULATORY APPROVALS

Helicoll Regulatory:

Helicoll was originally cleared by the FDA as a medical device on August 5, 2004 with 510(k) number K040314.

The product comes from USDA approved bovine sources with FDA required regulatory documentation to maintain and monitor the safety and quality of the procured animal derived raw materials.

Helicoll is a cleared FDA product and has been recognized as a high-cost skin substitute by Medicare continuously since 2017.

Helicoll may be the ONLY PRODUCT that is relatively highly bio-compatible and bio-active. It is made up of highly purified, non-crosslinked Type-I Collagen (U.S. Patented). Additionally, it is highly bio-active due to its controlled phosphorylation for maximum wound healing benefits. Helicoll is the ONLY PRODUCT that is relatively highly bio-compatible and bio-active. It is made up of highly purified, non-crosslinked Type-I Collagen (U.S. Patented).

FDA Cleared Indications for HELICOLL (FDA K# 040314, Aug. 2004)

- ✓ Partial and full-thickness wounds
- ✓ Pressure sores and venous ulcers
- ✓ Chronic vascular ulcers
- ✓ Diabetic ulcers
- ✓ Trauma wounds (abrasions, lacerations, 2nd degree burns)
- ✓ Surgical wounds, donor sites/grafts, Mohs' surgery
- ✓ Post laser surgery, podiatric surgery and wound dehiscence

Attachments at the end of this document:

- ✓ FDA Clearance
- ✓ FDA Letter to File Documents
- ✓ Medicare Approval as High Cost Skin Substitute
- ✓ MMS document to prove Helicoll as a Skin Substitute

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**).

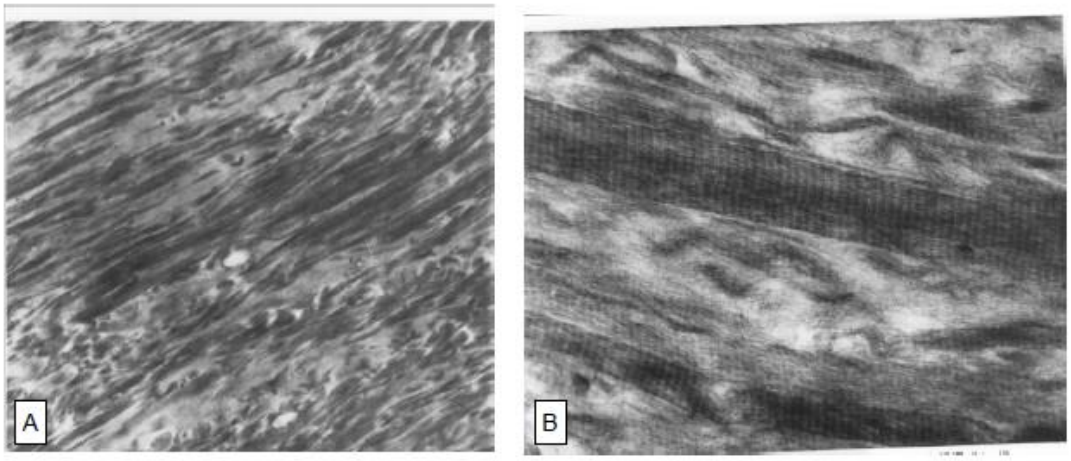


Figure 1: Microphotographs of Helicoll collagen fibrils[11]
Image of meshwork of collagen fibrils. A: 100X; B: 1000X, showing periodic banding.

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 clearance (See the FDA clearance 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 cleared technology focuses on "collagen - phosphorylation" - and exploit the same for extra-ordinary biomedical applications.

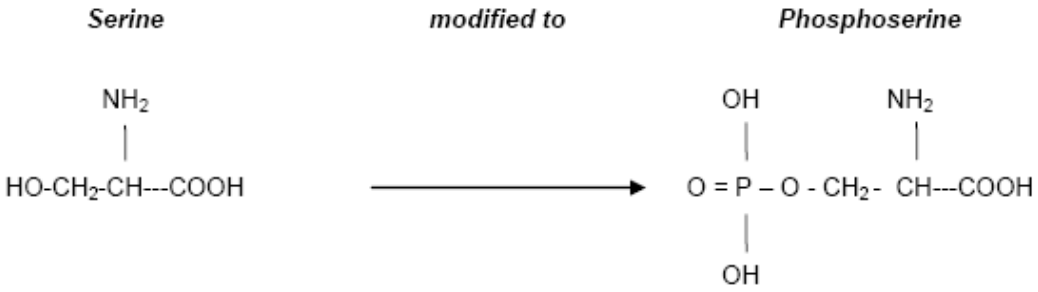


Figure 2: Serine phosphorylation

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]

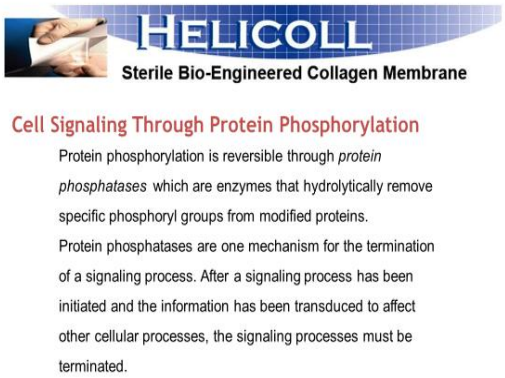
In one of the implant experiments, the modified collagen implants were analyzed for their alkaline phosphatase (an enzyme involved in new tissue formation) activity. The assay used[15,18] was a calorimetric method using the measurement of o-carboxy-phenyl phosphate (OCCP) following the hydrolysis by 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]

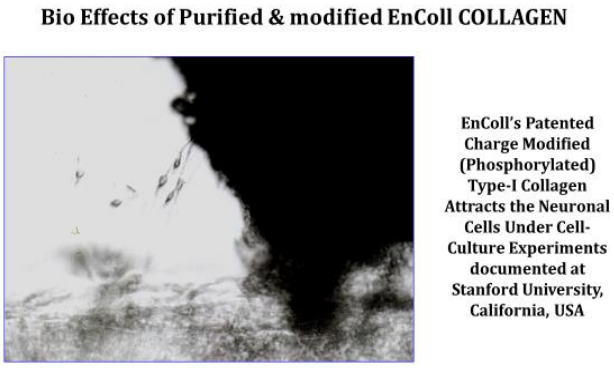
EnColl's patented technology includes chemical modifications in solution or solid form of collagen which can be used for a variety of purposes, including, but not limited to, biological implants[13,19-21], grafts[13,22], transplants[11,13], and drug delivery[13,23].

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.[11]

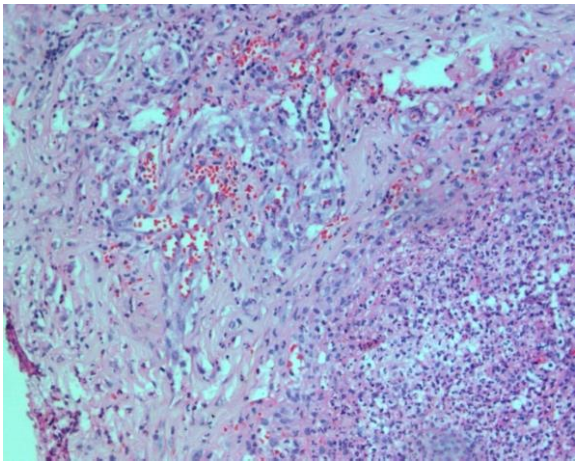
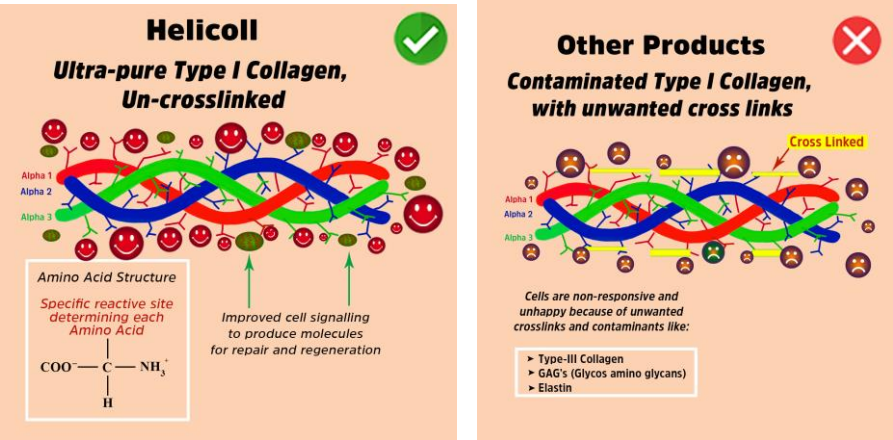


Figure 3: H&E staining after Helicoll application

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.

Clinical Experience with Helicoll

Clinical Situation	Helicoll Used Patients	Control Patients
Donor Site	81	77
2nd Degree Burns	10	11
Diabetic Ulcers	6	5
Chronic Venous Ulcers	10	10
Contracture release & Bare tendons, bones and joints	10	10

Figure 4: Clinical indications of subjects in Helicoll studies[11]

158 patients with split thickness skin grafts (STSG) were successfully treated with Helicoll in 2010. Study included measurement of pain reduction of patients treated with Helicoll compared to control patients.[11]

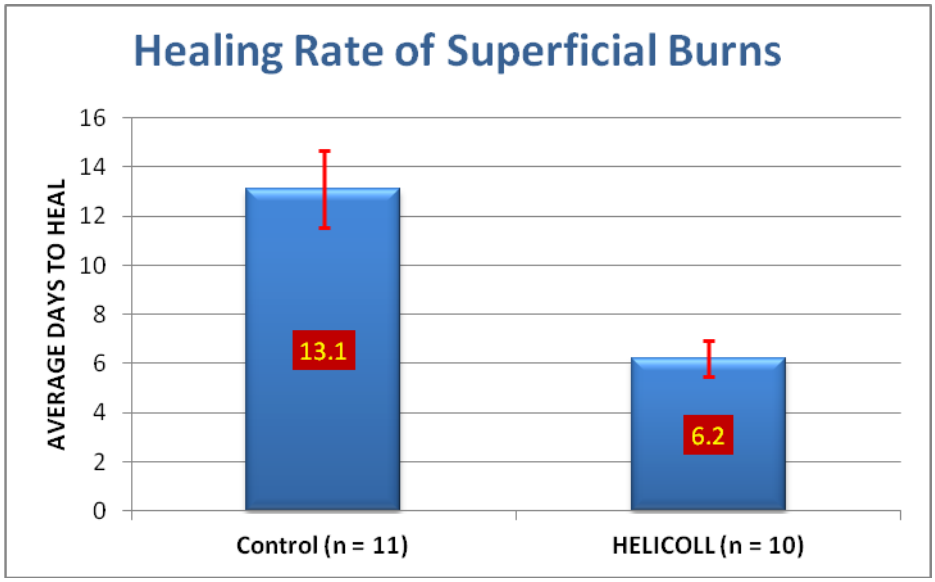


Figure 5: Healing rate of superficial burns treated with Helicoll or control[11]

Helicoll, in the clinical setting[11] significantly reduced burn healing time, provided rapid pain relief at the wound site, achieved 99.9% skin graft retention and reduced scarring, as well as return of native skin color to the patient after several months. Helicoll also significantly reduced the amount of hospital staff time required (dressing changes are less frequent as, Helicoll can remain on wound for several days, wound inspection simplified as Helicoll is semitransparent and wound can be assessed without removal of Helicoll), as well as total cost of care by up to 50% over current therapies (product cost is up to 92 less expensive than some competitors (Figure 10). Helicoll is available in large sizes so burns can be covered quickly.

