Human BioSciences Kollagen Technology

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Section I.
The Evolution of Wound Care

Prehistoric: It was observed that wounds bled less and perhaps historic healed faster when bandaged. Leaves and grass were used.[10]

1650 BC: Egyptians discovered that closed wounds healed faster than open wounds. They created the first adhesive bandage by applying gum to linen strips in order to draw wounds together.[10]

1600s to 1960s: Wound care treatments developed minimally between the 1600s and the 1960s. Dressings were used to cover 1960$ and protect the wound from exposure to the external environment.1600s Dressings used in treatment included cotton linters, paper, feathers and dust.[6]

1600s: Dressings used in treatment included cotton linters, paper, feathers and dust.[5,6]

1800s: Patients treated with dressings such as linters, cotton gauze, knitted fibers and fibers. 1900s Focus on covering the wound continues with the use of non-woven swabs, sleeve dressings, adhesive paper and planters/wipes.[6]

1867: Lister introduced antiseptics to wound care with the use of carbolic acid and phenol.[18,19]

1962: Landmark study by Winter finds that moist. Wounds were epithelialized more rapidly than dry wounds.[1]

1963: Polyethylene film first made available commercially.[2]

1970s: Medical research and practice continues to recognize moist wound healing as the standard of care.[6]

1971: Winter finds that “wound resurfaced faster when covered with a plastic material then those left open to air.”

1979: Hunt observes visible fibroblast activity in wound

within a couple days of injury.[1]

1980s: Moist wound healing continues to revolutionize wound care treatment. An influx of product innovations such as plastic films,hydrocolloids, gums and hydrogels reach the wound care market.[6]

1981: The dermis of superficial wounds exposed to air is more fibroblastic cibrotic and scarred than the dermal component of a similar wound maintained in a moist environment.[1]

1982: Hydrogels become commonly available. Research confirms moist healing as the most effective wound treatment."[13]

1983: - In studies using hydrocolloids, researchers confirm that the healing of normal wounds is significantly enhanced in a moist environment in carison to wounds that are allowed to dry or those treated with wet-to dry gauze.[14]

1985: Further investigation of Collagen's role in wound healing. Studies indicate that wounds subjected to a moist healing environment reepithelialized more rapidly than similar wounds exposed to air."[15]

1986: In wound healing cost-effectiveness studies, Trelease finds that "occlusive (moist) dressing have demonstrated to be more cost-effective than traditional dressings."[16]

1989: Alvarez finds that povidone-iodine, peroxide and Dakin are cytotoxic to wounds and impede healing. Update: *There is a non-cytotoxic Dakin's brand available.* Rodeheaver adds to prior research findings that antiseptic agents are reactive chemicals that are cytotoxic to normal tissue.[20]

1990s: In the past, product innovations demanded health care practitioners' attention. The trend in the 1990s is to focus on the impact of information on wound treatment. For example, outcomes-based wound healing - a philosophy introduced by the managed care system - demands that medical professionals provide a definition and comparison of what treatment or therapies work, at what cost and in what length of time.[7]

2000s: Researchers project increased development and use of biocompatible and bioabsorbable materials.[15,17] Dressings will actias delivery system for active agents, growth factor, debridement agents, etc. to create ideal environments for the healing process.

The future will focus on wound care information innovations rather than the product innovations and topical therapy revolutions that characterized the 1980s. The goal of the 1990s and beyond will beto stay updated on current data and technological advances.[7]

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Section II.
Collagen's Role in Wound Healing

Wound healing is a complex process that involves a number of chemical and biological factors. All wounds follow the same specific steps in the course of healing. These various overlapping stages include: hemostasis, vasoconstriction, vasodilation, phagocytosis, granulation, fibroblast formation, angiogenesis, reepithelialization, collagen secretion, removal, and remodeling.

Throughout most of these phases of healing, collagen serves as the key cellular component for skin tissue repair and restructuring.

Collagen's Properties

1. **Guiding Function** - Collagen fibers serve to guide fibroblasts. Fibroblasts migrate along a connective tissue matrix. 1302 *A* three dimensional collagenous framework can be provided by collagen sponges.
2. **Chemotactic Properties** - The large surface area available on the collagen fibers can attract fibrogenic cells. 102,104 The chemotactic properties of the breakdown products of collagen can also help in wound healing.
3. **Nucleation** - Collagen in the presence of certain neutral salt molecules can act as a nucleating agent causing formation of fibrillar structures.193 A collagen wound dressing might serve as a guide for orienting new collagen deposition and capillary growth.
4. **Reutilization** - Fibrous wound dressings can be solubilized and repolymerized to fibrous collagen in the extracellular space. 103
5. **Hemostatic Properties** - Blood platelets interact with the collagen to make a hemostatic plug.

Collagen Healing Process

Collagen is the most abundant family of proteins found in the human body. The function of nearly all systems and organs of the body is dependent on collagenous structures. Approximately 70% of the dry weight of the skin is collagen. As a result, use of collagen in wound healing has drawn tremendous interest from scientists in recent years. Due to the complexity of collagen's involvement and interaction between a myriad of chemical and biological factors, no single research work on collagen's role in wound healing can be complete and conclusions must be drawn from a number of references. A wound may be defined as an incision or a trauma to any of the tissues of the body. Wound healing is a complex continuous process. In order to simplify its complexities, the wound healing process is often divided into several overlapping stages.

Collagen Assists Hemostasis

Hemostasis (stoppage of bleeding) is the first step in the wound healing process. Blood platelets and soluble clotting factors play a major role as intravascular hemostatic factors.[163] Collagen is a very efficient hemostatic agent because platelets adhere to collagen, swell, and release substances which initiate hemostasis.[37,89,199] Furthermore, collagen can provide both positive and negative active polar sites, and, due to its size, a molecule of sufficient size for platelet aggregation.[190] The platelets adhere to collagen more closely than they adhere to undigested subendothelial surface.

Various studies have been done on the application of collagen as a hemostatic agent. In patients undergoing diagnostic catheterization or undergoing angioplasty, collagen use significantly lowered total hemostasis time when compared to conventional manual compression[156] Investigators using a vascular hemostatic device to deliver collagen to the arterial puncture site found that the hemostasis time decreased from 17.6+9.2 min. to 4.1+2.8 min. for patients undergoing

catheterization, and from 33.6+24.2 min. to 4.3+3.7 min. for patients undergoing angioplasty.[197] A low number of incidents of peripheral vascular complications were reported. In another study, collagen, sterilized gauze, and regenerated cellulose were used as hemostatic devices in thrombo genic patients. A significantly smaller number of hemorrhagic complications were found with the use of collagen," again demonstrating the superiority of collagen as a hemostatic device.

In oral surgery, the rate of secondary bleeding after 10 minutes was found to be lower with patients using collagen than patients using oxidized regenerated cellulose.* Bleeding from deep lacerations of the canine liver and spleen can be controlled within a few seconds when collagen flour is used.[73,74,75] Additional experiences with potentially lethal wounds resulted in the control of bleeding without sutures or death.13,74 A comparison of microcrystalline collagen dressings with gauze and oxidized cellulose showed that collagen dressings were superior in controlling suture-line hemorrhage and 50% more effective in incidences for additional sutures to control bleeding.

In oral wounds, Avitene (microfibrillar collagen) was found to be superior to other topically applied agents such as thrombin, Surgicel,$Gelfoam, and cyanoacrylate." In a dog wound model, collagen sheets more effectively controlled bleeding when compared to gelatin sponge or oxidized cellulose or powder-like collagen.“

Table I further illustrates collagen's blood sorption properties.[40,41]

Table I - Properties of Collagen Sponges

Type of Collagen	Sorption of Blood	Water Binding
Sponge	*(wt/*60 sec.)	(*wt/*5 minutes)
Native Collagen	5.3 + 1.9	20
Cross-linked Collagen	130	130
Fibrin	1.4 +3.8	45
Gelfoam	1.8 +0.1	21
Cotton tampon	2.5	6-7

Avitene is a registered trademark of Davol, Inc., Surgical" is a registered trademark of Johnson & Johnson, and Gellom* is a registered trademark of thc Upjohn Co.

