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].

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.

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 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 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

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 to one another. The presence of these respective amino acids gives type I collagen its structural

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

Specialty	Application
Dermatology	Soft-tissue augmentation[7]
Dentistry	Oral wounds[8] Biocoating for dental implants[9] Support for hydroxyapatite[10] Periodontal attachment[11]
General surgery	Hemostasis[12,13] Hernia repair[14,15]
Neurosurgery	Nerve repair[16,17] Nerve conduits[18]
Orthopaedic	Bone repair[19,20] Articular cartilage reconstruction[21]
Ophthalmology	Corneal graft[22,23] Tape for retinal reattachment[24]
Plastic surgery	Repair of tissue defects[25,26]
Urology	Ureter replacement[27] Dialysis membrane[28] Renal repair[29]
Vascular	Vessel replacement[30,31]
Other	Biocoating[32,33] Drug delivery[34,35]

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 self 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 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

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 sufficient quantity for widespread utilization. The material should be conducive for vascularization 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.

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	Plasma expander
gel	cosmeticum
flour	hemostatic agent
fibers	sutures, weaving of blood vessels, valve prosthesis
membrane	corneal replacement, wound dressing, hemodialysis
sponge	wound dressing, surgical tampons, vaginal contraceptive
tubing	vessel prosthesis, reconstruction of hollow organs such as the esophagus and trachea

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.

Cell Growth on Collagen Matrices

Wound repair involves many processes including cell migration, biosynthesis and deposition of connective tissue components, deposition and remodeling of granulation tissue. Type I collagen has been found to promote fibroblast 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 ulcers 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 I 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.

Clinical Applications

Type I collagen has been used in a wide variety of applications (Tables 1 and 2). The most successful applications of collagen exploit unique biological characteristics, for which no synthetic substitute 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

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

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

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 agents. 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.

Conclusions

Type I collagen is 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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