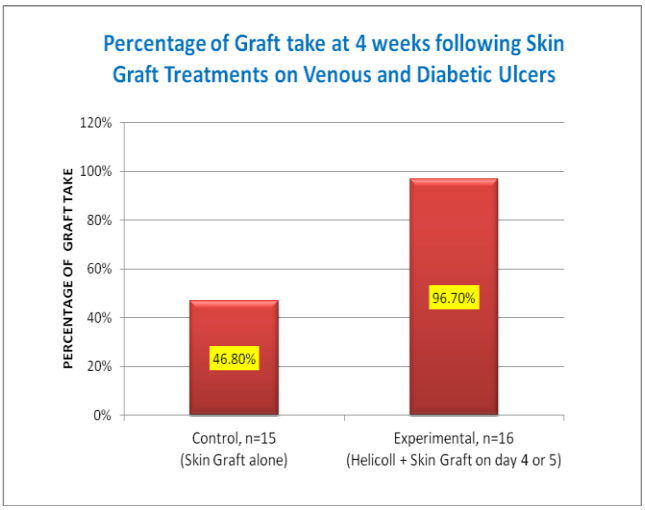


Figure 6: Graft take following skin grafts in venous and diabetic ulcers[11]

Helicoll is used for first and second degree burns, partial and full-thickness wounds, post laser treatment, as well as pressure, venous, vascular, and diabetic ulcers. Trauma wounds such as: Abrasions, lacerations, skin tears and donor sites are also indicated uses.

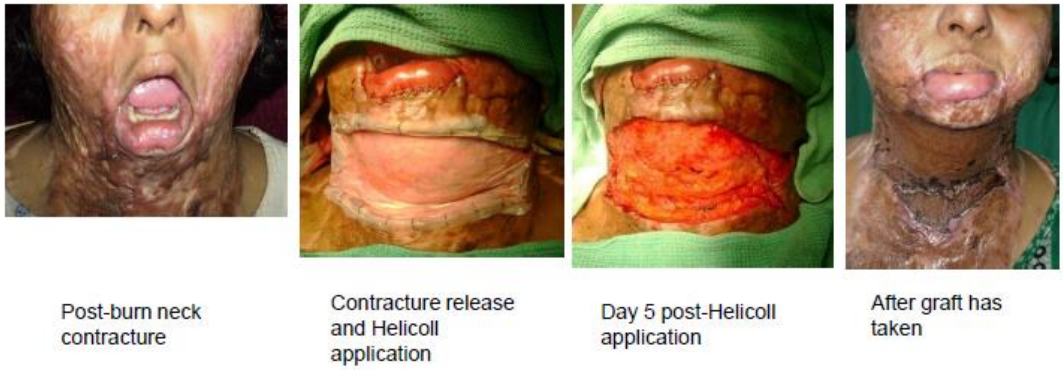


Figure 7: Treatment of Post-Burn Contracture with Helicoll[11]

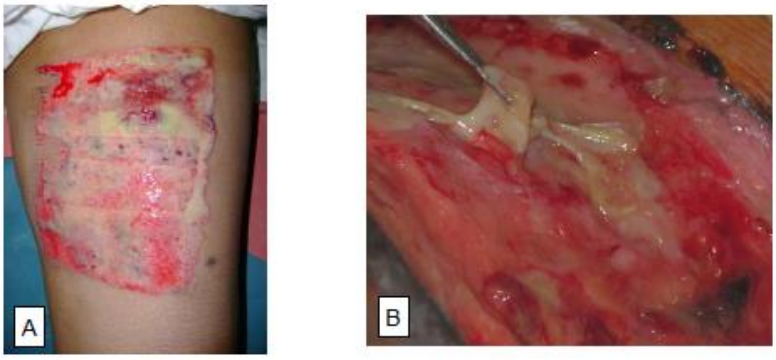
Helicoll can be placed on wounds caused by soft tissue necrosis secondary to radiation, chemical burns or corrosives. The Helicoll is moistened with sterile water or normal saline for six to ten minutes and placed in direct contact with the necrotic tissue. Daily dressing changes are recommended with mechanical debridement of the necrotic tissue to reduce the bioburden of the necrotic tissue and assist with autolysis.

Oxygen enhances the wound healing activity of collagen so Helicoll can be applied to wounds that are undergoing treatment with hyperbaric oxygen.

It does not matter which surface of the Helicoll Wound Dressings is placed against the wound surface. Helicoll must remain in contact with the wound by light pressure to ensure the contact of the wound surface with the collagen to ensure proper healing.

Only areas with skin damage will interact with Helicoll. Any excess collagen (see Figure 8) can be rinsed away with saline irrigation, so removal of the dressing does not interfere with healing granulation tissue nor does it cause a painful experience for the patient. Helicoll is also semi-translucent so that observation of the healing can be accomplished without disturbing the healing tissue.

Figure 8: Photographs of wounds treated with Helicoll[11]



Collagen consistently is incorporated into the wound by Day 4-5. Capillary bleeding and incorporation of Helicoll into deeper structures seen above. Induction of neo-vascularization and incorporation of collagen was assessed using histological and electron microscopic studies (not shown).

Figure 9: Photograph of wounds showing Helicoll incorporation[11]

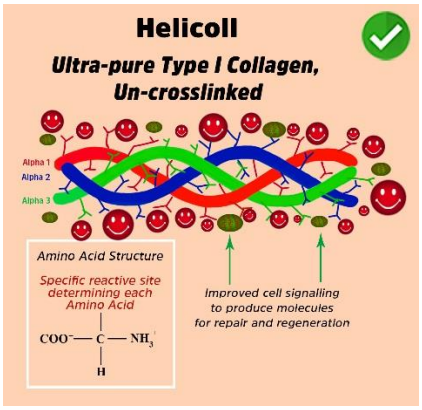
Helicoll Advantages:

The usefulness of Helicoll over any other dressings in the market is well documented for the treatment of diabetic ulcers, donor sites, burn treatments and other types of wounds. Refer to Fig 4 for distribution of types of wounds treated in clinical studies.

With Helicoll, epithelialization occurs even in the inner areas of the wound site. This did not happen with other collagen preparations used on the same wounds.[7]

As described in Section 1.3 of this document, native type-I collagen creates adhesion sites for growth factors and also triggers "cell signal transduction" through which floating stem cells convert into appropriate cell-lines to regenerate damaged tissue. Other collagen preparations may not maintain the native chemistry of type-I collagen. The high purity type I collagen dressing of Helicoll avoids any potential health risks normally caused by contaminating immunogenic molecules like type-III, type-II collagens, elastin, glycosaminoglycans, some proteolipids, oligopeptides etc. Accordingly, the other dressings are cross-linked to minimize the immunogenicity of contaminants at the expense of the needed bio-activity of collagen for enhanced wound healing.

Biochemical Advantages of Helicoll



EnColl Corporation's patented process uses a unique enzymatic process that result in a highly purified collagen that is relatively non-immunogenic. It also renders a native un-crosslinked collagen. Certain preparation methods of collagen products use crosslinking by chemicals such as aldehyde without realizing that the resulting collagen is cross-linked and no longer bioactive. If a collagen molecule is crosslinked, it loses the natural binding abilities to adhere to cell surface receptors, growth factors, and other potential active molecules necessary for the healing process to move forward. This impedes the natural cell-signaling properties of collagen and thereby the crosslinked collagen reduces the wound healing capabilities of un-crosslinked native collagen. If the collagen is native the cell-matrix interactions and the bioactivity of cells will increase. Helicoll collagen provides this environment. It works to reduce pain, scar formation and loss of pigmentation. Further it may also help to heal wounds with limited blood supply in cases of arterial insufficiency.

Another advantage of Helicoll is that it has been shown to be safe for use on patients of all ages from birth to centenarians. Helicoll provides hemostasis and accelerates tissue remodeling and acts as an acellular dermal replacement product similar to Integra. Like the Integra model, Helicoll promotes healing and neo-vascularization.

Some dressings are considered cytotoxic. Helicoll, however, has been shown to be extremely bioactive, biocompatible and non-cytotoxic in vivo and in vitro.

Common benefits of Helicoll over other commercially available collagens in the market are:

- ✓ Improved biocompatibility
- ✓ Non-immunogenicity
- ✓ Controlled bioresorbability
- ✓ Cell attractability
- ✓ Hemostatic ability
- ✓ Structural stability
- ✓ Target specificity

The disadvantages of using human skin allografts that do not apply to Helicoll include:

- ✓ fear of HIV and other human infections
- ✓ cross-linking or use of preservatives that can reduce the bioactivity of the graft
- ✓ biohazardous material disposal concerns
- ✓ immediate availability is quite difficult
- ✓ limited shelf life
- ✓ possible bacterial contamination
- ✓ many eventually are rejected, making them a temporary rather than permanent wound covering

CLINICAL TRIALS:

Use of Helicoll to treat skin ulcers:

64 patients with ulcers were selected at random from different centers and treated with varied acellular dermal replacement collagen dressings to compare the effectiveness of Helicoll dressing with other collagen dressings. Healing was visible as early as the 5th day after Helicoll treatment. There was no pain on opening the dressing and patients had no discomfort. No adverse events were reported.[6]

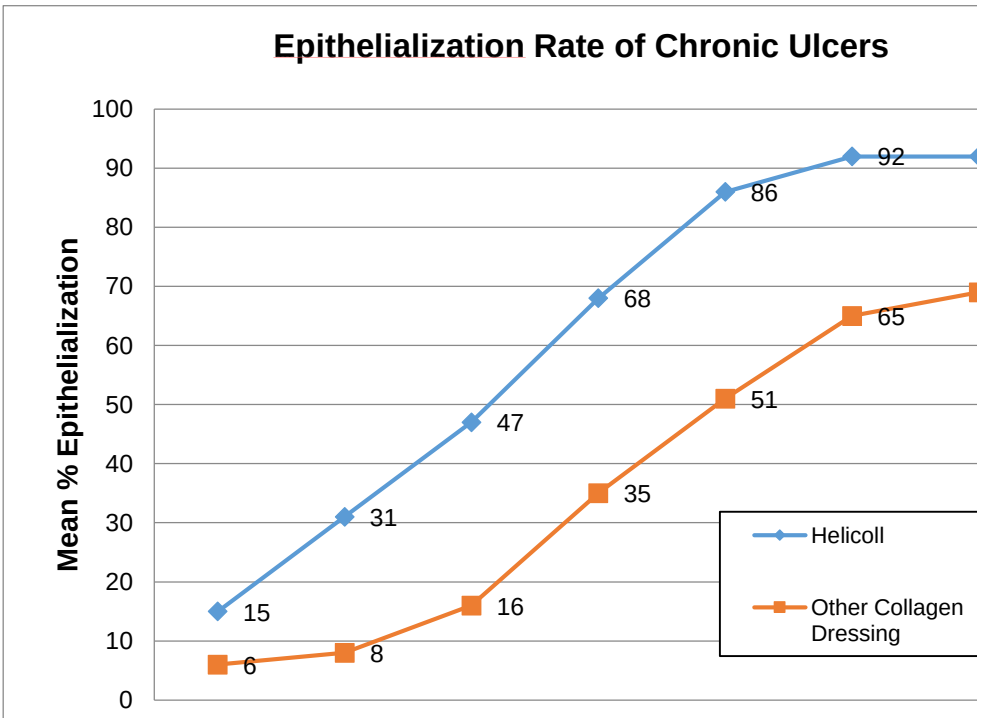


Figure 10: Rate of healing of wounds over 7 weeks in 64 patients with chronic ulcer wounds using Helicoll[6]

20 patients with ulcers were included to undergo treatment with Helicoll. In all cases, wounds closed after a few bi-weekly and weekly applications. The wounds remained closed for several months. It was noticed that epithelialization occurred even in the inner areas of the wound sites, which did not occur when other dressings were used.[7]

Helicoll was compared to traditional cotton gauze dressings for the management of lower extremity ulcers in 18 patients. Although both study groups were comparable at baseline, data indicate that the use of Helicoll resulted in faster re-epithelialization.[29]

The role of Helicoll collagens in foot care was demonstrated[25] in independent clinical studies showing at least 45% epithelialization of the foot ulcer wound in 6 days. Further 30% healing improvement was observed with Helicoll over other collagen products used for leg ulcer treatments.