The type of collagen used and its specific properties have a pronounced effect on the rate of hemostasis. Type III collagen has been reported to be more effective in inducing platelet aggregation than Types I and II collagen 10,91 Albeit there may be some variations in the amount of collagen required for aggregation, only one microgram/milliliter of collagen Type III was required to initiate platelet aggregation in platelet rich plasma." Collagen-platelet interactions depend on the degree of polymerization of the mature collagen and on the amount of positive charges found on the collagen molecule. 30 The rate of platelet agglutination has been attributed to electrostatic charges on the collagen molecule. [166,167*]*

The triple helix structure of collagen is essential for aggregation [34,133,147,161,188] and its quartemary structure is

essential for the collagen-platelet interaction.[22,25,33,*34*,*98*,147,188] Proline and hydroxyproline sites of collagen may also play an important role in collagen-platelet interaction.12 The primary (covalent) structure is also involved in the interactions. The collagen molecules may be required to have a minimum structural unit to cause platelet aggregation.[147] Specific binding sites for the one chain on isolated platelet membranes and intact platelets have been report ed.[35,36,38,39] Both one and two class collagen are active in platelet aggregation.[147] Collagen's ability to initiate platelet aggregation is greatly reduced if it is degraded by enzymatic diges tion.[33,34,88,199] Heat denaturation destroys the platelet aggregating ability of collagen.[91,147] The aggregating activity decreases by more than 90% if the free amine grouped are blocked. [118,190] Platelet aggregation does not depend on the free carboxyl groups.[31,118,189]

In other studies, free carboxyl groups were found to be critical for aggregation activity,[133,138] Carbohydrate residues may also play an important role in collagen-platelet interactions.[122] Oxidation of galactose residues linked to the hydroxylysine residues of collagen stops aggregation of platelets.[33,34] One study on the interactions of platelets with collagen found the adhesion to be dependent on the concentration of Mg2+ ions.[161] Another study has reported increased adherence in the presence of calcium.[32] Temperature and pH were found to be important factors in adherence. [32]

Platelets undergo a transformation from a disc to a sphere when they come in contact with exposed collagen.13,130 As a result of interaction with collagen, platelets release ADP5-hydroxytryptamine, prostaglandins, thromboxane A2, serotonin, and fibronectin.[1*6*,26,*87*,11*8*,133,136,16*6*,1*67*,200] The substances

then bind to other platelets to form platelet aggregates. Studies have shown that aggregation can be induced by ADP and it increased as the concentration of ADP increased [20,80,81,117,134,137,136] Endogenous prostaglandin could also be involved in aggregation.[76,77] ADP was found to induce platelet aggregation in washed rabbit platelets.[7]

Fibronectin released during collagen-platelet interaction has no effect on platelet aggregation.200 Thus formation of a hemostatic plug from platelet aggregation is primarily dependent on the interaction between collagen and platelets. The collagen molecules bind to specific receptor site(s) on platelet membranes to trigger aggregation and the release reaction.[35,36] Collagen assists in platelet aggregation because of its ability to bind the fibronectin. [10] Fibronectin is a large glycoprotein in the extracellular matrix and can provide a scaffolding for platelet adhesion and aggregation and other repair reactions. [142,176,196,197] In research studies, collagen-platelet interaction can be influenced by collagen's age, source, and conditions of its extraction, purification, use and even blood source.[66,114] It has been suggested that galactosylhydroxylysyl glucosyltransferase found in the platelet membrane forms an enzyme-acceptor complex with the galactosylhydroxylysyl residues found in collagen, thus mediating collagen-platelet interaction.[1,1321,24,101,165] However, the enzyme can not form an enzyme substrate complex with native collagen because it does not transfer the carbohydrate units to the native triple-helical collagen molecules, nor can the enzyme form an enzyme-inhibitor complex because the triple helical collagen is not an inhibitor of human gluco syltransferase.[6,120,121,131] Therefore, the theory of enzyme-acceptor complex formation cannot be accepted in this particular case because the enzyme cannot form an enzyme-substrate or an enzyme-inhibitor complex with native collagen, which is the proper medium for platelet adhe sion.120 One study has suggested that collagen accelerates aggregation by activating the Hageman factor. [188]

The exact mechanism of collagen-platelet interactions may not

yet be fully understood, but the data available clearly indicates that collagen interacts with platelets to initiate hemostasis. The importance of collagen in hemostasis is further exemplified by the fact that the bleeding time increases in the presence of abnormal collagen [30,31,174] Hemostasis is followed by vasoconstriction and vasodilation as Vasoconstriction lasts for approximately five to 10 minutes and slows down blood loss in the affected area.[65] During vasodilation, the non-injured vessels become more permeable and leak hormones, plasma proteins, electrolytes, antibodies, fluid, and polymorphonuclear leukocytes into the wound bed.[28]

Collagen Assists Phagocytosis

Vasoconstriction and vasodilation are followed by debridement of the wound, known as phagocytosis. There is a rapid accumulation of polymorphonuclear leukocytes and macrophages at the site of injury during this stage. Neutrophils infiltrate the site of injury and rid the wound of bacteria. Collagen has shown chemotactic properties for monocytes.[145] Monocytes, as macrophages, phagocytize the wound and scavenge tissue debris. [346,128] A reduction in the number of macrophages was found to delay wound debridement.[115]

Debridement is an essential prerequisite to fibroblast proliferation.[160] In vivo studies indicate that there is an intimate cell to cell contact between macrophages and fibroblasts in open wounds on the backs of rats during the healing process. 12 Investigators believe that macrophages play a role in both debridement and collagen synthesis. Along with macrophages, keratinocytes play a major role in the degradation of the extracellular matrix.[155] Keratinocytes migrate along Type I collagen and can be grown on collagen sheets. [122,155,171] Consequently, collagen plays a significant role during the

debridement stage by assisting with phagocytosis.

Collagen Enhances Fibroblastic Activity

The wound begins to rebuild after phagocytosis. Macrophages release cytokines and hydrolytic enzymes which further modify growth factors found at the site of tissue remodeling,resulting in the formation of granular tissue. With the release of angiogenic substances from the macrophages, there is a rapid growth of fibroplasia and angiogenesis. Granulation tissue consists of a large number of macrophages, fibroblasts, and neovasculature embedded in a matrix of fibronectin, collagen and hyaluronic acid. Collagen sponge dressings were found to enhance granulation in leg ulcers.[29]

The fibroblastic phase lasts approximately four to 20 days and provides strength to the wound.28 Fibroblast cells are involved in the fibroblastic phase. The activity of fibroblasts governs the restoration of tissue continuity and the strengthening of ensuing repair tissue, [152,153,191] Fibroblast replication, adhesion, spreading, chemoattraction, and migration are reported to be stimulated by fibronectin.[45,47,54] Fibronectins function as adhesive proteins that bind cells to other cells or to the substrata,[195] The ability of fibronectin to mediate cell attachment is dependent upon its interaction with other molecules. [154] Fibroblasts adhere well to the collagen. [59,106,142] It has been shown that fibroblasts possess membrane receptors for collagen.

