COLLAGEN USAGE

Regeneration in Periodontics: Collagen—A Review of Its Properties and Applications in Dentistry

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Abstract: Collagen is the most abundant protein in mammals, also making it the most important component of the body structurally and functionally. Collagen provides cell occlusiveness, biocompatibility, and resorbability. It is chemotactic for regenerative cells and may enhance the migration and attachment of fibroblasts through its space-making ability. Collagen also has the advantage of being a hemostatic agent with weak immunogenicity, easy manipulation, and the ability to augment tissue thickness. Additionally, upon breakdown through the resorption process, its byproducts are utilized by the host to form native tissue. Further, these proteins are elastic and enhance repair, properties that make the material useful for various biomedical applications. This review highlights and discusses some of the important aspects of collagen as a biomaterial in dentistry.

The word *collagen*, which was first used in 1865, originated from the Greek language and means "glue-maker." In mammals, every tissue contains collagen, making it the most abundant protein and the most important component of the body structurally and functionally. Its ubiquitous distribution throughout the human body involves collagen in all growth and remodeling processes, and because of its very specific structural, chemical, and biological characteristics, it may be readily studied from the viewpoints of morphology, biosynthesis, degradation, and geographic distribution as a function of time and physiologic change.[1] Collagen is also the oldest known protein. While less than 40 years ago, only one type of collagen was known, today 29 types of collagens and collagen-like proteins have been investigated.

The most defining feature of collagen is its structure, which gives it stability, functional ability, strength, and physical characteristics. It has three parallel polypeptide strands in a left-handed, polyproline II-type (PPII) helical conformation, which coil about each other with one residue stagger to form a right-handed triple helix. This elegant structural motif is the same for all of the 29 collagens. Astbury and Bell in 1940[2] were the first to describe it; however, with developments in science, improvements in its structure were made, and finally, in 1955, this structure was refined by Rich and Crick and by North and coworkers to become the triple helical structure accepted today.[3]

Collagen is composed of three chains—each of which is more than 1400 amino acids in length—that are wound together in a tight triple helix. This robust structure is formed by a repeating sequence of three amino acids, with every third amino acid being glycine; the other two are proline and hydroxyproline, which fill many of the remaining positions in the chain. Water present in the structure plays a role in maintaining the conformation of the native collagen molecule. The individual water bridges form and break within a few picoseconds to keep the imino-poor region from unwinding. However, the water molecules that form a single hydrogen bond to the backbone actually destabilize the triple helix, as they merely provide thermal energy to the structure. In addition, a hydration shell, which covers the entire triple helix, serves as an extensive cylinder of hydration surrounding the triple helix, and Hyp residues seem to act as "keystones" supporting the connection between the water network and the peptide molecules. The hydration shell is a biological lubricant in collagen self-assembly and determines the inter-helix distance. Without the hydration shell, collagen molecules would aggregate by forming nonspecific bonds, resulting in kinetically trapped amorphous states.[4]

Advances in the medical arena led to understanding the different properties of collagen and their application in both the

medical and dental fields. The most important of these properties are elasticity and repair, properties that have also been implicated in the collagenous products widely used in biomedical applications for regeneration of tissues. Collagen's association within the medical field as a biomaterial is not new; it was used as the first medical suture more than 5000 years ago in the form of hair.[5] Collagen catgut sutures were also developed and used for repair of wounds, such as those of gladiators treated by Claudius Galenus.[5] In dentistry today, collagen is used as a membrane, bone graft material, an agent for local drug delivery, and a hemostatic agent.