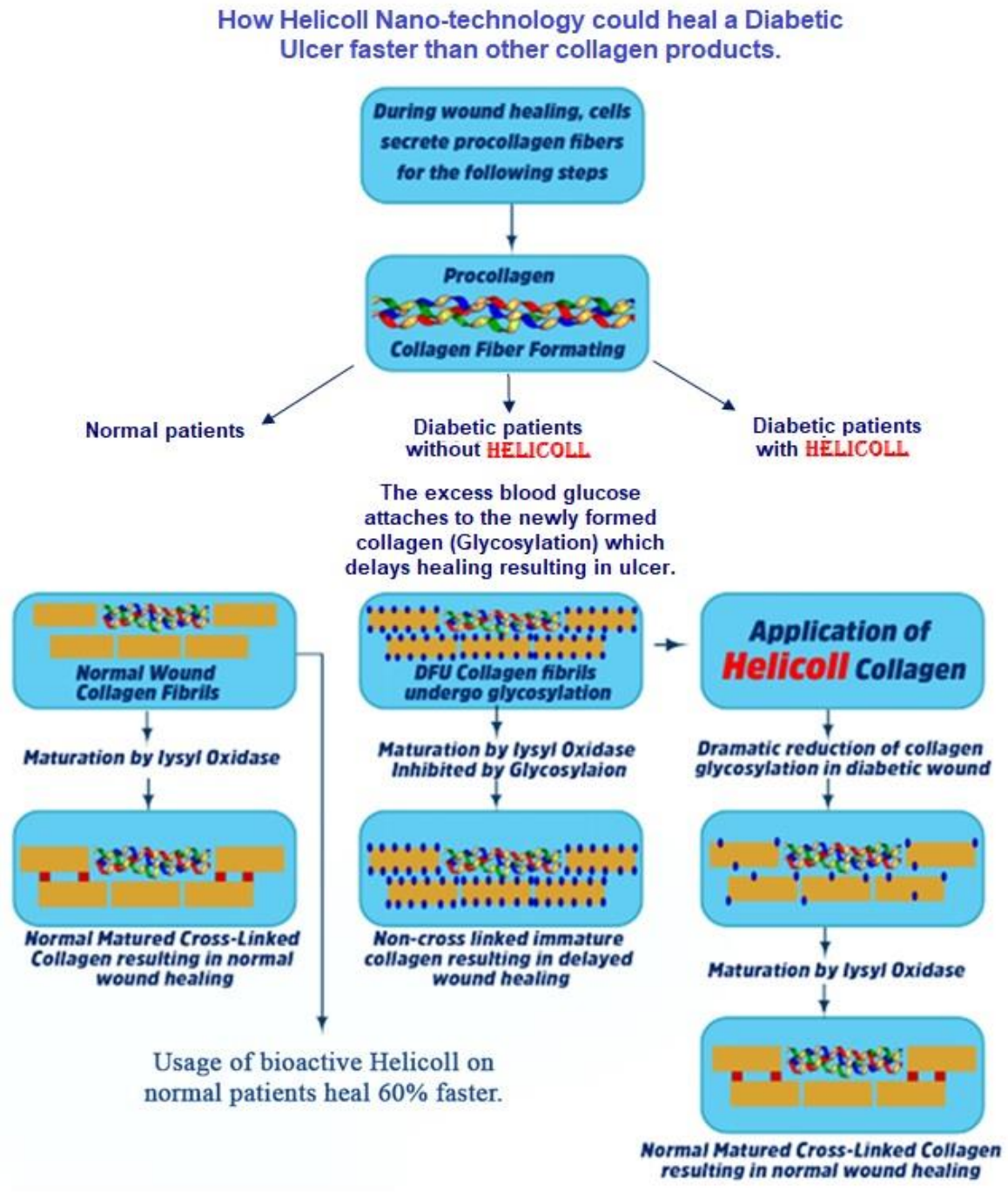
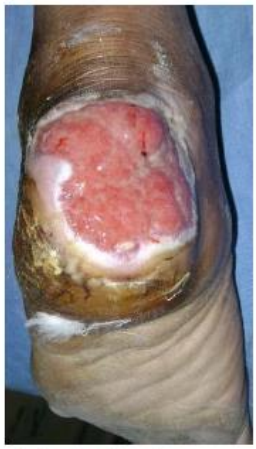
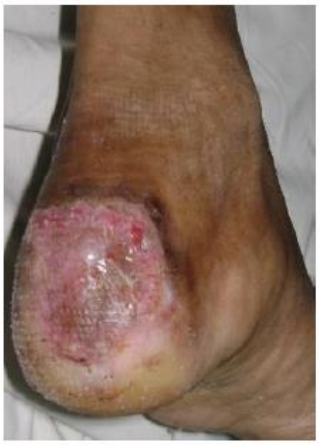


Figure 11: Diabetic Foot Ulcer Treated with Helicoll[25]

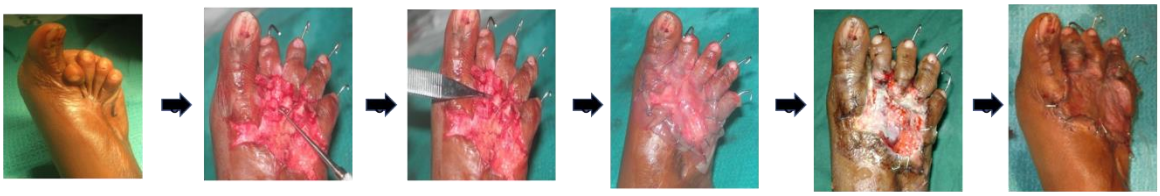


Before treatment



Post-Helicoll application, Day 9

Successful Usage of Helicoll on Exposed Bones



Bone-exposed wounds were successfully healed with Helicoll without the immediate need of for skin graft.

Sacral Pressure Sore

Stage 4 pressure sore

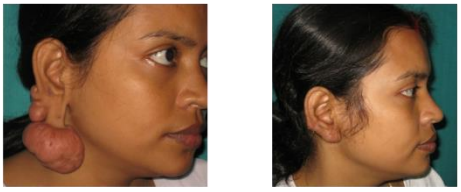
10 Days Post Helicoll application

Completely healed using Helicoll

Complete Recovery in 6 weeks

Courtesy: Vinoth Philip , MD, DNB, Plastic Surgeon

Keloid excision and closure



Before

After

No re-occurring of Keloid after healing with Helicoll

Usage of Helicoll in treating Burns

Clinical study of 43 patients with second degree burns, age range 1 to 57 years were randomized to receive Helicoll (n=23) or 1% silver sulphadiazine (n=22). Helicoll resulted in a statistically significantly shorter time to healing (7.2 days vs. 14.5 days, p=0.005). Healing was enhanced by 49.7% in the Helicoll group compared to the silver sulphadiazine group. Itching was significantly decreased in the Helicoll group (90.5% vs. 71.1% without itching).[8,30]

26 burn patients were treated with Helicoll, compared to conventional dressings in a multi-center study. There was a 4-fold increase in rate of healing in the Helicoll group compared to the control gauze group.[31]

Vishal Mago, MD, unpublished report on "First & Second-Degree Burn Treatment Trial of Collagen [Helicoll] Dressing vs. Silver Sulphadiazine Alone," as randomized, controlled study of efficacy and safety on 15 patients with clinical burns, 2007. Better wound pain control with Helicoll.

Helicoll usage in treating pediatric and adult burn cases: https://helicoll.com/case-reports/

Helicoll used to Heal Split Thickness Skin Grafts (STSG)

60 patients with donor sites were selected at random at different centers and treated with varied acellular dermal replacement collagen dressings to compare the effectiveness of Helicoll with other collagen dressings. There was no pain on opening the dressing and patients had no discomfort. Helicoll achieved a greater patient comfort level as well as an accelerated healing rate compared to other collagen dressings.[6]

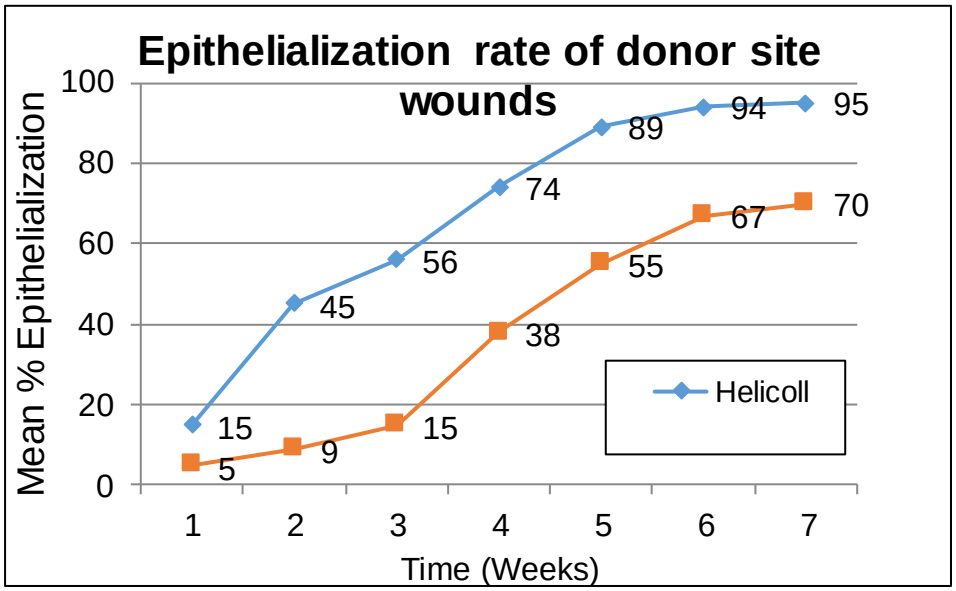


Figure 11: Rate of epithelialization in 60 patients whose donor sites were treated with Helicoll[6]

22 patients with skin graft donor site wounds were included to undergo treatment with Helicoll. Twenty of these patients had no pain, no restriction of mobility, no infection when used per the protocol and the time to heal was significantly faster when compared to other conventional dressings.[7]

158 patients with STSG were successfully treated with Helicoll in 2010. Study included measurement of pain reduction of patients treated with Helicoll compared to control patients.