Both fibronectin and fibroblast cultures have shown specific abilities to bind to collagen. [60,61,126] Studies also have demonstrated the binding of fibronectin to native collagen and the crosslinking of fibronectin to collagen.[104,127,154,176] Binding creates a matrix that can support cell adhesion and provide tissue integrity.[104] The ability of fibronectin to bind to collagen may ultimately influence the length and width of collagen fibers since fibronectin influences the rate of

fibrillo genesis. [108] Collagen,along with fibronectin, is closely involved in cell adhesion and attachment per both normal and fibrotic conditions, but not under conditions of malignant transformation. 19,85,107,176,177 Fibronectin is also associated with newly deposited collagen fibrils.[69]

The origin of fibroblasts is not yet fully understood. Fibroblasts may originate from the surrounding connective tissue, and from the keratinocytes, from the perivascular sheaths surrounding blood vessels, from blood monocytes undergoing metaplastic transformation.[14,17,82,164] Whatever the origin, the wound fibroblasts proliferate and migrate during the healing process. Active migration of fibroblasts has been shown in both in vitro and in vivo studies.[2,14,145] Studies indicate the chemotactic attraction of fibroblasts to Type I, II, and III collagen, collagen derived peptides, and binding of chemotactic collagen-derived peptides to fibroblasts. [37,146]

Morphological studies suggest that collagen based wound dressings enhance the deposition of oriented, organized fibers by attracting fibroblasts and assisting a directed migration of cells.ss This indicates the importance of collagen for fibroblast motility. Thus, collagen is involved in one of the major events of the wound healing process: the migration of fibroblasts to the site of injury. The fibroblasts synthesize collagen [92,191[

During angiogenesis, new capillary buds grow on preexisting small vessels. Angiogenesis occurs concomitantly with the ingrowth of fibroblasts and deposition of extracellular matrix (ECM) molecules into the wound space.[46] In a closed wound, the new capillaries meet similar cells from the other side and a network is formed across the entire wound.” In open wounds, the new capillaries form granulation tissue.[159] The growth of new capillaries depends on the level of support available at the wound site (migration of fibroblasts and production of new collagen). New tissue synthesis is dependent on the amount of nutrition supplied by new capillaries. There is a very delicate

balance between the formation of a fibroblastic network and growth of new capillaries. It is not clear if external collagen plays a direct role in the growth of new vessels, but collagen does play a big role in providing the necessary support.

Collagen Assists Wound Remodeling

When skin integrity is disrupted, reepithelialization occurs within hours after the injury.[46] During epithelialization, epithelial cells proliferate at the wound edges and migrate across the wound bed. [528,63] Wound debris, eschar, and blood clots obstruct epithelialization.[28] Two important factors in the reestablishment of skin integrity following an injury are the cohesion of epithelial cells (keratinocytes) and the adhesion of the epidermis to the dermis.[163] Collagen sheets were shown to support keratinocyte growth during in vitro studies and in vivo studies.[125] One study has reported a selective adherence of basal cells to a collagen substrate. Another study has reported attachment and differentiation of epidermal cells on collagen.[129] Mammary epithelial cells cultured on collagen membranes in a medium containing insulin, hydrocortisone, and prolactin were found to maintain differentiation through one month in culture.[60] Conversely, cell cultures on plastic, glass, or collagen gels formed a confluent epithelial sheet but lost secretory and myoepithelial specializations. The effects of collagen-coated substrates in tissue culture on cell adherence, differentiation, and protein synthesis are further indications that collagen is an important factor in the epithelial cell migration.[60,99,116,124,149] Fibronectin and fibrin have been found to provide a provisional matrix for epidermal cell migration during wound reepithelialization. Therefore, collagen may also play a role in reepithelialization because of its ability to bind with fibronectin.

In an ideal case of wound healing, lost or damaged tissue completely regenerates with no scar formation, Scarring results from the accumulation of collagen at the wound site and is

dependent on the rate of collagen synthesis as well as the rate of collagen degradation. Studies performed on white guinea pigs showed the amount of collagen in the wound increased rapid from five to eight days, but decreased thereafter.[68] Removal of excessive collagen is initiated by collagenase liberated from granulocytes, macrophages, or from epithelium and mes enchyme.[57,67,112,113,140,151,180,181] Collagenases are the only proteinases that can cleave the collagen molecule, and therefore the activity of the collagenase is essential for successful remodeling.[140] The collagenase cleaves the collagen molecule into two specific pieces, making it more susceptible to degradation by other proteases.[50,78,97,112,113,340,151] Cleavage is preceded by activation of the collagenase by other proteases.[178]

This process is known as the maturation or remodeling phase. It begins approximately 20 days after the injury and frequently occurs for more than a year.28 Electron microscopy revealed that collagen in rat wounds was in the form of irregular masses 100 days after an injury,[64] Collagen has an ability to bind collagenase inhibitors.[178,179] Also, keratinocytes grown on Type I collagen showed a significantly higher amount of collagenase as compared to keratinocytes grown on basement membrane proteins.[140,185] Thus, collagen dressings could play a major role in the degradation of extracellular matrix by helping keratinocytes produce increased levels of collagenase.

Collagen's interaction with collagenase inhibitors is thought to be responsible for a reduced scar size following third degree burns in guinea pigs as well as for reduced fibrous adhesion (excess scar tissue) following tendon surgery in chickens.[144,163] It may be noted that presence of a scar may be a result of lack of organization of collagen within the wound and not due to a lack of collagen formation." Research indicates that collagen-based wound dressings enhance the deposition of oriented and organized fibers which are characteristic of the remodeling phase.[54]

Research on the Application of Collagen Dressings

Stoop[7]summarized his experiences using collagen sponges in the treatment of pressure sores. After application of the collagen sponges, the following was observed:

1. The wounds were clean and bacterial infection was retarded.
2. The wound secretion drainage was reduced.
3. There was an improvement in the formation of new granulation tissue.
4. The undermined edges of the pressure sores were closed.
5. There was an increase in the formation of granulation tissue.
6. There were no contractures in the closed wounds.
7. Moist pressure sore fissures which showed no tendency to heal were closed.
8. There were no immunological reactions towards collagen.
9. The general condition of the patient improved.

A number of other researchers have drawn similar conclusions, for example:

- Collagen dressings were found to heal wounds more quickly than dextranomer. [139]
- Collagen dressings healed leg ulcers more quickly than hydrocolloids.[123]
- Collagen sponge dressings were significantly better than pigskin or Xeroform® in second degree burns in rabbits.[135] The collagen dressings were adherent to the burn wound and were actively permeated by the inflammatory cells.[135]

Comparisons of Collagen Dressings to

Conventional Dressings

Although numerous wound dressings have been developed and marketed, the majority of these products fail to provide the benefits of collagen dressings. Also, it has been concluded that therapeutic application of collagen wound care dressings poses no safety problems.[172] The following comparisons between collagen dressings and conventional wound care products illustrate this point:

***Xeroform" is a registered trademark of Sherwood Medical Industries, Ltd.**

Growth Factors vs. Collagen-Based Dressings

Growth factors are biologically active polypeptide molecules whose interaction with specific cell surface receptors leads to specific responses dictated by the receptor-mediated signal transduction pathways within target cells.[51,128] During the course of wound healing, the local release, expression, and concentration of growth factors changes markedly.[51] Numerous growth factors play a specific role in the healing process: epidermal growth factors, transforming growth factors, vascular endothelial growth factors, basic fibroblast growth factors, keratinocyte growth factors, and platelet-derived growth factors.[27,S1,71,128] It is interesting to speculate that growth factors may enhance the production of specific matrix components, which, once they are present, cancel the need for growth factors. Most studies on growth factors have emphasized the use of single growth factors, thus the synergistic effects of growth factors may not be fully understood. In contrast to growth factors, collagen-based dressings provide a suitable wound environment at each stage of healing.