Collagen functions as a kind of trigger that influences the proliferation and differentiation of specialized cells. From a biomedical view, collagen plays an important role in development, wound healing, platelet activation, and angiogenesis.[5] These properties of collagen have been used for various biomedical applications—for example, as drug delivery systems including: collagen shields in ophthalmology; sponges for burns/wounds; mini pellets and tablets for protein delivery; gel formulation in combination with liposomes for sustained drug delivery; a controlling material for transdermal delivery; nano particles for gene delivery; and basic matrices for cell culture systems. Collagen is also used for tissue engineering, including skin replacement, bone substitutes, and artificial blood vessels and valves.[6] Hydrolyzed collagen can play an important role in weight management. In cosmetic surgery, collagen is widely used as dermal fillers for treatment of wrinkles and aging skin.[7,8]

Collagen has wide applications in the dental field. For example, collagen plugs are used for control of bleeding, and resorbable forms of collagen are 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 defect areas.[8] Table 1 lists benefits and shortcomings of collagen as a biomaterial.[9-12]

TABLE 1

Benefits and Disadvantages of Collagen as a Biomaterial

BENEFITS

- Available in abundance and easily purified from living organisms (constitutes more than 30% of vertebrate tissues)
- Non-antigenic
- Biodegradable, bioresorbable, non-toxic, and biocompatible; biodegradability can be regulated by cross-linking
- Synergic with bioactive components
- Biological plastic due to high tensile strength and minimal expressibility
- Hemostatic – promotes blood coagulation
- Formulated in a number of different forms
- Easily modifiable to produce materials as desired by utilizing its functional groups
- Compatible with synthetic polymers

DISADVANTAGES

- High cost of pure type I collagen
- Variability of isolated collagen (eg, cross-link density, fiber size, trace impurities, etc.)
- Hydrophilicity that leads to swelling and more rapid release
- Variability in enzymatic degradation rate as compared with hydrolytic degradation
- Complex handling properties
- Side effects such as bovine spongeform encephalopathy (BSF) and mineralization

Collagen Membranes

In dentistry, membranes are used for periodontal reconstruction in the process known as guided tissue regeneration (GTR). Membranes are used to preclude 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 that have regenerative potential.[13] The first of these membranes to be commercially available were made of expanded polytetrafluorethylene (ePTFE) and were nonresorbable.[13]

Available now are a number of resorbable barrier materials that eliminate the need for a second surgery. There are various materials with which bioresorbable membranes have been prepared, such as polyglycolic acid, polylactic acid, dura mater, pericardium, oxidized cellulose, rubber dam, and laminar bone; however, collagen has shown to be particularly suitable for GTR applications because it is chemotactic for periodontal ligament fibroblasts,[14] acting as a barrier for migrating epithelial cells,[15] providing hemostasis,[16] and serving as a fibrillar scaffold for early vascular and tissue ingrowth.

Preparation of Collagen Membranes

Collagen can be prepared from a number of sources using a variety of techniques. However, collagen membranes typically are manufactured by demineralization of whole or pulverized bone, generally accompanied by lipid extraction.[17] The process begins with collagen being solubilized or dispersed, then purified and reconstituted. The noncollagenous materials subsequently are removed and the remaining collagen stabilized before implantation.[18] Lastly, cross-linking that occurs during biological maturation of collagen can be stimulated *in vitro* by several factors such as the type and concentration of the processing agent as well as the pH and temperature of incubation.[19]

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. Cross-links can be introduced by either physical or chemical reagents.[9] The most widely used cross-linking technique currently is glutaraldehyde (GA).[9] GA cross-linking of collagenous tissues significantly reduces antigenicity and biodegradation of the implant.[20] Essentially, GA blocks the lateral amino groups of collagen and achieves cross-links between peptide chains.[9]

Physical methods include drying or irradiating the collagen with ultraviolet or gamma radiation. Irradiation has two main effects on collagen: initiating random cross-links and breaking the tropocollagen molecule.[21] Sterilization methods for collagen include dry heat, ethylene oxide, and irradiation. Nevertheless, if collagen is carefully dried prior to heating, its stability is increased and sterilizing temperatures can be applied.

