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Use of collagen as an implantable material in the reconstructive procedures - an overview

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Abstract

Collagen is an important biomaterial in medical applications due to its special characteristics, such as biodegradability and weak antigenicity. Thus collagen, as a new type of biomaterial, has been used in drug delivery systems and tissue engineering. 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.

Keywords: Collagen; membranes; guided tissue regeneration.

Introduction

The manifold biomedical applications of proteins require preliminarily knowing of their thermal, chemical and mechanical stability under given employing conditions of them. More than a third of the proteins synthesized by the living cells of mammalian and human body consist of collagens and, among them, the collagen type I is the majority structural protein. As a consequence of an excellent biodegradability and a low immunogenic effect, the collagen type finds a lot of applications in biomedical field, cosmetics or pharmaceutical industry (Micutz, 2010).

The word collagen originated from the Greek word “kola” which means glue. This stems from the ancient process of boiling skins and parts of animals to produce the gelatin and protein rich tissue, which could then be manufactured into a type of adhesive. This original formula for using collagen as an adhesive has been carbon dated, and is currently known as the oldest formula for glue (Britannica Concise Encyclopedia).

The molecular and packing structures of collagen have eluded scientists over decades of research. The first evidence that it possesses a regular structure at the molecular level was presented in the mid-1930s (Clark, 1935). Since that time many prominent scholars, including Crick, Pauling, and Ramachandran, concentrated on the conformation of the collagen monomer. A number of other models, dealing with the conformation of each individual peptide chain, gave way to the triple-helical "Madras" model which provided a basic representation of the molecule's quaternary structure (Leonidas, 2001). The packing structure of collagen has not been defined to the same degree outside of the fibrillar collagen types, although it has been long known to be hexagonal or quasi-hexagonal (Fraser and MacRae, 1981). It has been alleged that the packing arrangement of collagen molecules is 'sheet-like' or microfibrillar. The microfibrillar structure of collagen fibrils in tendon, cornea and cartilage has been directly imaged by electron microscopy (Fraser and MacRae, 1981).

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 beneficial 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 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. Indisputably, collagen in almost all possible physical states (including solutions, gels, powder, fibers, membranes, sponges, tubing, etc.) has been used in medicine and dentistry.

Review of Literature

Mattson *et al.* (1995) in their study suggested that the use of type I bovine collagen as a membrane barrier did not elicit adverse clinical reactions, enhanced the formation of new bone in intrabony defects, resulted in a gain of clinical attachment, and reduced probing depths.

Bunyaratavej and Wang (2001) reported that the use of grafting material in combination with collagen membranes seems to improve clinical outcomes for furcation, but not intrabony, defects when compared to the use of membranes alone.

Vinholis *et al.* (2001) in a study evaluating the effect of subgingival irrigation with a 1% chlorhexidine collagen gel in periodontal pockets as an adjunct procedure to scaling and root planing (SRP) reported that there was an improvement in clinical parameters in all groups with a significantly greater decrease in GI and bleeding in the chlorhexidine group. The authors concluded that 1% chlorhexidine collagen gel is a promising adjunct to SRP in the treatment of adult periodontitis.

Hartman *et al.* (2004) evaluated an organic bovine-derived xenograft (Bio-Oss Collagen) in the treatment of human periodontal defects and reported that periodontal regeneration is possible following a bone-replacement graft of Bio-Oss.

Discussion

A) General uses of collagen

Purified and/or cross-linked collagen (in the form of powder or sponge) has been used as a hemostatic agent and biological dressing as well as in management of burn wounds, in combination with ophthalmologic and orthopedic procedures, and for oral, dental and plastic surgeries.

Collagen is used for soft-tissue augmentation and to correct scars, fine lines, and deep wrinkles for aesthetic purposes. 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 (Knapp *et al.*, 1977). Gamma-irradiated amnionic collagen from human placenta has been tested in animal studies as an injectable material, primarily for tissue augmentation procedures (Spira, 1994). 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 (Zeide, 1986).