Gel & Membrane Dressings vs. Collagen Sponge Dressings

Gels may promote bacterial growth and may cause biological effects on wound activities by possible leakage of gel constituents. Partially crosslinked collagen might be unstable in

the presence of wound exudates containing proteolytic enzymes. Membrane-type dressings lack a capacity to bind to fluids. The exuding fluid separates itself from the dressing and the pocket becomes prone to bacterial growth. Sponges form a unique matrix having a continuity of channels and a porous structure. Further interaction of collagen dressings with fibronectin and fibroblasts makes it an active dressing that increases mobility of the cells. If left on an uninfected wound, a collagen sponge dressing will be firmly attached by ingrowing cells and become loose only after the epithelialization underneath the dressing is complete. The high fluid binding ability of collagen helps it absorb cell exudates.

Other Polymer Sponge Dressings vs. Collagen Sponge Dressings

Polyurethane (PU) and polyvinyl alcohol (PVA) are two commercially available polymer sponges for burn treatments. Collagen has better fluid absorbing and retaining properties than these two polymers.

Monocomponent Dressings vs. Composite Dressings

It is almost impossible to duplicate the structural complexity of any tissue. Collagen sponges provide the optimum porosity for growth of fibrogenic cells and for formation of capillaries. Use of glycosaminoglycan (GAG) in collagen sponges may not affect the porosity, but may cause problems due to dissociation of GAG into the wound environment. Further, it will reduce the blood clotting capacity of the wounds. Still, collagen-gag matrices have been **fo**und to be superior to epidermal sheets alone."

Crosslinking of Dressing Materials

Uncrosslinked membrane is soft, pliable, and adheres nicely to a moist surface. When crosslinked, the dressings become stiffer, absorb less water, and attach poorly to the uneven surface of

the wound.

Conclusion

Wound healing is a complex process involving a number of stages. Collagen dressings facilitate an environment conducive to healing at each stage. The use of collagen dressings will negate the necessity of providing wound stage dependent, surface dependent dressings, or growth hormones. "

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Section III.

Results of Wound Treatments

Internal Use Only

A particular type of treatment is chosen for numerous reasons including: total healing time, ability to successfully heal the wound and overall treatment costs. The medical industry has strived to find the most appropriate methods of wound treatment. Currently, medical professionals use many types of different dressings to treat wounds, but many conventional methods result in recurring wounds. However, studies comparing traditional dressings to collagen dressings indicate that collagen dressings may significantly reduce wound healing time and facilitate wound closure.

Research Outcomes

Table I presents a comparison of the healing effectiveness of various dressings by average healing time and provides a percentage of reduced wound healing time by collagen

dressings.1,3-12,14-17 In many of these cases, collagen products were used to successfully heal wounds after other methods failed 3,5,7,10

Table I: Healing Effectiveness of Wound Dressings

Wound
Treatment
Average Healing Time
Reduction in Wound Healing Time by Collagen Dressings 56%
Diabetic Ulcers1-3,7,10,16-17
Collagen Dressing Mojst
Collagen Dressing Saline gauze
39.6 days 89 days 39.6 days 140 days
72%

Normalt
28%
Collagen Dressings Xeroform
7.75 days 10.62 days

30%
Acute Infected Collagen Dressings Paronychia Traditional
14.88 days 20.87 days

27.8%
Permanent Nail Surgery
Collagen Dressings Traditional
24 days 36 days

50%
Podiatric Surgical.9
Collagen Dressings Traditional
6.7 days 14.1 days

Ulcers!
39.2 %
Collagen Dressings Traditional

21.25 days 34.5 days

Verruca
34%
Collagen Dressings Traditional
10.5 days 34.5 days

50%
Venous Insuf." Skin Ulcers
Collagen Dressings Dextranomer
32 days 64 days

Leg Ulcers
Collagen Dressings Hydrocolloids.

** Collagen dressings healed wounds more rapidly than hydrocolloids ($p<0.05$).*

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Diabetic and Chronic Wounds

"Diabetic foot ulcers are typically some of the most difficult wounds to get closed and keep closed."10 Because no cure exists for diabetic and other chronic wounds, the effectiveness of wound dressings are best tested in these cases.

"Dramatic" results were found when using collagen treatments in cases of diabetic, non-healing, deteriorating ulcers. For example, researchers conducted on diabetic foot ulcerations studied a patient whose ulcer was not responsive to traditional treatments over several months. After four months of treatment using collagen dressings, the ulcer was reduced almost 100%.?

In another study, an insulin-dependent Diabetes Mellitus patient lived with chronic ulcers for about five years with little relief from several types of conventional treatment protocols. After utilizing collagen dressings for one month, the wound "presented with decreased amount of drainage, no undermining, sinus tracking, or odor."s In yet another study, traditional treatments failed to heal a diabetic patient's case of gangrene on his right foot. He received a skin graft and was treated with collagen dressings. Within five weeks, collagen dressings decreased the wound size from 8cm x 4cm x 2cm to 5.5cm x 3cm.

Donor Sites

Similar outcomes were reported in other studies involving donor sites. In one study, eight patients requiring dressings for two donor sites of equal size were treated with Xeroform (a stan dard fine mesh gauze dressing) on one site and with a collagen dressing on the other. The aver age size of the Xerofom donor site was *2*24.75 cm and the mean collagen donor site was 319.87 cmThe mean length of healing time was 7.75 days for the collagen dressing and 10.62 days for Xeroform®. The collagen dressings were considered less painful. The results indicate that collagen has a superior rate of healing than Xeroform. The difference in healing rates is most likely attributable to the characteristics of each dressing. Collagen has the ability to help in hemostasis and to act as a support for migration of fibroblasts. Exogenous collagen could also act as an immediate collagen source and provide an early matrix for reepithelialization.

Podiatric Cases

In other studies,8,9,10 300 podiatric cases (involving healthy and unhealthy patients) that were treated over a two-year time period demonstrated that the use of collagen significantly reduced healing time. The researchers suggested that collagen helps wound healing by assisting in hemo stasis, phagocytosis and

angiogenesis.&. In the following cases using other wound dressings were compared in healing time and it was found that the use of collagen products decreased healing time by:

• Permanent nail surgery
*2*7.8%

• Deep wart treatment after excision

• Acute infected paronchia 30%

• Standard surgical cases

50%

• Ulcers

39.2%

*4*7%

Skin Ulcers

Collagen was found to be a better healing agent than dextranomer in the treatment of skin ulcers.'s Seventy-two patients were treated with heterologous lyophilized type I collagen and with a dextranomer and healing times were compared. Twelve patients, each with venous insuf ficiency, diabetic gangrene, radiation ulcers, bed sores, burns or post traumatic wounds were examined as separate groups. In all cases, collagen treatment wounds healed significantly quicker than wounds receiving dextranomer treatment. No adverse reactions to collagen treat ment were observed.

Leg Ulcers

When lyophilized Type I collagen and hydrocolloids were used in the treatment of leg ulcers, 14 researchers found the following:

wounds healed significantly quicker with the use of collagen, in cases of arterial obstruction and thallesemia were effectively treated with collagen dressings, collagen yielded maximal increase in cicatrization difficulty, and no bacterial infection or sensi tization was detected. Telethermographic studies demonstrated increased blood perfusion when collagen was used. 14 Histological studies showed collagen therapy stimulates angiogenesis, fibropoiesis and epidermal growth.14 The investigators concluded that collagen's role in each of these stages contributed to faster wound healing.

Additional Research Findings

Baxter' applied collagen products to 18 patients with differing wound types. The treatments were compared with traditional dressings and researchers found that the use of collagen required fewer visits and less time to perform wound care per visit. Patient/family satisfaction increased and healing times improved after collagen treatment replaced traditional therapies.

In another medical evaluation, the use of collagen dressings was compared to conventional dressings in 10 individual cases. Findings indicated that the collagen products w to heal previously non-healing wounds, eliminated bleeding in hard-to-heal wounds, and added comfort and decreased trauma."

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Section IV.

Cost Benefits of Collagen Wound Dressings

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Cost Benefits of Collagen Wound Dressings

For health care professionals involved in wound management, achieving complete healing goals can be a long and costly process. Fortunately, modern wound care technology such as collagen dressings may improve treat ment cost effectiveness. As a result of these innovative new products, health care providers can better manage overall medical costs while successfully accomplishing their treatment goals.