Reduced itching, increased range of motion, and overall increased patient comfort were also experienced by patients treated with Helicoll for burns and STSGs in this study (see Figure 13).[11]

Helicoll collagen dressing treatment showed 41% improvement over other Standard Care Treatments in a Clinical Study of split skin graft donor sites. Helicoll treated wounds healed in 7-10 days compared with 10-12 days with a traditional treatment.[11]

Collagen reduces post-operative donor site pain. There was a significant reduction in post-operative pain in the collagen dressings upon application of the product, at days 1 and 2, and throughout the treatment process until complete healing when compared to the other gauze groups ($p < 0.02$).[11]

Figure 13 below shows slight increase in pain on Helicoll patients on days 4 and 5 which corresponds to the infiltration of the live tissue cells into Helicoll as part of normal healing process.

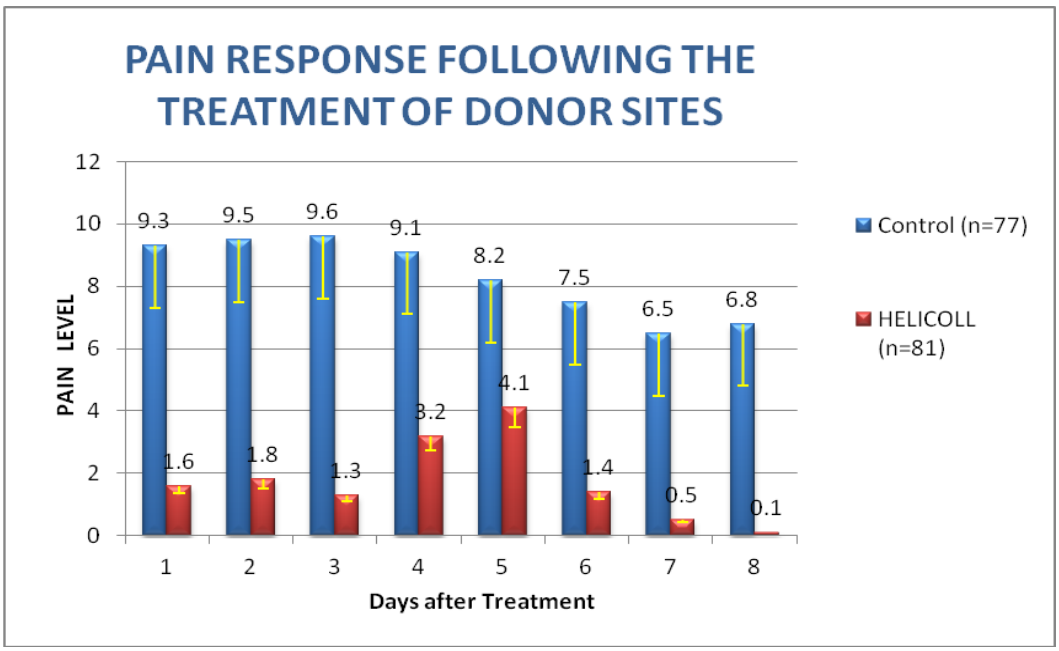


Figure 12: Pain Response in Helicoll and Control Wounds[11]

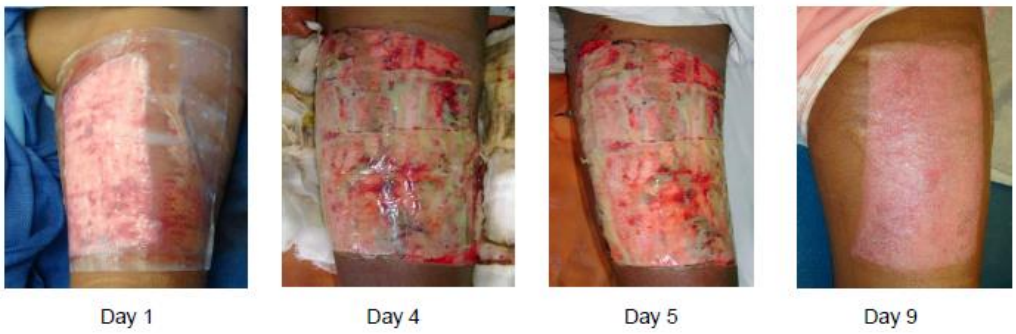


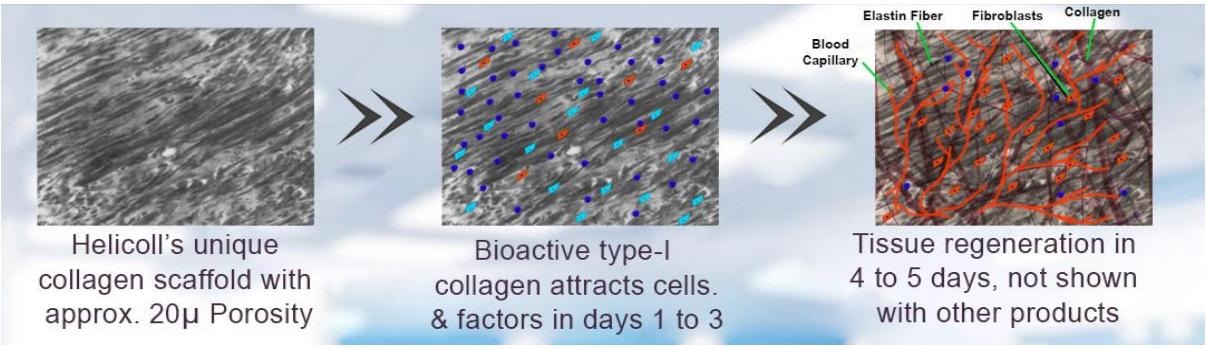
Figure 13: Donor site treatment using Helicoll[11]

Unique Features:

How Helicoll Collagen Differs From Other Collagen Products:

EnColl holds patented and proprietary technology and methods to manufacture highly-purified, medical-grade, non-immunogenic Type-I collagen, and to make other surface modifications to enhance the bioactivity of the protein. This method addresses the problems presented by commonly used other collagen preparations. EnColl's patented process removes all non-collagenous materials, while retaining the native molecular quaternary structure and other characteristic features of collagen (e.g., length, diameter, and periodicity of collagen Type-I fibrils, (Ref. US Pat # 6,548,077, Purifying type I collagen using two papain treatments and reducing and delipidation agents, 2003).

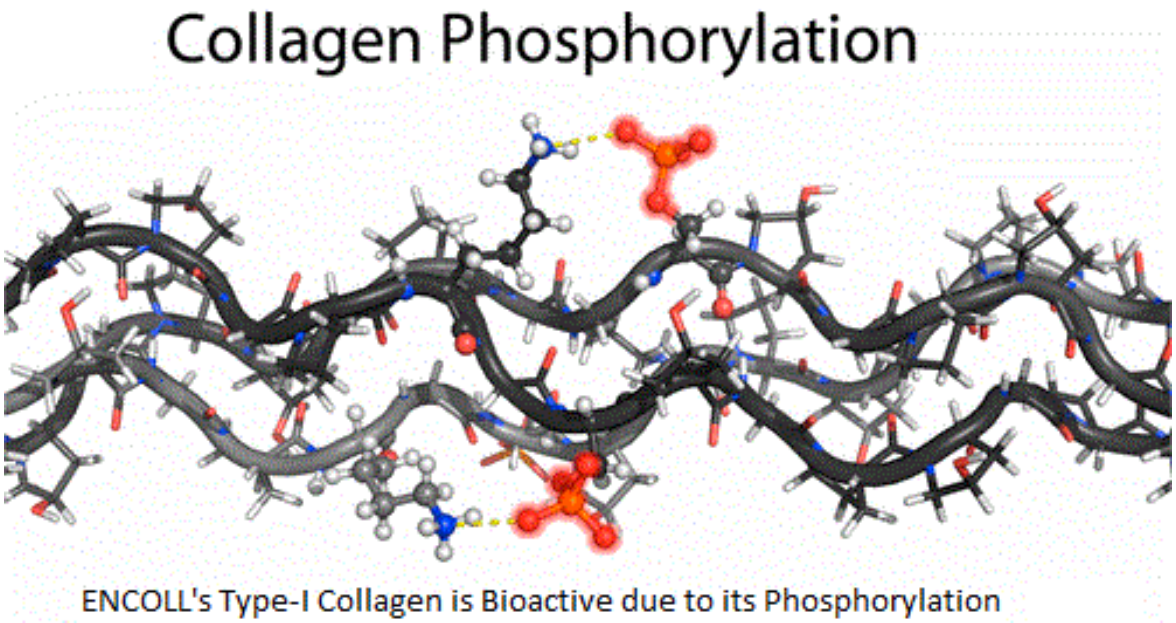
Structural Advantage of Helicoll Collagen Compared to Others



The clinical efficacy of our product Helicoll, is proven by the clinical studies conducted at the Dermatology Department of Stanford University to document the fast wound healing. Also the study shows when Helicoll is used, the patients expressed immediate pain relief upon the application to the wounded area. (see the Clinical data attached)

Helicoll's Unique Features: It contains native collagen that is pure and non-immunogenic, it will possess all the natural binding sites to all the cytokines, including epidermal growth factor (EGF), fibronectin, fibrinogen, histamine, platelet-derived growth factor (PDGF), serotonin, and von Willebrand factor etc…A cross-linked or contaminated Type III collagen cannot effectively achieve such wound healing characteristics. Additionally, a native collagen product also has cell membrane binding sites that would attract the neutrophil (for debris scavenging, and bacteria destruction) and leukocytes along with the macrophages/monocytes (for wound healing via secretion of enzymes and cytokines for tissue reconstruction). Subsequently, epithelialization, angiogenesis, granulation tissue formation, and collagen deposition are the principal steps in this anabolic portion of wound healing. Above all these unique features to Helicoll, in order to boost the product's bio-activity, the purified type-I collagen is further taken through a native biological surface modification process called phosphorylation. This is a normal physiological biochemical pathway of collagen during the process of tissue growth or repair or remodeling. This has been adopted to increase our collagen's bio-activity through cell-signal transduction commonly happening via protein phosphorylation methods.

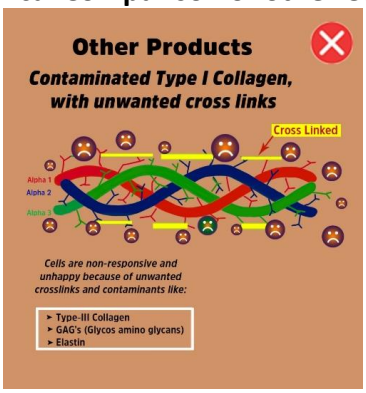
Unfortunately, most other collagen preparations or all of the intact tissue derived products made from amnion, pericardium, intestinal wall, urinary bladder etc. They all have to be contaminated with approximately 15% Elastin, a potential carcinogen, and type-III collagen lipids and other proteo-glycans which are all highly immunogenic. All such products to be tolerated by the host tissue, have to follow chemical cross-linking method to minimize their immunogenicity. While they are compelled to do chemical cross-linking to their



ENCOLL's Type-I Collagen is Bioactive due to its Phosphorylation

constructs, it damages the native chemistry of the biologically valuable type-I collagen also - and thereby then entire construct loses its bioactivity and also other binding abilities and significantly impaired with its wound healing abilities.

