COLLAGEN AS AN IMPLANTABLE MATERIAL IN MEDICINE AND DENTISTRY

Maria G. Patino, DDS, MS
Mirdza E. Neiders, DDS, MS
Sebastiano Andreana, DDS, MS
Bernice Noble, PhD
Robert E. Cohen, DDS, PhD

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***Maria G. Patino, DDS, MS,** is a clinical instructor at the Department of Periodontics and Endodontics, School of Dental Medicine, State University of New York at Buffalo, Buffalo, NY 14214.*

***Mirdza E. Neiders, DDS, MS,** is a professor with the Department of Oral Diagnostic Sciences, School of Dental Medicine, State University of New York at Buffalo, Buffalo, NY 14214.*

***Sebastiano Andreana, DDS, MS,** is a clinical assistant professor with the Department of Periodontics and Endodontics, School of Dental Medicine, State University of New York at Buffalo, Buffalo, NY 14214.*

***Bernice Noble, PhD,** is a professor with the Department of Microbiology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY 14214.*

***Robert E. Cohen, DDS, PhD,** is a professor at the School of Dental Medicine, State University of New York at Buffalo. Address correspondence to Dr Cohen at the Department of Periodontics and Endodontics, School of Dental Medicine, State University of New York at Buffalo, Buffalo, NY 14214.*

Collagen is a highly versatile material, extensively used in the medical, dental, and pharmacological fields. Collagen is capable of being prepared into cross-linked compacted solids or into lattice-like gels. Resorbable forms of collagen have been used to dress oral wounds, for closure of graft and extraction sites, and to promote healing. Collagen-based membranes also have been used in periodontal and implant therapy as barriers to prevent epithelial migration and allow cells with regenerative capacity to repopulate the defect area. It has been hypothesized that membrane regenerative techniques facilitate the natural biological potential by creating a favorable environment for periodontal and peri-implant regeneration. Due to the enormous potential of collagen-based regenerative barriers, clinicians may benefit from a review of potential applications of implantable collagen and knowledge of collagen preparation and membrane types as well as from as awareness of the functional and degradation properties of those materials.

INTRODUCTION

Collagen, the most abundant protein in animals, is the major component of connective tissue; represents the major protein of tendons, ligaments, and the cornea; and forms the matrix of bones and teeth. Collagen consists of a protein with 3 polypeptide chains, each containing about 1000 amino acids and having at least 1 stretch of the repeating amino acid sequence Gly-X-Y (where X and Y can be any amino acid but usually are proline and hydroxyproline, respectively). Collagen assembles into different supramolecular structures and has exceptional functional diversity. A resorbable, naturally occurring substance, collagen has been incorporated into a variety of medical devices and has been used for multiple purposes. As a result, a review of current and potential applications of collagens, their chemical and physical properties, and preparation of collagen membranes all may be of benefit to clinicians who use those materials in surgical and reconstructive procedures. Clinicians also should be aware that basic studies on materials implanted into periodontal tissues can provide information on the behavior of those materials in vivo. The purpose of this article is thus to provide a brief overview of collagen as an implantable device.

Collagen used for medical applications is readily available in large quantities from several animal sources,

including bovine skin, tendon, or intestine, which makes it an obvious choice to be used extensively as a biomedical device. Indeed, collagen-based materials have been in use since the early part of last century and, since that time, collagen in almost all possible physical states (including solutions, gels, powder, fibers, membranes, sponges, tubing, etc) has been used in medicine and pharmacology.

Purified and/or cross-linked collagen have been used as a hemostatic agent and biological dressing as well as in management of burn wounds, in conjunction with ophthalmologic and orthopedic procedures, and for oral, dental, hand, and plastic surgeries.[1] Dermatologic and cosmetic surgeons have used collagen for soft-tissue augmentation and to correct scars, fine lines, and deep wrinkles.[2] For modest tissue augmentation, a solubilized injectable dermal collagen preparation has been developed that takes advantage of the ability of solubilized collagen to form fibrils under physiological conditions.[3,4] Gamma-irradiated amnionic collagen from human placenta also has been tested in animal studies as an injectable material, primarily for tissue augmentation procedures.[5,6] However, bovine collagen (Zyderm) is the most commonly used injectable material for soft-tissue augmentation, with major indications including the elimination of wrinkles and acne scars.[7]

In addition, an intact fibrous dermal collagen preparation has been employed to reconstruct tissue contour defects resulting from loss of dermis, subcutaneous fat, and connective tissue. Those preparations generally are obtained by cutting skin to the desired thickness and treating it with a solution of crystalline trypsin, which removes all cells and other structures, leaving the insoluble Type I collagen component unaltered even at the fibril level.[8,9] In burn and leprosy patients, collagen can be used as a wound dressing to protect skin surfaces. The resulting bandage simulates some of the basic properties of skin, controls fluid loss, maintains thermoregulation, and prevents contamination until healing occurs or a skin replacement can be grafted.[10] Collagen in powder[11] or sponge[1] form also has been utilized extensively as a hemostatic agent, interacting directly with platelets.