Cost Benefit
Research

It is often difficult to determine treatment cost benefits or cost

effectiveness because few universally accepted definition or calculation methods exist and standards for outcome measurement for cost calculation vary from study to study. However, numerous studies comparing collagen wound dressings to conventional wound care products indicate that collagen dressings decrease total treatment costs. The cost benefits include the follow ing:

- A cost decrease due to diminished healing time.
- A cost decrease due to less care (thus less cost) during the healing process.
- A cost decrease because wounds achieve closure.

Decreased Healing Time

Depending on the wound, healing time can vary from a few weeks to a few months. A recent study comparing collagen dressings to other conventional dressings showed that collagen products decreased overall healing time. 12,13 In the following cases, collagen products diminished healing time: 27.8 percent for permanent nail surgery, 30 percent for acute infected paronchia, 50 percent for standard surgical cases, 34 percent for superfi cial warts, 47 percent for deep wart treatment after excision and 39.2 percent for ulcers. 12,13

In another study, collagen and dextranomer were used in the treatment of venous insufficiency, diabetic gan grene radiation ulcers, bed sores, bums and post traumatic wounds.21 In all cases, collagen treatment healed wounds more rapidly.21 Healing time decreased by more than 50 percent in cases of venous insufficiency.21 In cases of chronic leg ulcers, collagen healed faster than hydrocolloids, increased blood perfusion, and stimulat ed angiogenesis, fibropoiesis and epidermal growth." In addition, cases of bum wounds showed that collagen significantly increased the rate of granulation tissue formation in the wound bed.'

Although wound treatment may be costly, only 10 percent to 15 percent of the total cost is attributed to wound dressings alone." More than 70 percent of the total cost results from care provider salary and other staff

expenses." For example, cost of treatment of pressure ulcers has been reported from $5,000 to $60,000 depending on the stage of ulcer and patient condition.24,820 The average cost of a primary amputation is more than $40,000.12,13 In cases of diabetic foot ulcers, costs could range from approximately $22,000 to $36,000 depending on the method used. Another study reports that the average cost of treating one of the 2.5 million cases of leg ulcers is about $40,000 per year.24 In cases of burns covering 30 percent of body surface, estimat ed costs range between $37,000 and $41,000.8

Because total treatment cost is directly proportional to the duration of wound healing, it important to consider using dressings that will quickly facilitate wound closure. For example, research indicates that healing time may be reduced by as much as 50 percent depending on the type of the wound.14 In cases of pressure ulcers and diabetic ulcers alone, this would amount to about $30,000 savings (per pressure ulcer) and approximately $18,000 (per diabetic foot ulcer).

Less Frequent Care Required During Wound Healing

The cost of total wound treatment using collagen products may also be reduced because collagen dressings require less frequent changes. As a result, patients need less nursing care and office visits. For example, one study reports that Kollagen" dressings needed to be changed an average of once a day and yielded better results than conventional dressings. In contrast, traditional dressings need to be changed up to four times daily.23 Consequently, any additional cost due to using Kollagen" dressings rather than conventional treatments is offset by the decrease in nursing care needed for Kollagen" dressings.

Wounds Achieve Closure

There are 2.4 million non-healing or slow healing ulcers in the U.S. today.21 Only 70 percent of the pressure ulcers are able to heal in the first 12 months and diabetic and other chronic wounds may take years to heal or may not heal at all.3,20 In one

study, only 38.8 percent of the diabetic foot ulcer patients healed using the saline method.“ In another study, half the healed ulcers recurred within three months of discharge.19 Diabetic com plications account for approximately 50 percent of all non-traumatic amputations in the Unites States.? In one hospital, pressure ulcers accounted for 62 percent readmission cases. Each of these wounds would have cost approximately $40,000 annually per patient. In many instances, collagen products were used when the con ventional methods failed and collagen dressings yielded positive results.

Quality of Life Issues

Leaders in wound management recommend that health care professionals focus on the "big picture" of wound healing when calculating dressing and treatment costs. Instead of addressing specific wound dressing costs, care givers should also consider the costs associated with their patients' quality of life during the healing process.

In many cases, providing an improved quality of life for patients is secondary to the more immediate need for wound healing." However, medical professionals should consider psychological impacts such as loss of inde pendence, feeling of fear, lack of social contact and loss of self-confidence." In one study, patients undergoing wound healing suffered from anger, anxiety, and depression.24 Treatments that insure a faster patient recovery may alleviate these problems.

Conclusion

Exact cost studies on the use of collagen for wound dressings are not available. However, a cost decrease can be estimated from a comparative decrease in healing time and in care costs. From arguments above, the treat ment costs may decrease by more than 50 percent because of the reduced healing time and by at least 25 per cent because of a reduction in required nursing. Consequently, treatment costs will be decreased by a signifi

cant amount in cases that achieve wound closure, especially in chronic wounds.

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Section V.

Wound Care Market Demographics

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Table 1; Projected Age Distribution for Various Wounds *All numbers expressed in thousands* VENOUS STASIS ULCER
PRESSURE ULCER 1997 1998 1999 2000 1997 1998 0 1 0
DIABETIC ULCER 2000 1997 1998
BURN 2000 1997
AGE
1999)
1999
1998
1999
2000
200
1270
1264
1 256
1248

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432
440
120
5-9 10-14 15-19 20-24 25-29 30-34 35-39 40-44 45-49 50-54 55-59 *6*0-64 65-69 70-7*4* *75*-79 80-84 85+
10.7
15.2 20.2
10.4 14.5 19.5 27.8 43.7 *6*3.5 77.*9*
11,2 16.6 *2*1.7 28.8
DO
28.1

432
440
456
464
43.2
10.9 15.9 20.9 28.5 42.7 63.1 80.9 *79.*7 110.3
*42.*2
15
1
14
63.3 79.4 78.2 106.8
*6*3 82.5 81.1 113.8
192 184 144 144
192 184 152 152
192 192
152 T152
192 192 152 160 129
20 13
103.3
1
T
T
30
31
32
18

Tablc 1 shows projected age distribution for patients with venous stasis ulcers, pressure ulcers, diabetic ulcers and burn wounds from 1997-2000.

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Table 2: Distribution of Patients at Care Facilities for Various W*ounds All numbers expressed in thousands* TYPE OF WOUND " HOSPITAL NURSING HOME

(Stage 7 and III)
1994-1995
1997 1998 1999 2000 1994-1995
PRESSURE ULCERS 2792 281628322856
.
VENOUS STASIS
SURGICAL 2699
33,000
BURNS
T 79T79 1801
1997
04
1998
1999
COMMUNITY CARE 2000 1994-1995
1997
400
107
1101
111
1998
4061
1999
411
2000)
416

TV 1
529
533
536
539

Table 2 shows projected distribution of patients in Hospitals, Nursing Homes and Community Care Centers with pressure sores, venous stasis ulcers, surgical wounds and bums from 1997-2000.