Biochemical Comparison of other Collagens



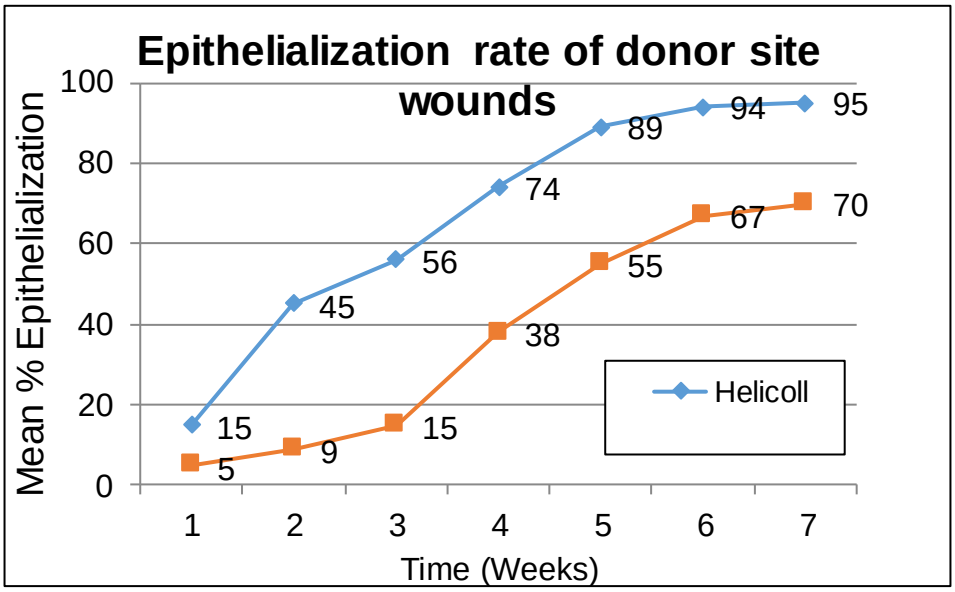
Helicoll is highly biocompatible, non-immunogenic and bioactive. US patents prove the purity of type-I collages as well as the surface chemistry modification through phosphorylation enhances the cell signaling practically reduces the healing time and would have better patient care and safety by default compared to other products.

HELICOLL IS UNIQUE IN ITS CLINICAL OUTCOME AS IT IS BASED ON PATENTED HIGH PURITY TYPE-I COLLAGEN AND DOES NOT CONTAIN ANY IMMUNOGENIC & POTENTIALLY CARCINOGENIC ELASTIN OR OTHER POTENTIAL IMMUNOGENIC MOLECULES, LIKE ALL OTHER INTACT TISSUE DERIVED PRODUCTS POSSESS. AS A RESULT, HELICOLL IS THE ONLY PRODUCT CLINICALLY PROVEN TO HAVE THE NEW BLOOD CAPILLARIES FORMED IN THE MATRIX WITHIN 4 TO 5 DAYS UPON APPLICATION. (SEE USP MONOGRAPH ATTACHED). ANOTHER STUDY ALSO HAS PROVEN THE CHEMOTACTIC CELL ATTRACTION TOWARDS HELICOLL COLLAGEN IN A CELL CULTURE MODEL.

EVIDENCE OF BENEFITS OF HELICOLL

Based on the above Preclinical and CLINCIAL EVIDENCE, Helicoll skin substitute membranes offers the following benefits:

- biocompatible and hypoallergenic, US Patents – see below.
- Clinical evidence for product efficacy, shown by the above clinical studies
- faster wound healing[11]
- wound granulation and epithelialization in 4-5 days instead of 21–28 days (see)
- reduced pain (see
-)
- lesser scar formation, (Clinically shown)
- return of native skin pigmentation, (see Figure 14)
- Helicoll expedited healing in all cases and contained infection,
- Promoted healthy granulation tissue, and stimulated a wound bed that better supported a skin graft.
- Itching was reduced.
- Time to healing was hastened, and hence, total cost of treatment was also lessened (see
- ,



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Reduced itching, increased range of motion, and overall increased patient comfort were also experienced by patients treated with Helicoll for burns and STSGs in this study (see Figure 13).[11]

Helicoll collagen dressing treatment showed 41% improvement over other Standard Care Treatments in a Clinical Study of split skin graft donor sites. Helicoll treated wounds healed in 7-10 days compared with 10-12 days with a traditional treatment.[11]

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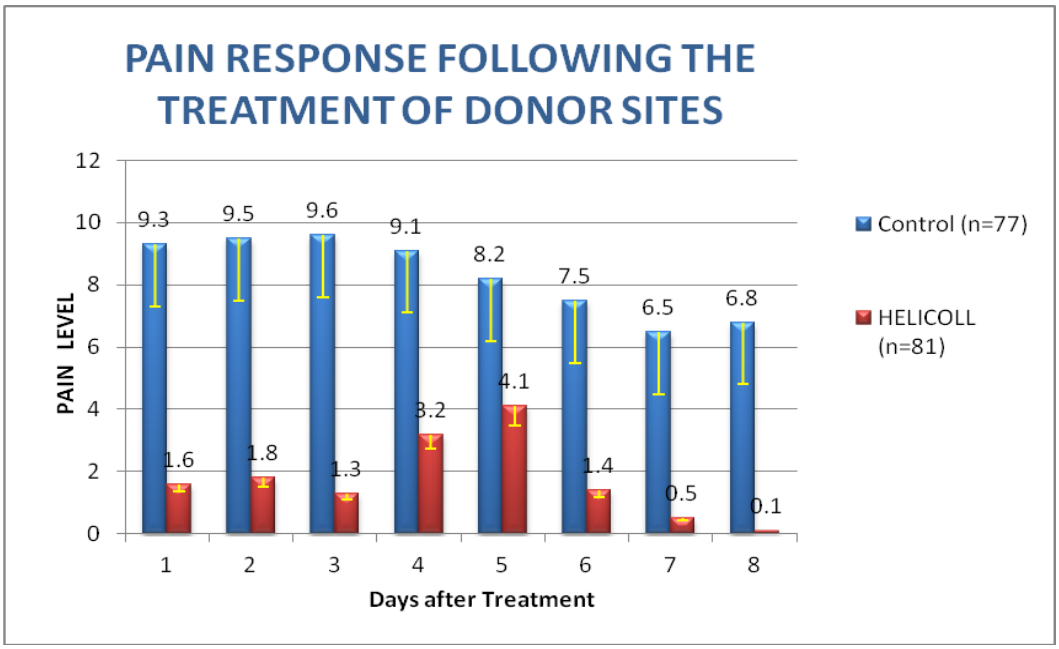

Figure 12: Pain Response in Helicoll and Control Wounds[11]

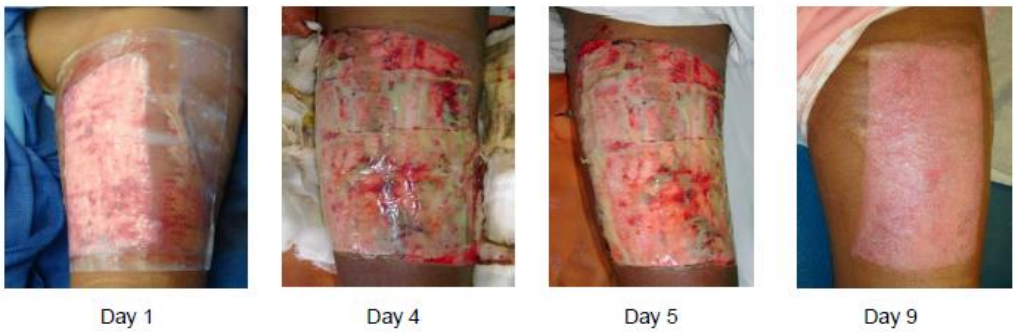

Figure 13: Donor site treatment using Helicoll[11]

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Helicoll, biological skin substitute collagen membrane normally comes in sizes from 0.5 in dia disc to 2x4 inches. Smaller or Larger sizes to cover larger body areas can be easily produced.

To date, Helicoll has been used on over 97,000 patients (by May 2018) primarily by private and university hospital professional health care providers. There have been no signs of adverse reactions. We believe that the product Helicoll is the most effective (faster wound healing), efficient (shorter time to apply and less dressing changes are required), durable (has high tensile strength) and easy to use (training physicians, nurses, medical assistants, patients and care givers takes less than 15 minutes) wound-healing product on the market. It is safe for neonates and infants or geriatrics and is currently used on wounds and burns.

Unique Benefits or Advantages to Clinical Outcomes:

- **High purity Type-I Collagen:** Healicoll is a patented reconstituted bioactive collagen sheet, free of immunogenic proteins, lipids, and elastin. The native structure of collagen is not altered or cross-linked which maintains its high bioactivity.
- **Faster Healing:** Collagen phosphorylation attracts cells, regenerates tissue, and stimulates blood capillaries/granulation within 4 to 5 days.
- **Innovative Technology:** Better than intact tissue-based membranes like amnion, intestinal wall, urinary bladder etc. which contain 15% elastin.
- **Pain Control:** Effectively reduces pain.
- **Easy Application**: No washing needed prior to use. The overall clinical usage of Healicoll is simple and easy as it can be cut, sutured or stapled.
- **Cost-Effective**: Accelerated wound healing and tissue remodeling with minimal applications reduce the treatment cost by over 40%.
- **Various Sizes:** Choose from standard or customized dimensions.
- **Long Shelf Life:** Remains clinically usable for 3 years when stored in room temperature conditions.
- **High Reimbursement:** Medicare recognizes Helicoll as a high-cost skin substitute continuously since 2017.