In addition to their usefulness for skin augmentation and as a dressing, several types of vascular prostheses have been derived from collagen (those devices generally have been further treated with heparin to create antithrombogenic surfaces[12,13]). Collagen tube allografts have been used to guide peripheral nerve regeneration and for vascular prostheses,[14] while biological structures with a high collagen content have been studied as autogenous transplants in vessel surgery. The diversity and utility of collagen also has been demonstrated in the development of composite collagen prostheses, where a fibrocollagenous tubing is formed around subcutaneously implanted silicone rubber or Teflon.[15]

Implantable collagen hydrogels have been examined as agents for delivery of chemotherapeutic agents,[14] and new ocular drug delivery systems are being evaluated using collagen inserts as a controlled-release system. For example, pilocarpine currently is under investigation in a collagen drug carrier because it can be used as a topical miotic for controlling elevated intraocular pressure associated with glaucoma.[16] In that case, the kinetics of drug release can be manipulated based on modifications made to the collagen carrier. Additional medical applications include male contraceptives, disposable contact lenses, otologic repair, and transdermal drug-delivery systems. For oral applications, homogenized reconstituted collagen mixed with cell culture media has been used for burn treatment and for endodontic repair.[17] Resorbable collagen wound dressings have been used in oral wounds and closure of grafted areas or extraction sites because they stabilize blood clots, protect surgical sites, and accelerate the healing process.[18] Perhaps more importantly, collagen-based membranes have been widely used in periodontal and implant therapy as barriers that prevent the migration of epithelial cells and encourage wound repopulation by cells with regenerative potential.[18]

Use of Collagen in Guided Tissue Regeneration

Goals of periodontal therapy include the regeneration of lost tissues that surround the teeth or implants as well as the elimination of periodontal or periimplant defects by forming new bone, new cementum, and (around teeth) a new periodontal ligament. Guided tissue regeneration (GTR) is a procedure that attempts to reconstitute the lost tissues and is based on the concept of selective repopulation. That is, the type of cells that first repopulate the wound will influence the type of attachment that will form on root or implant surfaces.[19] Although the periodontium is formed by 4 tissue types (epithelium, connective tissue, alveolar bone, and periodontal ligament), regenerative cells are derived only from pluripotential cells of the periodontal ligament or the alveolar bone.[20]

The first report of a human tooth treated by guided tissue regeneration was by Nyman et al[21] in 1982, with the term GTR coined by Gottlow et al in 1986.[22] To exclude the fast-growing cells of the gingival epithelium from migrating to the wound, GTR procedures use barrier devices that are placed between the periodontal flap and the osseous defect to maintain a space for repopulation of the defect with cells having regenerative potential.[23] The first of those membranes to be commercially available were made of expanded polytetrafluorethylene (ePTFE) and were nonresorbable.[24] Those membranes maintain their structural integrity during the entire implantation. Expanded polytetrafluorethylene is biocompatible and cell occlusive and provides control over the length of time the membrane will re-

main in place. The nonabsorbable membranes provide predictable results and simplified management, but a second surgical procedure is required for removal. This involves a potential risk to the newly regenerated tissues as well as additional surgical trauma to the patient.[24]

A number of resorbable barrier materials have been introduced that offer advantages over traditional nonresorbable materials. Resorbable barriers generally eliminate the need for a second surgical procedure and thus eliminate some of the problems associated with nonresorbable barriers.[25] Bioresorbable membranes have been prepared from various materials, such as polyglycolic acid, polylactic acid, and collagen. Other natural products that have been investigated as potential GTR materials include dura mater, oxidized cellulose, rubber dam, and laminar bone. However, collagen may be particularly suitable for GTR applications because the material is chemotactic for periodontal ligament fibroblasts,[26] acts as a barrier for migrating epithelial cells,[27] provides hemostasis,[28] and serves as a fibrillar scaffold for early vascular and tissue ingrowth.[28]