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2000

Table 3: Treatment Type for Various Diseases from 1997-2000 *All numbers expressed in thousands* PRESSURE ULCER PATIENTS
VENOUS STASIS PATIENTS
DRESSING TYPE 1997 1998 1999
1998 1999
WET-TO-DRY 2951 270248 230 T 67
*5*7
ABSORP, DRESSING
88 TL 84
81
79 120
18
NEW, INCLUDING COLLAGE 56
584
597 609 119 125 128
STANDARD 904 979 1029 1065 206
237
2829 129063002 3111
1581
596 61*4*
BURN PATIENTS 2000 1997 1998
53
18
131 38
245 341
637
19
DLABETIC PATIENTS 1999 2000 1997 1998
72 T 66

21 20 42 42 129 134 380T384 222
626
642
19
1999
61
19
138
254
663
224
241
141
263
686
MOIST
Table 3 projects how many wounds are treated by different dressing methods for pressure ulcers, venous stasis ulcers, burns and diabetic patients from 1997-2000. The numbers show current trends and can change if new treatments arrive.
References: 1. Medştrat, Inc., *Wound and Burn Management 2000 1989*. 2. POV Inc., P*OV Reports: The Professional Market for Wound Care Products in the U.S.*, 1992. 3. Allman, RM, "Epidemiology of Pressure Sores lo Different Populations," *Decubitus* 1989;2(2):30-33.
Table 4: Average Cost of Treatmet Per Day for Various Wounds
WOUND TYPE
NUMBER OF PEOPLE COST TO HEAL NUMBER OF DAYS DAILY COSTS
DIABETIC WOUNDS
3,900,000 1 $22,000-$36,000[4] L 240 (8 months) $90-$150
VENOUS STASIS
550,000
40000[7] 255 (8.5 months)
$156
PRESSURE ULCERS
2,500,00 $5,000-$60,000[2,3,5,6] 17-120
$100-$500
1,250,000

$1500-$5000
BURNS
Probability: 000
30
Table 4 shows typical costs for treatment of diabetic wounds, venous stasis ulcers, pressure ulcers and burns using tradtional methods from 1997-2000.

References: 1. Medstrat, Inc., *Wound and Burn Management 2000 1*989, 2. Allman, R.M., Laprade, C.A., Nocl, L.B., Walker, J.M., Moorer, C.A., Dear, MR., Smith, CR., "Pressure Sores Among Hospitalized Patients," A*nnals of International Medicine* 1986;105(3):337-342. 3. Baker, J., Medicaid Claims History of Florida Long-Term Care Residents Hospitalized for Pressurt Ulcers," *JWOCN* 1996;23(1):23-25. 4. Bentkover, J.D., Champion, A.H., "Economic Evaluation of Alternative Methods of Treatment for Diabetic Foot Ulcer Patients: Cost effectiveness of Platelet-Relcasate and Wound Care Clinics," *WOUNDS* 1993;5(4):207-215. 5. Carroll, P., "Pressure Ulcers and Matertal Managment: Cost Effective Prevention and Care," *Hospital Material Manag.* 1993;15(2):38-49. *6.* Olson, B., Langemo, D., Burd, C., Hansom, D., Hunter, S., Silberberg, T.C., "Pressure Ulcer Incidence in an Acute Care Setting," *JWOCN* 1996;23:15-22. 7. Van Rijswijk, Lt, Polansky, M., "Predictors of Time to Healing Deep Pressure Ulcers," *WOUNDS* 1994;6(5):159-165. 8. Brigham, P., McLoughlin, E., "Burn Incidence and Medical Care Use in the United States: Estimate, Trends, and Data Sources," *J BURN CARE REHABIL* 1996;17:95-107.

Section VI.

Review of Collagen and Kollagen TM Products

A) Technical Summary

Rev.1/April 1996/Updated February 2021

COLLAGEN: BIOMATERIAL PROPERTIES AND APPLICATIONS IN MEDICAL DEVICES

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Connective tissues are composed of fibrous and non fibrous components. Collagen and elastic fibers are the fibrous components. The primary non fibrous components of connective tissues are proteoglycans and glycoproteins. These are found in the interfibrillar spaces and are composed of proteins and sugars. Further, there is the presence of cells such as fibroblasts, osteoblasts, chondrocytes and other cell types found in various tissues. These components are found in all connective tissues in varying amounts and in different structural arrangements [1,2].
tissues. Collagen, a natural biomaterial extracted from animal tissues has been purified and used in various forms for medical applications (Table 1) [4 52]. The medical device industry has recently shown increasing interest in collagen-based biomaterials as medical devices. Reasons for this interest are based upon advent of new technology for collagen purification and processing and economical sources for raw material. Another factor affecting use of collagen as a biomaterial is recently published research indicating that collagen materials improve wound-healing characteristics. Further, by modeling tissues as composites, technology now exists to design biomaterials based upon function as found in native connective tissues. Recent advances in cell and enzyme immobilization have documented new uses for collagen and collagen-composite materials. This article

Rev.1/April 1996/Updated February 2021

describes the properties which make collagen a useful biomaterial and the current state of research on medical devices that utilize collagen.

Collagen Structure and Properties

Generally, composition, distribution and orientation of connective tissue components reflect function of the tissue [2]. For example, tendon is used for the transmission of tension. Therefore, tendon is composed of large collagen fibers that are alligned parallel to the tendon axis which limit deformation and provide tensile strength. Tendon contains only small amounts of glucoproteins and proteoglycans which are generally used to dissipate or store energy. In comparison, the aortic wall contains collagen, elastin and proteoglycans [3]. The collagen and elastic fibers in aorta form independent structural networks that are directed in both the circumferential and longitudinal directions. This allows for the aorta to act as a secondary pump for blood, while limiting deformation of the vessel, This concept of design based upon function in tissues is critical when viewing connective tissues as biomaterials.

The primary functions of native collagen are to contain, support and interconnect body tissues [1]. Characterization of different collagen types present in connective tissues such as skin, tendon, bone, cardiovascular tissue etc. [3,53] has been done extensively. To date, twelve distinct types of collagen have been characterized based upon chemical data and can be classified by functionality and distribution in tissues [53]. Type I collagen is the most abundant in connective tissues. It is a unique fibrous protein found in connective tissues such as skin dermis and bone. It consists of three alpha chains with a repeating GLY-X-Y sequence where X and Y are often proline and hydroxyproline respectively staggered by one amino acid relative toone another. The presence of these respective amino acids gives type 1 collagen its structural

Biomaterials have been used in the past for tissue repair and replacement caused by thermal, chemical, or mechanical trauma. These materials included amnion, placenta, human cadaver and porcine skin, and fasciae. These body tissues are composed

primarily of collagen, which is the principal structural protein found in mammalian.

Table 1: The Use of Coilagen devices in various medical applications

polymerized, a characteristic banding pattern every 6.90 nm [53] exists which lends strength and resilience to collagen fibers. The usefulness of collagen in medical devices is primarily a consequence of this ability to aggregate in vitro into various strong structures.

Purification, Processing, and Crosslinking of Collagen
Speciality Application Dermatology Soft-tissue augmentation
Dentistry Oral wounds
Biocoating for dental implants" Support for hydroxyapatite"
Periodontal attachment General surgery Hemostasis 19:26
Hernia repair-22 Neurosurgery Nerve repairaz
Nerve conduits Orthopaedic : Bone repaira-27
Articular cartilage
reconstructions Ophthalmology Corneal grafta
Tape for retinal reattachment Plastic surgery Repair of tissue defects3-37 Urology
Ureter replacement Dialysis membranex
Renal repair Vascular Vessel replacements Other
Biocoatingus Drug deliveryone

Ideally, certain characteriatics in a biomaterial are crucial. Biomaterial sterilization must be relatively easy and effective to avoid infection at the time of implantation. The material should be non inflamatory, non immunogenic and available in a sufficient quantity for widespread utilization. The material should be conducive forvascularization and cellular ingrowth. Finally, the material and its degradative products should be free of any mutagenic or carcinogenic potential (54,55). Collagen is used today as a biomaterial partly due to its adherance to the above criteria.