Collagen Membranes Used for GTR

The various types of membranes used for GTR treatment are derived from different animal sources—eg, bovine, porcine, or equine—and obtained from a variety of sites, such as tendon or dermis. They are obtained from type I or type III collagen or both. Some commonly available membranes are: BioMend® (Zimmer Dental, www.zimmerdental.com), Bio-Gide® (Geistlich Pharma, www.geislich-na.com), OraMem® (Salvin, www.salvin.com), RCM6 Resorbable Collagen Membrane and conFORM™ (Ace Surgical, www.acesurgical.com), and Periogen® (Collagen Corp.). The suitability of other collagen types such as rat collagens, Avitene™ (Davol, Inc., www.davol.com), and dura mater also have been investigated, with varying results.[19]

Studies have indicated that bone growth into the pores and interstices of the membrane may occur at the sites where the membrane has come into close contact with bone. Bone is directly deposited on the pore wall, filling the pores almost completely and even penetrating through the membrane consisting of a perfectly bioinert material with osteoconductive properties.[22] This phenomenon is found in collagen-based barrier membranes, particularly

TABLE 2

Collagen Bone Substitutes

COMMERCIAL NAME (COMPANY)	COMPOSITION	COMMERCIALLY AVAILABLE FORMS
HEALOS® Bone Graft Replacement (DePuy Spine, Inc.)	Mineralized collagen matrix	Variety of strip sizes
Integra Mozaik™ (Integra OrthoBiologics)	80% highly purified b-TCP/20% highly purified type-1 collagen	Strip and putty
Infuse® Bone Graft (Medtronic Spinal & Biologics)	rhBMP-2 protein on an absorbable collagen sponge	Multiple kit sizes
MasterGraft® Matrix (Medtronic Spinal & Biologics)	Biphasic calcium phosphate and collagen	Compression-resistant block
MasterGraft® Putty (Medtronic Spinal & Biologics)	Biphasic calcium phosphate and collagen	Moldable putty
MasterGraft® Strip (Medtronic Spinal & Biologics)	Biphasic calcium phosphate (15% HA and 85% b-TCP) and collagen	Compression-resistant strip
Progenix® Plus (Medtronic Spinal & Biologics)	DBM in type-1 bovine collagen and sodium alginate	Putty with demineralized cortical bone chips
Progenix® Putty (Medtronic Spinal & Biologics)	DBM in type-1 bovine collagen and sodium alginate	Ready-to-use injectable putty
Vitoss® (Orthovita)	100% b-TCP and 80% b-TCP/20% collagen and 70% b-TCP/20% collagen/10% bioactive glass	Putty, strip, flow, morsels, and shapes
OP-1® Implant (Stryker Biotech)	rhBMP-7 with type-1 bone collagen	Lyophilized powder reconstituted with saline to form wet sand-like consistency
OP-1® Putty (Stryker Biotech)	rhBMP-7 with type-1 bone collagen plus carboxymethyl-cellulose (putty additive)	Lyophilized powder reconstituted with saline to form wet sand-like consistency
CopiOs® Bone Void Filler (Zimmer)	Dibasic calcium phosphate and type-1 collagen	Cancellous chips, cancellous cubes, and cortico-cancellous wedges

if they are cross-linked.[22] Although the ideal GTR membrane is yet to be developed, those made of collagen currently appear to provide many of the desired characteristics.[9]

Hemostatic Collagen

Collagen is also used for control of bleeding; various products used for this purpose are available, including CollaPlug®, CollaCote®, and CollaTape® (Zimmer Dental) and Helistat® Absorbable Collagen Hemostatic Sponge (Integra Lifesciences Corp., www.integralife.com). These agents are soft, white, pliable, nonfriable, coherent, sponge-like structures. They are fabricated from bovine collagen (usually from deep flexor tendons) and are nontoxic and nonpyrogenic. The products are highly absorbent and able to hold many times their own weight of fluid. Their indications are for wound protection and for control of oozing or bleeding from clean oral wounds. They may be cut to shape and applied to a bleeding surface. When placed, they rapidly absorb blood, creating an artificial clot-like structure, thereby slowing or stopping bleeding at the site. Regarding their application, these products should be held in place for approximately 2 to 5 minutes to achieve hemostasis and then may be removed, replaced, or left *in situ*. These collagen materials completely resorb within 14 to 56 days, depending on how cross-linked the material is.[23]