A fibrous dermal collagen preparation has been employed to reconstruct tissue contour defects resulting from loss of dermis, subcutaneous fat, and connective tissue. These preparations 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 (Oliver *et al.*, 1976).

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. In addition to their usefulness for skin augmentation and as a dressing, several types of vascular prostheses have been derived from collagen, these devices generally have been further treated with heparin to create anti-thrombogenic surfaces (Gill *et al.*, 1989)

Collagen tube allografts have been used to guide peripheral nerve regeneration and for vascular prostheses, while biological structures with high collagen content have been studied as autogenous transplants in vessel surgery.

Implantable collagen hydrogels have been incorporated as agents for delivery of chemotherapeutic agents (Chvapil *et al.*, 1973) 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 where the kinetics of drug release can be manipulated based on modifications made to the collagen carrier (Vasantha *et al.*, 1988).

For oral applications, homogenized reconstituted collagen mixed with cell culture media has been used for burn treatment and for endodontic repair (Bashutski and Wang, 2009). 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. Notably, 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 (Wang, 1998).

B) Use of collagen in guided tissue regeneration

The principle of Guided Tissue Regeneration (GTR) involves the use of a physiological barrier which is placed over the denuded lesions in such a way that all periodontal tissue except the periodontal ligament (PDL) cells and the alveolar bone are prevented from reaching contact with the root. The cells of PDL are the only ones which seem to have the capacity to form new attachment. Cells of PDL and differentiate faster than those of bone, thus even though bone cells are allowed to migrate to the area along with the cells of PDL, it is the cells of PDL which repopulate along the root surface.

Several investigators have examined type I collagen as a possible membrane barrier for use in GTR procedures. Collagen is absorbable, does not require a second surgical procedure for removal, and has some unique properties (Hyder *et al.*, 1992).

Collagen is used as a membrane due to following reasons: It is the major extracellular macromolecule of the periodontal connective tissue and is physiologically metabolized by these tissues, it is chemotactic for fibroblasts, it has been reported to act as a barrier for migrating epithelial cells in vitro, and it is a weak immunogen that has been used experimentally in animals and human. Pitaru *et al.* in a series of experiments, concluded type I collagen has the capacity to support regeneration of periodontal tissues (Pitaru *et al.*, 1988).

A study comparing the use of collagen and expanded polytetrafluoroethylene (ePTFE) in treating human mandibular Class II furcations reported that the collagen membrane evoked a lower inflammatory response than did the ePTFE, the material is pliable when moist and conforms well to the surgical area, collagen provides a thrombogenic surface that is sealed coronally to the root surface by a fibrin clot and there was no allergic response to the collagen (Blumenthal, 1993).

Formation of the fibrin clot on the root surface is a critical event for new attachment formation. The use of a membrane stabilizes the wound and protects the root surface-adhering fibrin clot from tensile forces acting on the wound margin. It was observed that collagen is chemotactic for fibroblasts. The collagen membrane barrier may act to enhance and protect the initial clot formation onto the root surface by acting as a scaffold for cell adhesion and ingrowth. It may also attract fibroblasts to the area which may aid in the formation of new attachment and regeneration during GTR procedures (Wikesjö *et al.*, 1992).

In a study by Numabe *et al.* (1993) using a telocollagen in the rat palate, it was found that it inhibited apical migration of the regenerating epithelium as well as accelerates connective tissue reattachment, in part by inhibiting the mitotic function of the basal epithelial cells during the early stages of wound healing.

In another study by Anderson (1991), type I collagen membranes were used as a barrier in the treatment of Class II furcations to controls with no barriers. The mean vertical defect fill was 1.63 mm for the test and 0.30 mm for the controls. The mean horizontal defect fill was 1.76 mm and 0.68 mm for test and controls respectively. In the test group, a firm nonprobeable reparative connective tissue was found that was consistent with open probing new attachment.

C) 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. 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 (Tatakis *et al.*, 1999).

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. 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 (Bell *et al.*, 1979).