Native collagen extracted from animal tissues is inherently impure, Purification of native collagen must maintain the integrity of collagen in terms of molecular and fibrillar

structure. The use of collagen as a biomaterial is linked to its low potential for immunological complications when purified [56]. Some antigenic sites of collagen reside in the non helical or telopeptide region (57]. Removal of these regions by enzymatic treatment may result in decreased antigenicity. Purification of collagen from tissues such as bovine skin using enzymatic techniques reduce the collagen to single molecules. Purification using non enzymatic techniques exist which maintain the fibrillarstructure of collagen [57]. Structural glycoproteins as well as other miscilleneous tissue components must also be removed during the purification process.
rigidity. Each individual chain exists as a left handed helix and has a molecular weight of approximatley 100,000 (3]. These left handed helices intertwine giving rise to a right handed super helix with an axial rise of approximatley 39 residues and molecular wieght of approximatley 285,000 (1,3]. The super helix is stabilized by inter and intramolecular interactions between side chains and by hydrogen bonded water bridges. The collagen molecule has characteristic dimensions of 300 nm x 1.5 nm. There also exists two nonhelical regions termed carboxy and amino terminals important in crosslinking and seif assembly of collagen. Aggregation of collagen molecules results in the formation of collagen fibrils which in turn give rise to collagen fibers. This ability to self assemble is directly related to the unique amino acid sequence found in collagen. When
Increased knowledge of collagen chemistry has lead to the creation of many collagen based biomaterials with a wide range of functions. A list of existing biomaterials using reconstituted collagen in various forms can be seen in table 2 (5,53). These biomaterials require high purity of collagen to minimize implant antigenicity. Collagen based biomaterials degrade with time due to the presecnce of degradative enzymes [3]. Crosslinking of collagen
Table 2: Forms and applications of collagen in medical devices (5)

Form of Collagen

Application

Solution

gel

flour fibers

membrane
Plasma expander cosmeticum hemostatic agent sutures, weaving of blood vessels, valve prosthesis

corneal replacement, wound dressing, hemodialysis wound dressing, surgical tampons, vaginal contraceptive vessel prosthesis, reconstruction of hollow organs such as the esophagus and trachea
cell migration, biosynthesis and deposition of connective tissue components, deposition and remodeling of granulation tissue. Type I collagen has been found to promote osteoblast proliferation in vitro (64). It has also been shown to be a good scaffold for growth of hard tissue indicated by increases in alkaline phosphatase activity, presence of mineral and increased mechanical properties of the matrix upon cell infiltration (64]. For Soft tissue applications, migration, growth, and proliferation of fibroblast cells has been enhanced using collagen matrices [65-67]. It has been shown that collagen matrices are effective in closing skin uicers which would normally lead to exposure of tendon, muscle, and bone if left untreated [17). It has been recognized that growth, differentiation, and replication of many cell types in culture is assisted by collagen and collagen-containing substrates. The majority of work to date has been done with type ! collagen isolated from bovine hide. Type I collagen alone has proved to be a useful matrix for many cell types eventhough in vivo, a wide dispersity of collagenous and non collagenous components exist [53,66-69). To date, various uses for type I collagen matrices exist such as scaffolds for artificial skin, bone, and cell-seeded burn dressings [68,69). Immobilization ofenzymes or cells onto the collagen provides a "living" bioreactor [70].

This use of collagen matrices may have great commercial value with the increase in collagen technology.
sponge

tubing

Clinical Applications
can control the degradation rate of collagen in vivo [3]. Native collagen molecules are crosslinked after an enzyme catalyzed modification during molecular packing into native fibrils in vivo. However, collagen crosslinking can be controlled through the use of physical techniques or chemical agents in vitro. By controlling crosslink density, one can control biodegradation time of a subcutaneous collagen implant, the capacity of collagen to absorb water, the solubility of collagen, the tensile strength of the collagen fibers, and the rate of collagen degradation by enzymes. Methods for crosslinking include drying, ageing, anhydrous heating and chemical treatment using formaldehyde, glutaraldehyde, sucinaldehyde, glyoxal, acrolein, carbodiimides, and diisocyanate compounds [58-63). The type of crosslinking, which is directly related to the biodegradation rate in vivo, is usually selected based upon tissue ingrowth and biomaterial properties required for the particular application. Also, the use of collagen based implants to serve as a template or scaffold for tissue regeneration is useful due to its inert products of degradation.
Type I collagen has been used in a wide variety of applications (Tables 1 and 2). The most successful applications of collagen

exploit its unique biological characteristics, for which no synthetic sustitute material currently exists. These characteristics for medical devices include minimal antigenicity, which can be reduced further through crosslink control, attachement sites for many cell lines, hemostatic capability, a mild inflammatory response when implanted; and the relative abundance of natural sources. Taking advantage of some of the biological characteristics described above, one of the most commercially successful medical uses of collagen in the past has been the subcutaneous implantation of soluble collagen for the repair of dermatological defects. The physical characteristics of soluble collagen allow it to polymerize at body temperatures

Cell Growth on Collagen Matrices

Wound repair involves many processes including

conclusions

and thus from a stable subcutaneous gel. In approximately 95% of clinical cases, the implant produces a limited celular response, becoming rapidly populated by host fibroblasts (6,7). The material becomes vascularized and remains histologically stable for 6 to 18 months.

Collagen Composite Technology

Type I collagenis a very useful natural biomaterial for various applications. It can be used for wound healing and wound care where a scaffold is required for tissue regeneration such as artificial skin. It is useful in promoting cellular migration, growth and proliferation. It can be used for hemostasis. It can also be used in a composite form to provide a biomaterial with more complex properties such as for induction of bone growth and for dental applications. its usefulness stems from its material and biological properties. New techniques are being developed to purify, process and manufacture collagen based materials economically in order to make most effective use of the unique properties of collagen.

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The most recent advances have come in collagen composite technology. Collagen composites used for orthopaedic, dental, and wound care show great promise for repair and replacement of hard and soft tissues (53,64,71). Again, the key characteristic of these materials is the use of collagen as a scaffold material for cellular ingrowth and proliferation. The other composite components are important in mineral deposition, and cell migration. In dental application, collagen can be used as a carrier substance for hydroxyapatite delivery. Use of a collagen matrix for this purpose makes application easier and insures that the hydroxyapatite does not travel to other body sites along with speading the healing process. Mathematical modeling now exists to predict mechanical properties of collagen based composites making material design and selection easier for a specific application (53,64,72). This is particularly useful for design of bone implants where material properties vary greatly depending upon location in the body. Biological composites are now being developed and at present seem to be the most promising materials for the future.

Collagen As a Hemostatic Agent

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An established use of collagen has been as a hemastatic agent. It has been shown that a crosslinked collagen sponge can serve as an excellent hemostatic device stopping blood flow within 2 to 5 minutes (18). Collagen sponges can hold upto 40 times thier weight in fluid (18). Collagen powders are currently marketed as hemostatic agerits. Collagen has been used with great sophistication in hemostasis in an increasing number of procedures, including hepatic resectioning, splenic repair, laparoscopy, and oral surgery (13-19]. Use of these materials is increasing due to the advent of newer more economical processing proceedures for collagen devices.

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B) Photographic Review

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Comparisons page 1

Figure 1 shows a textbook picture of collagen fibrils at a magnification of x18,000. Figures 3, 5 and 7 show microscopic photos of Skin Tempo, a collagen dressing, at magnifications of

x10,000, X2,500 and x750, respectively. Notice the similarity in fibrillar structure between the textbook collagen picture (Figure 1) and SkinTemp* (Figure 3).

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At lower magnifications, the fibrillar structure of SkinTempo (Figure 3) evolves into an ordered sheet like structure (Figure 5) and forms an ordered tis sue-like structure (Figure 7).

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Figure 1. Collagen fibrils: Magnification x18,000

Figures 2, 4 and 6 show Hemotene, a collagen hemostat, at magnifications of x10,000, X2,500 and X750 respectively. Notice the lack of ordered struc ture and the presence of irregular strands of fibers. At lower magnifications (Figures 4 & 6), Hemotene's irregular strands become more promi nent and lack organization.

Figures 8 and 9 show oxidized regenerated cellu lose (Surgicel*). The fibers and the weave pattern are clearly synthetic materials different in nature and composition than animal tissues.