In addition to serving as a mechanical obstruction to bleeding, these materials affect the coagulation process. When in contact with blood, collagen causes aggregation of platelets, which bind in large numbers to the collagen fibrils. The aggregated platelets degranulate, releasing factors such as thromboxane A2,[24] which assists in the formation of a clot. The collagen sponge also provides a 3-dimensional (3-D) matrix for strengthening the blood clot.[25]

As with most hemostatic agents, collagens are not to be used in infected or contaminated wounds. The agents may serve as a nidus for abscess formation and may potentiate bacterial growth. Possible adverse reactions are formation of adhesions, allergic reactions, foreign body reactions, wound dehiscence and the formation of subgalealseroma, which is an accumulation of blood serum beneath the scalp.[25] Precautions include possible interference with wound healing, and placement in extraction sockets has been associated with increased pain. In an animal model, incision sites inoculated with *Staphylococcus aureus* demonstrated more infection when collagen was used, as compared to a control.[25] Besides sponges and plugs, these products are available in microfibrillar form. This form is generally less useful for oral surgical procedures.

Collagen as Bone Substitutes

Collagen has been used as implantable carriers for bone-inducing proteins such as bone morphogenetic protein-2 (rhBMP-2).[26] Collagen, in combination with other polymers or chemicals, is used for bone augmentation procedures (Table 2). Demineralized bone collagen is used as a bone graft material for the treatment of acquired and congenital defects either by itself or in combination with hydroxyapatite.[27]

Collagen for Local Drug Delivery System

Different types of collagen-based membranes have been tested for local drug delivery.[28] A degradable controlled-release device based on formaldehyde cross-linked bycoprotein matrix containing chlorhexidine was described by Steinberg et al 1990.[28] PerioCol® (Eucare Pharmaceuticals Private Limited, www.eucareindia.com) is the most commonly used collagenous membrane for local drug delivery system and has two contents: chlorhexidine and collagen.

The source of collagen is from the air bladder of fresh water fish. The air bladder is an accessory respiratory organ used for real respiration.[28] Fish collagen, which is also called Piscean collagen, is a protein extracted from fish waste, including the scales, skin, or air bladders; it is predominantly sourced from fresh water carp, Labeo rohita.[29] Purifying collagen from fish involves alkaline hydrolysis, returning the collagen to a neutral pH, and subjecting it to acid hydrolysis to stabilize it. The collagen is freeze-dried into particles, sponge, and sheets that can be cut into the required shape

CLAIMED MECHANISMS OF ACTION

Osteoconduction; Creeping substitution; Osteoinduction; Osteogenesis when mixed with bone marrow aspirate; Bone void filler, must be used with autogenous bone marrow
Osteoconduction; Bioresorbable; Bone void filler
Bioresorbable carrier; Osteoinduction; Chemotaxis of stem cells; indirect osteogenesis
Osteoconduction; Bioresorbable; Bone void filler, must be used with autogenous bone marrow
Osteoconduction; Bioresorbable; Bone graft extender, must be used with autograft; Bone void filler, must be used with autogenous bone marrow and/or autograft and/or sterile water
Osteoconduction; Bioresorbable
Osteoconduction; Bioresorbable; Osteoinduction; Bone graft substitute; Bone graft extender; Bone void filler
Osteoconductoin; Bioresorbable; Osteoinduction; Bone graft substitute; Bone void filler; Bone graft extender, must be used with autograft bone
Osteoconduction; Bioresorbable; Bioactive/osteostimulation; Osteogenesis and osteoinduction when mixed with bone
Bioresorbable scaffold; Osteoinduction
Bioresorbable scoffold; Osteoinduction
Osteoconduction

b-TCP = beta-tricalcium phosphate
HA = hydrofluoric acid
DBM = demineralized bone matrix

and size.[30] The film membrane preparation is cast onto slabs and is not freeze-dried.