PREPARATION OF COLLAGEN MEMBRANES

Collagen can be prepared from a number of sources using a variety of techniques. However, collagen implants typically are manufactured by demineralization of whole or pulverized bone, generally accompanied by lipid extraction.[1] Initially, collagen is solubilized or dispersed, then purified and reconstituted. (Collagen will solubilize by degradation, and most resistant types can be converted to soluble fragments by acid or base hydrolysis at elevated temperatures.) The noncollagenous materials subsequently are removed and the remaining collagen stabilized before implantation.[23] Ideally, methods of dispersion and reconstitution account for the anatomical source and age of tissue since the ratio of soluble to insoluble collagen varies accordingly.[15] The resulting membranes generally are formed by reconstitution. In that process, collagen derived from a rich source such as skin dermis or tendon is isolated and purified, then precipitated into fibrillar form by changing the ionic strength, pH, or by elevating the temperature to 37°C followed by air evaporation and freeze drying.[29] Collagen may be further treated with pepsin for removal of the terminal telopeptides of the molecule, which is the major inflammatory component.[28]

Cross-linking that occurs during biological maturation of collagen can be stimulated in vitro by several agents. Factors that control the extent of cross-linking include the type and concentration of the processing agent as well as the pH and temperature of incubation.[30] Normally, most barrier membranes are cross-linked to extend the absorption time and to reduce antigenicity. Moreover, the degree to which collagen barriers are cross-linked also may influence therapeutic outcomes. In animal studies, Minabe[31] demonstrated increased regenerative tissue formation with use of cross-linked (vs non–cross-linked) collagen barriers, whereas Brunel et al[32] found increased bone formation in rat calvarian defects when cross-linked barriers were employed for guided bone regeneration.

Cross-links can be introduced by either physical or chemical reagents. For example, chemical reagents such as acetaldehyde, acrolein, formaldehyde, glyoxal, glutaraldehyde, and diphenylphosphoryl-azide (DPPA) all react with collagen to produce additional intramolecular and intermolecular bonds. However, the most widely used cross-linking technique currently is glutaraldehyde (GA). GA cross-linking of collagenous tissues significantly reduces antigenicity and biodegradation of the implant.[33] Essentially, GA blocks the lateral amino groups of collagen and achieves cross-links between peptide chains. In a study using porcine collagen membranes in 2 treatment protocols, 1 involving microwaving and glutaraldehyde and the other using glutaraldehyde treatment at room temperature, microwave cross-linking resulted in less reactive inflammation when implanted in rats.[33] Other studies have shown that cross-linking of a porcine dermal collagen membrane with different concentrations of glutaraldehyde (0.01%, 0.05%, 3%) can retard its resorption rate in tissue and still preserve its biocompatibility.[34] Thus, the extent and method of cross-linking may have important effects on biological properties.

Physical methods include drying or irradiating with ultraviolet or gamma radiation. Irradiation has 2 main effects on collagen: initiating random cross-links and breaking the tropocollagen molecule.[35] Recently, a new cross-linking process has been developed.[36] This process, known as the diphenyl-phosphoryl-azide, or DPPA, technique, achieves natural cross-links between peptide chains without leaving any foreign product in the cross-linked collagen.

Sterilization methods for collagen include dry heat, ethylene oxide, and irradiation. Irradiation is the most frequently used method because it does not appear to affect structural stability. Such methods typically employ doses of approximately 2.5 megarads, frequently from cobalt-60 gamma sources.[37] However, with ethylene oxide, the physical and biological properties are affected due to a reaction between ethylene oxide and collagen. Similarly, moist heat (autoclaving) cannot be used to sterilize collagen because the hydrated protein is labile to thermal denaturation, with even low concentrations of water causing significant disruption of the helical structure. Nevertheless, if collagen is carefully dried prior to heating, its stability is increased and sterilizing temperatures can be applied.[15]

TYPES OF COLLAGEN USED IN BARRIER MEMBRANES

The collagen comprising current GTR barriers are of various subtypes (usu-

ally type I collagen predominates), derived from different animal sources (eg, bovine or porcine), and obtained from a variety of sites (eg, tendon or dermis).[25] Some of the membranes commercially available for use in the United States include Biomend (Sulzer Calcitek, Carlsbad, Calif), Bio-Gide (Geistlich, Wohlhusen, Switzerland), and Periogen (Collagen Corporation, Palo Alto, Calif). Biomend is formed by 100% type I collagen derived from bovine deep flexor (Achilles) tendon. The material is semiporous and resorbs in 4 to 8 weeks.[25] Bio-Gide is a bioresorbable collagen bilayer membrane consisting of type I and III porcine collagen manufactured with a process that includes additional purification steps for removal of lipoproteins.[38] This membrane maintains its barrier function for 4 to 6 months.[39] Periogen is prepared from highly purified bovine collagen and resorbs in 4 to 6 weeks.[28]