	HELICOLL® COMPARISION WITH OTHER FDA APPROVED PRIME PRODUCTS							
PRODUCT	**HELICOLL®**	**APLIGRAF® /DERMAGRAFT®**	**PURAPLY® /PURAPLY® AM**	**XWRAP®**	**OASIS™**	**INTEGRA™ /PRIMATRIX**	**EPIFIX™ /AMNIOFIX™**	**CYTAL™**
Manufacturer	**ENCOLL Corp.**	**Organogenesis Inc.**	**Organogenesis Inc.**	**Applied Biologics**	**Smith & Nephew**	**Integra LifeSciences**	**MiMedX**	**Acell**
Matrix	Patented high purity bovine Type-I collagen	Human fibroblast - on bovine Type I collagen/polyglactin mesh	Porcine intestinal cross-linked type-III collagen	Amniotic Membrane Derived Allograft with Carcinogenic Elastin[1]	Porcine small intestinal submucosa (SIS) with 10% Carcinogenic Elastin[2]	Collagen with or without glycosaminoglycan and a silicone layer	Dehydrated Human Amnion/Chorion Allograft with 42% Carcinogenic Elastin[1]	Porcine urinary Bladder Xenograft with 9% Carcinogenic Elastin[3]
Size/shape	5x5 cm to 60x60 cm & Custom sizes	Circular, 8 cm dia, disc / 5 cm x 7.5 cm	1.6 cm disc to 8x16 cm sizes	2x2 cm to 4x8 cm sizes	3x3.5 cm to 7x20 cm in sizes	2x2 cm to 20x25 cm in sizes	2x2 cm to 4x6 cm in sizes	3x3.5 cm to 10x15 cm in sizes
Sterilization	Terminal sterilization	Aseptically processed	Terminal sterilization using gamma that might denature/cross-link collagen	Terminal Sterilization	Terminal sterilization	Aseptically processed	Terminal sterilization	Terminal sterilization
Shelf life	3 years at room temperature	5 days at room temperature	Greater than 2 years	2 years & Requires Refrigeration	2 years at room temperature	1 year at room temp.	5 years at room temperature	2 years at room temperature
Handling	Rehydrates in saline in 5 min.; easily handled, sutured & stapled	Shipped on a nutrient medium/frozen; difficult to handle; fragile	Rehydrates in saline	Rehydrates in saline	Rehydrates in saline; easily sutured & stapled	Can be sutured & stapled; easily handled	Can be sutured & stapled; easily handled	Can be sutured & stapled; easily handled
Large presence of immunogenic Elastin/ adverse biomolecules	No	No	Yes (significant amnt of Type-III Collagen)	Yes (>15% elastin presence)	Yes (>15% elastin presence)	Yes (significant amnt of GAGs)	Yes (>15% elastin presence)	Yes (>15% elastin presence)
Bioactivity expressed via neo-vascularization & granulation	**Within 4 to 5 days after application (Clinically proven)**	No report indicates lesser than 9 days	No such fast infiltration of blood vessels is reported	No report indicates lesser than 9 days	No report indicates lesser than 9 days	No report indicates lesser than 9 days	No report indicates lesser than 9 days	No report indicates lesser than 9 days
Applications to Heal	1-4 applications	Up to 5 applications	variable	variable	variable	variable	variable	variable
Control of hyper glycosylation of Diabetic Foot Ulcer Wounds to heal fast	Yes	No	No	No	No	No	No	No
Total Advantages of the product	**9 of 9**	**1 of 9**	**3 of 9**	**2 of 9**	**2 of 9**	**1 of 9**	**3 of 9**	**2 of 9**

Note: Intact tissue-based membrane products (like OASIS™, EPIFIX™, AMNIOFIX™, CYTAL™) naturally contain at least 15% of high immunogenic compound namely Elastin, besides other allergenic biological molecules like glycosaminoglycans and certain types of collagen other than Type-I collagen.

Ref 1. https://pubmed.ncbi.nlm.nih.gov/16968153/ 2. https://link.springer.com/article/10.1007/s10029-020-02238-y 3. https://pubmed.ncbi.nlm.nih.gov/9852359/

Unique Benefits to Safety:
All of the following aspects are well addressed with proper production and management documents to comply the Medical Product regulations.
(i) Periodic monitoring of the results of the risk analysis
(ii) Specification of materials, and manufacturing/special processing
(iii) Specifications, drawings and circuit diagrams for components, sub- assemblies and the complete product including packaging, where appropriate.
(iv) The specifications of the checks, tests and trials that are intended to be carried out as part of routine production
(v) The performances and compatibilities intended by the manufacturer
(vi) Labeling, including any instructions for use
(vii) Identification of 'shelf-life' reflected by any 'use by' date, or other 'lifetime' of the device(s)
(viii) Results of Bench Testing
(viii) Clinical data
(ix) Post market surveillance
(x) Documentation and reporting Design Changes

Additional Non-clinical Benefits:
HELICOLL is a better cost-effective product than most other advanced skin substitutes on the market. Also, the easy use, storage and long shelf life are all added features to the product.

Our team of Wound Care specialists are extremely happy to assist your team of experts to bring the public awareness to the regenerative aspects of wound care that uses nanotechnology that bio-mimics the physiological biochemical pathways of wound healing process.

Names of close Competitors:

Q4101 Apligraf	Q4134 hMatrix
Q4102 Oasis Wound Matrix	Q4135 Mediskin
Q4104 Integra BMWD	Q4136 Ezderm
Q4106 Dermagraft	Q4150 Allowrap DS or Dry 1 sq cm
Q4107 GraftJacket	Q4151* AmnioBand, Guardian 1 sq cm
Q4110 Primatrix	Q4152* Dermapure 1 square cm
Q4111 Gammagraft	Q4153 Dermavest 1 square cm
Q4115 Alloskin	Q4154* Biovance 1 square cm
Q4116 Alloderm	Q4160 NuShield 1 square cm
Q4121 Theraskin	Q4161 Bio-Connekt per square cm
Q4122 Dermacell, awm, porous sq cm	Q4163 Woundex, bioskin, per sq cm
Q4123 Alloskin	Q4164 Helicoll, per sq cm
Q4124 Oasis Tri-layer Wound Matrix	Q4165 Keramatrix, kerasorb sq cm
Q4126 Memoderm/derma/tranz/integup	Q4186 Epifix
Q4127 Talymed	Q4195+ Puraply 1 sq cm
Q4133 Grafix stravix prime pl sq cm	Q4203 Derma-gide, 1 sq cm
	Q4204 Xwrap 1 sq cm
	Q4221 Amniowrap2 per sq cm

What Differentiates Encoll's Helicoll product from the Competitors:
Encoll has over thirty years expertise in the field of surgical applications of collagen. The founder and CEO of Encoll has technical contributions in the development of standards for manufacturing surgical grade collagen. This fact is evidenced by Dr. Gunasekaran's active involvement in the steering committee along with the FDA group headed by Dr. David Kaplan and other experts at ASTM (American Standards for Test Methods). Resulted in 1995 a ASTM as the guidelines to the manufacturers of Type-I collagen. (Ref: ASTM: F 2212–09 Standard Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products).

Encoll's expertise in the technical aspects of the development of non-immunogenic biological construct is enormous. Among the other fifty products in the market that are Medicare approved, almost half of them are manufactured from intact tissues. The manufacturers of such products are given authority to claim their products are "Biological skin substitutes". Unfortunately, these manufacturers don't realize that 15% elastin is still present in their final product which is NOT bio-compatible & may be a potential carcinogen. Thereby, they have to cross-link the finished product to eliminate the rejection of those products. This action results in the loss of bio-activity for the entire product. This is the MAJOR difference when compared to Helicoll that is not endpoint cross-linked in any manner.

Another set of manufacturers who produce products with cultured cells are also having difficulties to manage the incompatibility or immunogenicity of certain structural proteins synthesized by those cells harvested from another individual. Furthermore, all such cell seeded products have an extremely high risk in maintaining sterility of their end product with a very short shelf life and an enormous cost of production.

I prefer the reviewer of this document to consider the pros and cons of intact tissue based skin substitutes. Recently approved innovative technology products namely ACell and MiMedx are derived from intact tissues. ACell MS2550 (derived from porcine urinary bladder) & MiMedx MS3680 (derived from amniotic tissue). Naturally all such intact tissue based products do have at least 15% of high immunogenic & potentially carcinogenic compound namely Elastin, besides other allergenic biological molecules like glycosaminoglycans and certain types of collagen other than type-I collagen.

Both the above products containing potential immunogenic molecules have to be surface charge modified (cross-linked) to minimize their immunogenicity. As a result, the above products are not capable to be a bio-active construct for faster healing and repair of the damaged tissues.

HELICOLL:
On the other hand, Helicoll is nothing but highly bioactive and bio-compatible type-I collagen construct. That's why, Helicoll has been clinically documented for faster wound healing. (see the recent Stanford University Dermatology clinical study publication)
It encourages the formation of **new blood capillaries within 4 to 5 days** upon the application of the product over the wound.
No other skin substitute has shown such an advanced clinical result.

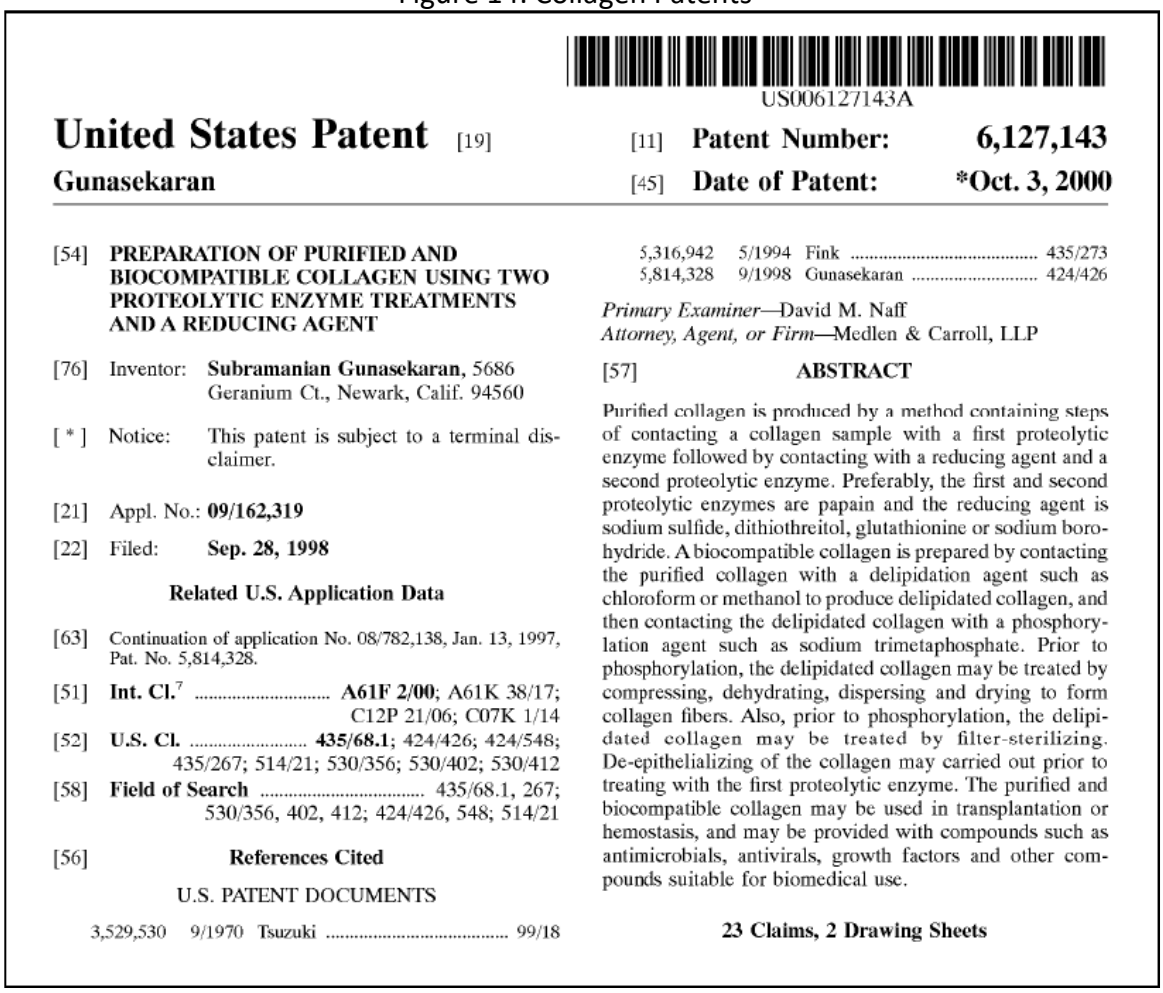
Name of Reference Clinical Users:

Dr. Christopher Cox Dayton, OH 45402 Travis Perry, MD Dayton, OH 45409 Morris Brown, MD Dayton, OH 45402 Dr. Tanisha Richmond, DPM Charles Drew Health Center Dayton, OH 45402 Dr. Sekhar Sompalli The Perry Orthopedic & Sports Med Clinic Chicago, Il 60611 Dr. Walter F. D'Costa Santa Rosa, CA 95403 Dr. Howard Sutkin Advanced Aesthetics and Plastic Surg Clinic Los Gatos, CA 95032 Dr. Marilyn Kwolek Danville, CA 94526 Dr. Prasad Kilaru Wound Clinic Washington Hospital Fremont, CA 94538 Dr. Tariq Mirza Ariba Healthcare Group San Jose, CA 95112 Stanford University Dept. of Dermatology Redwood City, CA 94063	CCS Medical Englewood, CO 80112 O' Conner Hospital San Jose, CA 95128 Dr. Benninghoven Scott W MD St. Louise Hospital, Gilroy, CA 95020 St. Francis Mem. Hospital San Francisco, CA 94109 Healthy Living Foundation Pinole CA 94564 Dr. Robert Beer Balfour Dermatology Brentwood, CA 94513 Capitol Logistics Muscat, Oman Dr. Renuka Bhatt Fine Skin Orland Park, IL 60467 Dr. Larry Woodcox Podiatric Foot and Ankle Surgery Oakland, CA 94612 Dr. Mark Miller Vista, CA 92081

Mfg. Product/NDC #:	Helicoll® Bioengineered Skin Substitute 0.5 in dia disc/1.27 cm dia disc (1 sq cm) NDC # 74745-0052-02 1.0 in dia disc/2.54 cm dia disc (5 sq cm) NDC # 74745-0102-02 0.8 in x 1.6 in/2 cm x 4 cm (8 sq cm) NDC # 74745-0082-02 1.2 in x 1.6 in/3 cm x 4 cm (12 sq cm) NDC # 74745-0122-02 1.6 in x 1.6 in/4 cm x 4 cm (16 sq cm) NDC # 74745-0162-02 2in x 2in (5cm x 5cm=25 sq cm) NDC # 74745-0225-05 2in x 4in (5cm x 10cm=50 sq cm) NDC # 74745-0245-05 (Other sizes can also be provided)
FDA Clearance:	Yes FDA 510K (attached) Following FDA Letter to File to recognize the product as a Biological Skin Substitute (attached) Medicare approval as a Bio-engineered skin substitute with HCPCS code Q4164. (attached)
PMA, 510(k), NDC or Exempt:	K040314 followed with FDA Letter to File to refer as Bioresorbable Skin Substitute
When the Technology Product made available for sale in USA:	Made available from 2006. Medicare approved as High Cost Skin Substitute (code Q4164) continuously since 2017.
Reimbursement:	Yes
Code:	Q4164
Peer Reviewed Clinical Trials:	Yes (attached)
Certified Diverse Minority / Small Business	YES. Registered with SAM.gov (monitored through SBA) as a minority owned small business.

Patent / Intellectual Property	U.S. Patents associated with the Helicoll Technology are: 5,814,328 - 1998; 6,127,143 - 2000; 6,548,077- 2003
Distributor in the US	Yes. Also we sell Directly
Website(s):	www.encoll.com, www.helicoll.com
Email comments to:	guna@encoll.com or murugan@encoll.com

Figure 14: Collagen Patents



US006127143A

United States Patent [19]

Gunasekaran

[11] **Patent Number: 6,127,143**

[45] **Date of Patent: *Oct. 3, 2000**

[54] **PREPARATION OF PURIFIED AND BIOCOMPATIBLE COLLAGEN USING TWO PROTEOLYTIC ENZYME TREATMENTS AND A REDUCING AGENT**

[76] Inventor: **Subramanian Gunasekaran**, 5686 Geranium Ct., Newark, Calif. 94560

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **09/162,319**

[22] Filed: **Sep. 28, 1998**

Related U.S. Application Data

[63] Continuation of application No. 08/782,138, Jan. 13, 1997, Pat. No. 5,814,328.

[51] **Int. Cl.**[7] **A61F 2/00**; A61K 38/17; C12P 21/06; C07K 1/14

[52] **U.S. Cl.** **435/68.1**; 424/426; 424/548; 435/267; 514/21; 530/356; 530/402; 530/412

[58] **Field of Search** 435/68.1, 267; 530/356, 402, 412; 424/426, 548; 514/21

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,529,530 9/1970 Tsuzuki 99/18
5,316,942 5/1994 Fink 435/273
5,814,328 9/1998 Gunasekaran 424/426

Primary Examiner—David M. Naff
Attorney, Agent, or Firm—Medlen & Carroll, LLP

[57] **ABSTRACT**

Purified collagen is produced by a method containing steps of contacting a collagen sample with a first proteolytic enzyme followed by contacting with a reducing agent and a second proteolytic enzyme. Preferably, the first and second proteolytic enzymes are papain and the reducing agent is sodium sulfide, dithiothreitol, glutathionine or sodium borohydride. A biocompatible collagen is prepared by contacting the purified collagen with a delipidation agent such as chloroform or methanol to produce delipidated collagen, and then contacting the delipidated collagen with a phosphorylation agent such as sodium trimetaphosphate. Prior to phosphorylation, the delipidated collagen may be treated by compressing, dehydrating, dispersing and drying to form collagen fibers. Also, prior to phosphorylation, the delipidated collagen may be treated by filter-sterilizing. De-epithelializing of the collagen may carried out prior to treating with the first proteolytic enzyme. The purified and biocompatible collagen may be used in transplantation or hemostasis, and may be provided with compounds such as antimicrobials, antivirals, growth factors and other compounds suitable for biomedical use.

23 Claims, 2 Drawing Sheets

US006548077B1

(12) **United States Patent**
Gunasekaran

(10) **Patent No.: US 6,548,077 B1**
(45) **Date of Patent: *Apr. 15, 2003**

(54) **PURIFYING TYPE I COLLAGEN USING TWO PAPAIN TREATMENTS AND REDUCING AND DELIPIDATION AGENTS**

(76) Inventor: **Subramanian Gunasekaran**, 5686 Geranium Ct., Newark, CA (US) 94560

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/677,646**

(22) Filed: **Oct. 3, 2000**

Related U.S. Application Data

(63) Continuation of application No. 09/162,319, filed on Sep. 28, 1998, now Pat. No. 6,127,143, which is a continuation of application No. 08/782,138, filed on Jan. 13, 1997, now Pat. No. 5,814,328.

(51) **Int. Cl.**[7] **A61F 13/00**; A61F 2/00; A61K 38/17; C12P 21/06; C07K 1/14

(52) **U.S. Cl.** **424/422**; 424/426; 424/548; 435/68.1; 435/267; 514/21; 530/356; 530/402; 530/412

(58) **Field of Search** 424/422, 426, 424/548; 435/68.1, 267; 514/21; 530/356, 402, 412

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,202,566	A		5/1940	Schulte 18/54
3,113,568	A		12/1963	Robins 128/156
3,157,524	A		11/1964	Artandi 106/122
3,491,760	A		1/1970	Braun et al. 128/334
3,526,530	A	*	9/1970	Tsuzuki 426/32
3,664,844	A		5/1972	Miller 99/18
3,800,792	A		4/1974	McKnight et al. 128/156
4,148,664	A		4/1979	Cruz, Jr. 106/161
4,350,629	A		9/1982	Yannas et al. 260/123.7
4,407,787	A		10/1983	Stemberger 424/28
4,409,332	A		10/1983	Jefferies et al. 435/188
4,412,947	A		11/1983	Cioca 260/123.7
4,424,208	A		1/1984	Wallace et al. 424/177
4,485,095	A		11/1984	Fujisaki et al. 424/94
4,485,097	A		11/1984	Bell 424/95
4,488,911	A		12/1984	Luck et al. 106/161
4,511,653	A		4/1985	Play et al. 435/69
4,536,716	A		9/1985	Bell 623/1
4,546,500	A		10/1985	Bell 623/1
4,557,764	A		12/1985	Chu 106/161
4,597,762	A		7/1986	Walter et al. 623/1
4,600,533	A		7/1986	Chu 530/356
4,604,346	A		8/1986	Bell et al. 435/1
4,655,980	A		4/1987	Chu 264/102
4,687,518	A		8/1987	Miyata et al. 106/161
4,689,399	A		8/1987	Chu 530/356
4,703,108	A		10/1987	Silver et al. 530/356
4,725,671	A		2/1988	Chu et al. 530/356
4,772,288	A		9/1988	Borner et al. 8/94.11

(List continued on next page.)

OTHER PUBLICATIONS

Junqueira and Carneiro, *Basic Histology*, 4th ed., Lange Medical Publications, Los Altos, CA, pp. 89–119[1983].

Prudden, "Wound Healing Produced by Cartilage Preparations," *Arch. Surg.* 89:1046–1059 [1964].

Peacock et al., "Use of Tanned Collagen Sponges in the Treatment of Liver Injuries," *Ann. Surg.* 161:238–247 [1965].

Beachey et al., "Collagen–Platelet Interaction," *in International Review of Connective Tissues*, (Eds. Chvapil et al.) Academic Press, 6:1–55 [1979].

Smith and Kimmel, "Papain (with a Section on Ficin)," *in The Enzymes*, vol. 4, 2d ed., (Eds. Boyer et al.) Academic Press, NY, p. 138 [1960].

Coulson, "The Effect of Proteolytic Enzymes on the Tensile Strength of Whole Aorta and Isolated Aortic Elastin," *Biochim. Biophys. Acta* 237:378–385 [1971].

Smith et al., "Pharmacology: Enzyme Dissolution of the Nucleus Pulposus," *Nature* 198:1311–1312 [1963].

Nimni et al., "Bioprosthesis derived from crosslinked and chemically modified collagenous tissues," in *Collagen*, vol. 3, (Eds. M.E. Nimni et al.) CRC Press, Inc., Boca Raton, FL, pp. 1–39 [1988].

Amiel et al., "Biochemistry of tendon and ligament," in *Collagen*, vol. 3, pp 223–229.

Soskel et al., "Isolation and Characterization of Insoluble and Soluble Elastins," *Meth. Enzymol.* 144:196–215 [1952].

Hill and Harper, "Quantitation of Types I and III Collagens in Human Tissue Samples and Cell Culture by Cyanogen Bromide Peptide Analysis," *Anal. Biochem.* 141:83–93 [1984].

(List continued on next page.)