PerioCol is prepared by incorporating 2.5 mg chlorhexidine from a 20% chlorhexidine solution in a collagen membrane. The collagen-based chip, which is 4 mm x 5 mm in size, 0.25 mm to 0.32 mm in thickness, and 10 mg in weight, is sterilized by gamma irradiation at 2.5 mega rads and individually packed.[28] Application of this chip with chlorhexidine in chronic periodontitis as an adjunct to scaling and root planing procedures has shown reduction in probing pocket depth, gingival bleeding, and clinical attachment level compared to scaling and root planing alone.[28]

Another material commonly used for local drug delivery is Periodontal Plus AB™ (EnColl, www.encoll.com). This product comprises tetracycline fibers impregnated in collagen fibers, which are available in vials. These brownish-colored fibers are resorbable. They are soaked in saline and packed into periodontal pockets with a cotton forceps or curette until the pocket is filled up to or slightly below the gingival margin.31 This allows diffusion of the drug as the collagen undergoes resorption, releasing the drug from the matrix in a controlled manner.

Collagen Dressings

Collagen in dentistry is also used for wound dressing. Several major features of collagen led to the development of collagen dressings. First is its structural and functional significance in wound repair. Then, too, is its hydrophilic nature, which can be attributed to a molecular structure characterized by a high content of diaminodicarboxylic amino acids and carbohydrate moieties, which provide a surface geometry very suitable for cell adhesion. Another factor, which promotes attraction of fibrogenic cells to collagen implants, is the presence of a glucoprotein-like fibronectin on the surface of the cells. These molecules have a high affinity for collagen and link specifically with the definite regions on the collagen surface.

There are two primary forms of collagen wound dressing. The first is collagen sponges. Sponges form a 3-D structure that composes large pores or channels, interchannel communications, and combinations of macromolecules of the connective tissue to enhance wound tissue infiltration and cell growth *in vivo*. Sponges have been used as both temporary and permanent coverings. Cellular ingrowth within a sponge depends on the porosity and the presence of a fibrous structure. Various collagenous sponges are available, including those found in Table 3.

The other form of collagen wound dressing is collagen films, which are made by casting collagen onto methacrylate surfaces. These are cross-linked with ultraviolet irradiations to improve their handling properties.

Conclusion

Collagen's abundance, its importance, and its high-resolution crystal structure and modern biophysical approaches have

TABLE 3

Collagen Sponges

COMMERCIAL NAME (COMPANY)	FUNCTION
Helisorb® Sponge (Eucare Pharmaceuticals Private Limited)	Hemostatic agent
OraPLUG® (Salvin)	Extraction and biopsy sites Bleeding control and blood clot stabilization Wound bed protection Provides matrix for tissue ingrowth
Avitene™ Ultrafoam™ Collagen Sponge® (Davol, Inc.)	Topical hemostat
CollaPlug® (Zimmer Dental)	Extraction and biopsy sites Four-wall sockets
CollaTape® (Zimmer Dental)	Localized ridge defects Socket grafting Schneiderian membrane tears Subantral augmentations Soft tissue donor sites
CollaCote® (Zimmer Dental)	Soft tissue recontouring Sinus graft containment Guided bone regeneration Sinus membrane perforation
Hémocollagène (hemostatic sponge with collagen of bovine origin) (Septodont USA)	Local hemostasis after dental surgical procedures

enabled detailed study of the structure and stability of collagen triple helices, simplifying the means to synthesize long collagen triple helices and fibrils. The resultant materials are poised for use in biomedicine and nanotechnology. The functional significance due to elasticity and repairability has been implicated in collagenous biomaterials' ability to enhance regeneration of periodontal tissues and restoration of esthetics and function.

These unique mechanical properties are predominantly dependent on the formation of intermolecular cross-links between the collagen molecules within the fibers to prevent slippage under load and by the varied alignment of these cross-linked fibers within a tissue.[32]

Thus, it has become necessary to systematically study the mechanical behavior of collagen and understand its microscopic design details, as these would influence the mechanical strength and other properties when viewed on a larger scale. Understanding these properties has shed light on their behavior in higher bodily structures and how different types of collagen influence the mechanical properties of different types of tissues. This thorough understanding supports the use of collagen for manufacturing biomaterials that provide superior quality of biomedical agents, especially for the reconstruction and regeneration of an ailing periodontium.

DISCLOSURE

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