Collagen may be further treated with pepsin for removal of the terminal telopeptides of the molecule, which is the major inflammatory component. 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 (Chvapil and Krajicek, 1963).

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 (1991) reported increased regenerative tissue formation with use of cross-linked (vs. non cross-linked) collagen barriers, whereas Brunel *et al.* (1996) 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 crosslinking technique currently is glutaraldehyde (GA). GA cross-linking of collagenous tissues significantly reduces antigenicity and biodegradation of the implant. Essentially, GA blocks the lateral amino groups of collagen and achieves cross-links between peptide chains. In a study using porcine collagen membranes in two treatment protocols, one 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 (Vardaxis *et al.*, 1994).

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. 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 two main effects on collagen: initiating random crosslinks and breaking the tropocollagen molecule (Miyata, 1971).

Recently, a new cross-linking process has been developed, known as the iphenyl-phosphoryl- azide, or DPPA, technique, which achieves natural cross-links between peptide chains without leaving any foreign product in the cross-linked collagen (Petite *et al.*, 1990).

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. 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.

D) Types of collagen used in barrier membranes

The collagen comprising current GTR barriers are of various subtypes, derived from different animal sources (e.g., bovine or porcine), and

obtained from a variety of sites (e.g., tendon or dermis) (Wang and MacNeil, 1998).

Some of the membranes commercially available for use in the United States include Biomend (SulzerCalcitek, 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 (Wang and MacNeil, 1998).

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. This membrane maintains its barrier function for 4 to 6 months (Camelo, 1998).

The suitability of other collagen types such as rat collagens, Avitene, and dura mater also has been investigated, with varying results. Avitene, a microfibrillar collagen hemostat derived from bovine corium, has been evaluated histologically in humans (Tanner *et al.*, 1988). 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 (Busschop and de Boever, 1983).

E) Properties and design of barrier membranes

Membranes used for GTR procedures ideally should offer biocompatibility, cell exclusion, space maintenance, and reasonable manageability (Scantlebury, 1993). Biocompatibility allows the material to function in a specific situation without adversely and significantly affecting the body (or the body tissue affecting the material). 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 requiring mechanical properties that allow the barrier to withstand forces exerted by or through the periodontal tissues. Bioabsorbable membranes also should maintain the underlying space long enough to allow the coagulum to mature and allow selective repopulation. Although the optimum time period may vary, cell repopulation is greatest during the first 2 weeks of healing but subsides during the third week (Scantlebury, 1993). Other studies have suggested that 3 to 4 weeks is enough time for allowing repopulation to occur (Minabe, 1991).

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. Membranes should be easy to cut and shape, with no sharp edges to perforate tissue, and be pliable enough to allow close adaptation to a variety of defect morphologies. In addition to those general characteristics, resorbable barriers must also be nontoxic, non-antigenic, and produce a minimal inflammatory response to the bioresorption process without interfering with regeneration. Indeed, creation and maintenance of a space is a critical requirement for bone formation. Although the ideal GTR membrane has yet to be developed, those made of collagen currently appear to provide many of the desired characteristics.

F) 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 (Chvapil, 1979). 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. In general, the activity of collagenase appears to be high for processed, denatured proteins. 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 [3H]-labeled collagen as a tracer, providing a method to quantitate the amount of collagen present as a function of time. Kronenthal (1975) 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 space making capacity. Strength loss occurs due to initial cleavage of the polymer backbone, resulting in a decrease in space making 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. Cross-linking of collagen with glycosaminoglycans (GAGs) results in polymers that are more resistant to collagenase degradation than was GAG-free collagen and the rate of degradation decreases with increasing GAG content (Yannas, 1988).

Conclusion

In summary, collagen appears to be a good material for use as a biomedical implantable device. Collagen is used to form a matrix for regenerating tissue outside of the body, for example in regenerating skin for use in burns treatment, but increasingly it is also used in the development of other tissues offering the prospect of growing replacements for damaged organs. 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.

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