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gure 2. Hemotene. Magnification x10,000
Figure 3. Skin Temp: Magnification x10,000

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Figure 4. Hemotene: Magnification x2500
Figure 5. SkinTemp. Magnification X2500

Comparisons page 2
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Figure 6. Hemotene®: Magnification X750
Figure 7. Skin Temp®. Magnification X750
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Figure 8. Surgicel®: Magnification X25
Figure 9. Surgicel*: Magnification X750

Hemotone® is a registered trademark of Astra Pharmaceutical Products, Inc. Surgicel® is a registered trademark of Johnson & Johnson.

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C) Histological Review

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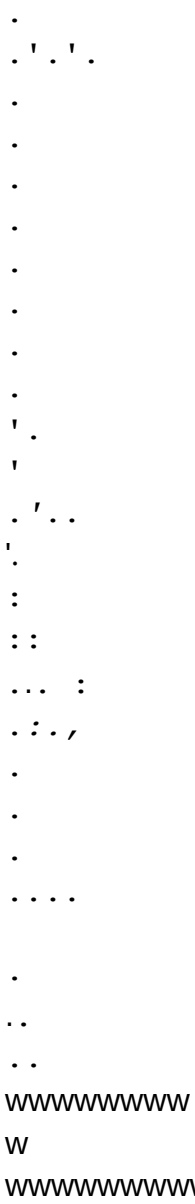

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Comparative Study of the Inflammatory Response to Collagen and Alginate Wound Dressings in Adult Miniswine

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Maui, Hawaii

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Comparative Study of the Inflammatory Response to Collagen and Alginate Wound Dressings in Adult Miniswine

INTRODUCTION

The course of time and degree of inflammation at a wound site directly effect the healing response, and ultimately the length of time required for re-epithelialization of a wound. For instance, a prolonged inflammatory response can extend healing time and delay final resolution of the wound. In this study, three different wound dressing materials (collagen, alginate and a collagen-alginate composite) were implanted in adult Yucatan miniswine to compare the extent of inflammation associated with each of these materials. The inflammatory response to these materials in an open wound was obtained five days after placement in a biopsy punch hole. Samples were also implanted subcutaneously and retrieved after 2 months in the same animal.

METHODS

Samples of collagen (0.5 -0.7 g), alginate (0.5 -0.7 g) and a collagen-alginate composite material (0.12 -0.14 g) were implanted subcutaneously in the abdomen of an adult Yucatan miniswine and retrieved after 2 months. Five days before retrieval, the same materials plus a collagen gel were used to fill biopsy punch wounds (lcm deep and 6mm diameter) on the miniswine dorsum. The wound site and surrounding tissue were excised and histologically processed with a Brown-Brenn stain for bacteria, and H&E stain to show the cellular response and a Masson's trichrome stain to show collagenous deposition. Immunocytochemistry with an anti smooth muscle actin antibody was also used to reveal the extent of neovascularization into each implant.

RESULTS

The tissue response to all of these materials was solely due to the placement of material since analysis with the Brown-Brenn stain indicated an absence of infection for each implant.

1) After five days, a wide band of inflammatory cells has accumulated along the edge of the biopsy filled with alginate. Alginate particles are dispersed within this inflammatory region. Cellular infiltration into the central portion of the biopsy punch hole is still minimal after five days.

2) At a higher magnification, alginate particles along the biopsy edge are surrounded by inflammatory cells.

3) A low magnification view of the collagen-alginate composite material reveals a similar, though somewhat reduced response along the edge of the biopsy punch hole.

4) A particle of alginate from the composite material can be seen at a higher magnification surrounded by inflammatory cells, granulocytes and lymphocytes.

5) The inflammatory response to collagen after five days is minimal relative to that seen with the alginate.

6) At a higher magnification, the response to collagen can be seen to consist of primarily macrophages and smooth muscle cells.

7) The inflammatory response to collagen gel is minimal and not as densely packed as seen with the alginate.

8) Macrophages and smooth muscle cells predominate within the collagen gel matrix after five days.

9) Immunocytochemistry reveals a complete absence of neovascularization into the alginate after five days.

10) Immunocytochemistry reveals a complete absence of neovascularization into the alginate-collagen composite material after five days.

11) Immunocytochemistry reveals the presence of neovascularization into the collagen sheet after five days.

12) Immunocytochemistry reveals the presence of neovascularization into the collagen gel after five days.

13) After two months, there is still a heavy inflammatory response to the alginate as seen in this Mason's trichrome stain. Necrotic debris and blood products like fibrin and red blood cells stain red. Bands of collagen (blue) are encapsulating pockets of the necrotic debris.

14) At a higher magnification, particles of alginate can be seen which are surrounded by very large multi-nucleated giant cells that are trying to engulf these particles. Thin bands of collagen are dispersed throughout this granuloma but inflammatory cells and necrotic debris predominate.

15) The collagen-alginate also exhibits a heavy inflammatory response after two months with extensive regions of necrotic debris and very little collagen deposition within the implanted material.

16) At a higher magnification, alginate particles remaining from the collagen-alginate can be seen to be responsible for this continuing inflammation.

17) This small nodule is all that remains of the collagen after two months. Bands of collagen are widely dispersed throughout the nodule.

18) At a higher magnification, the few remaining multinucleated giant cells are encapsulated by bands of collagen. This collagen sample is almost completely healed after two months.

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Comparative study of the inflammatory response to collagen and alginate wound dressings in adult miniswine.

-C. Baxter, M.D. and B. Fowler

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2. At a higher magnification, alginate particles along the biopsy edge are surrounded by inflammatory cells.

1. After five days, a wide band of inflammatory cells has accumulated along the edge of the biopsy filled with alginate, Alginate particles are dispersed within this inflammatory region. Cellular infiltration into the central portion of the biopsy punch hole is still minimal after five days.

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3. A low magnification view of the collagen-alginate composite material reveals a similar, though somewhat reduced response along the edge of the biopsy punch hole.
4. A particle of alginate from the composite material can be seen at a higher magnification surrounded by inflammatory cells, granulocytes and lymphocytes.

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5. The inflammatory response to collagen after five days is minimal relative to that seen with the alginate.
6. At a higher magnification, the response to collagen can be seen to consist of primarily macrophages and smootlı muscle cells.

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7. The inflammatory response to collagen gel is minimal and not as densely packed as seen with the alginate.
8. Macrophages and smooth muscle cells predominate within the collagen gel matrix after five days.

9. Immunocytochemistry reveals a complete absence of neovascularization into the alginate after five days.
10. Immunocytochemistry reveals a complete absence of neovascularization into the alginate-collagen composite material after five days,

11. Immunocytochemistry reveals the presence of neovascularization into the collagen after five days.
12. Iinmunocytochemistry reveals the presence of neovascularization into the collagen gel after five days.

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13. After two months, there is still a heavy inflammatory response to the alginate as seen in this Masson's trichrome stain. Necrotic debris and blood products like fibrin and red blood cell stain red. Bands of collagen (blue) are encapsulating pockets of the necrotic debris.
14. At a bigber magnification, particles of alginate can be seen which are surrounded by very large multi-nucleated giant cells that are trying to engulf these particles. Thin bands of collagen are dispersed throughout this granuloma but inflammatory cells and necrotic debris predominate.

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15. The collagen-alginate also exhibits a heavy inflammatory response after two months with extensive regions of necrotic debris and very little collagen deposi tion within the implanted material.
16. At a higher magnification, alginate particles remaining from the collagen-alginate can be seen to be responsible for this continuing inflammation.

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17. This small nodule is al that remains of the collagen after two months. Bands of collagen are widely dis persed throughout the nodule.
18. At a higher magnification, the few remaining multinucleated giant cells are encapsulated by bands of collagen. This collagen sample is almost completely healed after two months.