The suitability of other collagen types such as rat collagens, Avitene, and dura mater also has been investigated, with varying results.[23] In one study, rat tail collagen membrane produced a chronic inflammatory infiltrate after implantation in dogs but disappeared within a few days after membrane resorption.[40] Avitene, a microfibrillar collagen hemostat derived from bovine corium, has been evaluated histologically in humans.[41] However, that material proved to be an inefficient barrier for epithelial migration, did not facilitate GTR, and was relatively difficult to use. Dura mater consists of an irregular network of collagen fibers, processed to eliminate antigenic and pyrogenic activity, then lyophilized and sterilized. Histologic observations in humans showed limited tissue integration with this material, although it did inhibit epithelial apical migration.[42]

Properties and Design of Barrier Membranes

Membranes used for GTR procedures ideally should offer biocompatibility, cell exclusion, space maintenance, and reasonable manageability.[43] Biocompatibility allows the material to function in a specific situation without adversely and significantly affecting the body (or the body tissue affecting the material).[23] A GTR device also should have the ability to exclude tissues or cells so that those originating from periodontal ligament and bone can repopulate the defect area.[43] Indeed, creation and maintenance of a space is a critical requirement for bone formation,[24] requiring mechanical properties that allow the barrier to withstand forces exerted by or through the periodontal tissues.[23] Bioabsorbable membranes also should maintain the underlying space long enough to allow the coagulum to mature and allow selective repopulation.[44] Although the optimum time period may vary, cell repopulation is greatest during the first 2 weeks of healing but subsides during the third week.[45] Other studies have suggested that 3 to 4 weeks is enough time for allowing repopulation to occur.[31]

Materials for GTR must have acceptable handling properties, be malleable yet support tissue, preserve and maintain space, conform to the defect shape, and have the ability to be customized for unique situations.[25] Membranes should be easy to cut and shape, with no sharp edges to perforate tissue,[43] and be pliable enough to allow close adaptation to a variety of defect morphologies.[45] In addition to those general characteristics, resorbable barriers must also be nontoxic, nonantigenic, and produce a minimal inflammatory response to the bioresorption process without interfering with regeneration.[25] Although the ideal GTR membrane has yet to be developed, those made of collagen currently appear to provide many of the desired characteristics.

Degradation of Barrier Membranes

Implanted collagenous material is degraded by the action of a series of collagenolytic enzymes present primarily in inflammatory cells such as granulocytes and macrophages.[46] In one system, the rate of enzymatic degradation can be assessed in vitro by measuring the average molecular weight between cross-links of implants before and after incubation of collagen in bacterial collagenase. That procedure detects changes in the triple helical structure of insoluble collagen implants resulting from interaction with tissue.[47] In general, the activity of collagenase appears to be high for processed, denatured proteins.[48] In vivo models to quantitate collagen resorption rates include subcutaneous implantation of the material in guinea pigs, followed by surgical excision of implants at different intervals and determination of wet weights. Another method employs [^{3}H]-labeled collagen as a tracer, providing a method to quantitate the amount of collagen present as a function of time.[49]

Kronenthal[50] has reported 4 stages of polymer degradation in vivo: hydration, strength loss, loss of integrity, and mass loss. Hydration results in lubrication of the polymer chains, resulting in loss of membrane stiffness that affects spacemaking capacity. Strength loss occurs due to initial cleavage of the polymer backbone, also resulting in a decrease in spacemaking capability. Loss of mass integrity occurs when strength loss progresses to a point where the material structure is no longer cohesive, and the material breaks into fragments. Mass loss is characterized by final breakdown of the material into its component units such as amino acids. Absorbable membranes undergo a disintegration process that starts at the time of tissue placement but varies significantly between individuals. The rate at which collagen products are resorbed in vivo depends on the extent of cross-linking as well as on the site of implantation.[51] In one study, observations of various collagens that were implanted subcutaneously in guinea pigs revealed that the physicochemical structure of the implant could be used to control both the resorption rate as well as implant–host

tissue interactions.[47] Further studies revealed that cross-linking of collagen with glycosaminoglycans (GAGs) resulted in polymers that were more resistant to collagenase degradation than was GAG-free collagen and that the rate of degradation decreased with increasing GAG content.[47]

In summary, collagen appears to be a good material for use as a biomedical implantable device. In periodontal and implant therapy, collagen barriers may be particularly useful due to their cell occlusiveness, biocompatibility, and resorbability (with the advantage of avoiding a second-stage surgery for their removal). Collagen membranes are also chemotactic for regenerative cells and may enhance the migration and attachment of fibroblasts through its space-making ability.[34]

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