Primary Examiner—David M. Naff
(74) *Attorney, Agent, or Firm*—Medlen & Carroll LLP

(57) **ABSTRACT**

Purified collagen such a type I collagen is produced by a method containing steps of contacting collagen with a first proteolytic enzyme followed by contacting with a reducing agent and a second proteolytic enzyme. Preferably, the first and second proteolytic enzymes are papain and the reducing agent is sodium sulfide, dithiothreitol, glutathionine or sodium borohydride. In a further step, the purified collagen may be contacted with a delipidation agent such as a mixture of chloroform and methanol to produce delipidated collagen. The delipidated collagen may be filter-sterilized and contacted with a phosphorylation agent such as sodium trimetaphosphate to produce phosphorylated collagen. The delipidated collagen may also be treated by compressing, dehydrating, dispersing and drying to form collagen fibers. De-epithelializing of the collagen may carried out prior to treating with the first proteolytic enzyme. The collagen may be solubilized using a solubilizing agent such as acetic acid. The purified collagen is biocompatible and may be used in transplantation or hemostasis, and may be provided with compounds such as antimicrobials, antivirals, growth factors, anti-dehydration compounds and other compounds suitable for biomedical use.

20 Claims, 2 Drawing Sheets

U.S. PATENT DOCUMENTS

4,789,401	A		12/1988	Ebinger et al. 106/122
4,835,102	A		5/1989	Bell et al. 435/29
4,837,379	A		6/1989	Weinberg 424/101
4,841,962	A		6/1989	Berg et al. 128/156
4,883,864	A		11/1989	Scholz 530/356
4,894,441	A		1/1990	Menicagli 530/356
4,931,546	A		6/1990	Tardy et al. 530/356
4,948,540	A		8/1990	Nigam 264/28
4,950,483	A		8/1990	Ksander et al. 424/422
4,969,912	A		11/1990	Kelman et al. 623/66
4,975,527	A		12/1990	Koezuka et al. 530/356
5,002,071	A		3/1991	Harrell 128/897
5,028,597	A		7/1991	Kodama et al. 514/56
5,028,695	A		7/1991	Eckmayer et al. 530/356
5,043,426	A		8/1991	Goldstein 530/356
5,064,941	A		11/1991	Davison 530/356
5,081,106	A		1/1992	Bentley et al. 514/5
5,104,660	A		4/1992	Chvapil et al. 424/445
5,104,957	A		4/1992	Kelman et al. 527/201
5,106,949	A		4/1992	Kemp et al. 530/356
5,116,389	A		5/1992	Mitz 8/127.5
5,162,506	A		11/1992	Hadden 530/412
5,229,497	A		7/1993	Boni 530/356
5,316,942	A	*	5/1994	Fink 435/273
5,332,802	A		7/1994	Kelman et al. 530/356
5,374,539	A	*	12/1994	Nimni et al. 435/68.1
5,383,930	A		1/1995	Brannan et al. 623/11
5,814,328	A	*	9/1998	Gunasekaran 424/426
6,127,143	A	*	10/2000	Gunasekaran 435/68.1

OTHER PUBLICATIONS

Folch et al., "A Simple Method for The Isolation and Purification of Total Lipides From Animal Tissues," *J. Biol. Chem.* 226:497–509 [1957].

Skipski et al., "Quantitative Analysis of Phospholipids by Thin–Layer Chromatography," *Biochem. J.* 90:374–378 [1964].

Rouser et al., "Quantitative Analysis of Brain and Spinach Leaf Lipids Employing Silicic Acid Column Chromatography and Acetone for Elution of Glycolipids," *Lipids* 2:37–40 [1967].

Stegeman, "Mikrobestimmung von Hydroxyprolin mit Chloramin–T und p–Dimethylaminobenzaldehyd," *J. Physiol. Chem.* 311:41–45 [1958].

Blumenkrantz and Absoe–Hansen, "An Assay for total Hexosamine and a Differential Assay for Glucosamine and Galactosamine," *Clin. Biochem.* 9:269–274 [1976].

Fiske and Subbarow, "The Colorimetric determination of Phosphorus," *J. Biol. Chem.* 66:375–400 [1925].

Bradenberger and Hanson, "Eine spektrophotometrische Bestimmungsmethode für sauer und alkalische Phosphatasen," *Helv. Chim. Acta* 36:900–907 [1953].

Hofstee, "Direct and Continuous Spectrophotometric Assay of Phosphomonoesterases," *Arch. Biochem. Biophys.* 51:139–146 [1954].

Gunasekaran et al., "Collagen/Phospholipid Interaction: Possible role in Skin Wound Dressings," *Proc. Soc. Biomater.* 20:311 [1994].

Simmons et al., "Corneal Epithelial Wound Closure in Tissue Culture: An *in Vitro* Model of Ccular Irritancy," *Toxicol. Appl. Pharmacol.* 88:13–23 [1987].

Jumblatt and Neufeld, "A Tissue Culture Assay of Corneal Epithelial Wound Closure," *Invest. Ophthamol. Visual Sci.* 15:8–13 [1986].

Gunasekaran, "Collagen–Based Wound Dressings: How to Control Infection and Immune Response," *in Encyclopedia of Biomaterials and Bioengineering*, 2(37):1267–1284 [1995].

Brown, *Hematology: Principles and Practice*, 3d ed., Eds. Lea & Febiger, Philadelphia, pp. 125–126 [1980].

* cited by examiner

STATE OF CALIFORNIA

DEPARTMENT OF PUBLIC HEALTH
FOOD AND DRUG BRANCH

MEDICAL DEVICE MANUFACTURING LICENSE

Encoll Corporation
4576 Enterprise St
Fremont, CA 94538

LICENSE NUMBER: 50637
EXPIRATION DATE: 9/17/2026

The person named herein is licensed to manufacture devices through the expiration date of this license. This license is issued in accordance with the California Health and Safety Code and is not transferable to any other person or place. The licensee is required by law to immediately notify the California Department of Public Health of any change in the information reported in the application.

Food and Drug Branch, 1500 Capitol Avenue, MS 7602, PO Box 997435, Sacramento, CA 95899-7435 (916) 650-6500

References

1. Wild T, Rahbarnia A, Kellner M, Sobotka L, Eberlein T. Basics in nutrition and wound healing. Nutrition. 2010;26(9):862-866.
2. King M. The Extracellular Matrix 2012; http://themedicalbiochemistrypage.org/extracellularmatrix.php. Accessed July 2, 2012.
3. Wilner GDN, H.L.Lerocy, E.C. Activation of Hageman factor by collagen. The Journal of Clinical Investigation. 1968;47(12):2608.
4. Rosenberg L. dlTJ. Wound Healing, Growth Factors. 2006; www.emedicine.com. Accessed January 20, 2008.
5. Calne S, ed International consensus. Acellular matrices for the treatment of wounds. An expert working group review. London: Wounds International; 2010.
6. Gunasekaran S KM, Dhanraj P. Bioactive Collagen Dressing for the Treatment of Burns, Donor Sites, and Ulcers. World Biomaterials Congress Meeting. 2008.
7. Dhanraj P GS, DeWeese J, Sutkin H. How Native, Pure Type-I Collagen Dressing Cures Ulcers Better Than Other Comparable Skin Substitutes. Association of Plastic Surgeons of India. 2008.
8. Gunasekaran S KM, Dhanikachalam A, Narayan R. A comparative second-degree burn treatment trial collagen dressing vs. silver sulphadiazine alone. American Society for Dermatological Surgery. 2005.
9. Cho Lee A-R, Leem H, Lee J, Chan Park K. Reversal of silver sulfadiazine-impaired wound healing by epidermal growth factor. Biomaterials. 2005;26(22):4670-4676.
10. Koempel JA, Gibson SE, O'Grady K, Toriumi DM. The effect of platelet-derived growth factor on tracheal wound healing. International Journal of Pediatric Otorhinolaryngology. 1998;46(1–2):1-8.
11. Dhanraj P MR, Herndon D. A Clinical Breakthrough in Wound Cover "Bioengineered Collagen" - A Cost Effective and Expeditious Permanent Skin Substitute. International Society for Burn Injuries. 2010.
12. Nimni ME. Collagen: Biochemistry, biomechanics, biotechnology. Vol 3. Boca Raton, FL: CRC Press, Inc; 1988.
13. Gunasekaran S, Inventor. US Patent 5814328. Preparation of collagen using papain and a reducing agent. 1998.
14. Smith EL, and J.R. Kimmel. Papain. In: P.D. Boyer HL, and K. Myrback, ed. The Enzymes. Vol 4. 2 ed. New York: Academic Press, Inc.; 1960:138.
15. Brandenberger H. RH. Spectrophotometric determination of acid and alkaline phosphatases. Helv. Chim. Acta. 1953;36:900-906.
16. Nimni ME, Cheung, D.T., Inventor. U.S. Patent 5374539. Process for purifying collagen and generating bioprosthesis. 1994.
17. Gunasekaran S. Collagen/Phospholipid Interaction: Possible role in skin wound dressing. Proc. Soc. Biomater. 1994;20:311.
18. Hofstee BHJ. Direct and continuous spectrophotometric assay of phosphomonoesterases. Archives of Biochemistry and Biophysics. 1954;51(1):139-146.
19. Simmons SJ, Jumblatt MM, Neufeld AH. Corneal epithelial wound closure in tissue culture: An in vitro model of ocular irritancy. Toxicology and Applied Pharmacology. 1987;88(1):13-23.

20. Jumblatt MM, Neufeld AH. A tissue culture assay of corneal epithelial wound closure. Investigative Ophthalmology & Visual Science. January 1, 1986 1986;27(1):8-13.
21. Kuwabara T, Perkins DG, Cogan DG. Sliding of the epithelium in experimental corneal wounds. Investigative ophthalmology. 1976;15(1):4-14.
22. Coulson WF. The effect of proteolytic enzymes on the tensile strength of whole aorta and isolated aortic elastin. Biochimica et biophysica acta. 1971;237(2):378-386.
23. Gunasekaran S, Inventor. US Patent 6127143. Preparation of purified and biocompatible collagen using two proteolytic enzyme treatments and a reducing agent. 2000.
24. Gunasekaran S, Inventor. US Patent 6548077. Purifying type I collagen using two papain treatments and reducing and delipidation agents. 2003.
25. Gunasekaran S KM, Dhanikachalam A, Kilaru PG. Clinical review of a new bio-engineered collagen dressing on diabetic ulcer wounds. Society for Biomaterials. 2007.
26. Nelson D.L. MC. Lehninger Principles of Biochemistry, 5th edition. 5 ed2008.
27. Rodkey W, DeHaven K, Montgomery W, et al. Comparison of the collagen meniscus implant with partial meniscectomy. A prospective randomized trial. Journal of Bone and Joint Surgery; American volume. 2008;90(7):1413-1426.
28. Pentlow A, Smart NJ, Richards SK, Inward CD, Morgan JDT. The use of porcine dermal collagen implants in assisting abdominal wall closure of pediatric renal transplant recipients with donor size discrepancy. Pediatric Transplantation. 2008;12(1):20-23
29. Rajendran MK ST, Sivakumar M, Gunasekaran S. Improved healing of lower-extremity ulcers using advanced collagen membrane. Southern California Tissue Engineering Symposium. 2002.
30. Gunasekaran S KM, Dhanikachalam A, Narayan R. A Comparative Second-Degree Burn Treatment Trial of Collagen Dressing vs. Silver Sulphadiazine Alone. Society for Biomaterials. 2006.
31. Gunasekaran S RM, Swaminathan T, Dhawan S. Modified Collagen for Burns Enhances Healing Rate Possibly by Cell Signaling. 2003 American Society for Dermatological Surgery Annual Meeting. 2003.