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Review

Biomedical applications of collagen

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Abstract

Collagen is regarded as one of the most useful biomaterials. The excellent biocompatibility and safety due to its biological characteristics, such as biodegradability and weak antigenecity, made collagen the primary resource in medical applications. The main applications of collagen as drug delivery systems are 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, as controlling material for transdermal delivery, and nanoparticles for gene delivery and basic matrices for cell culture systems. It was also used for tissue engineering including skin replacement, bone substitutes, and artificial blood vessels and valves. This article reviews biomedical applications of collagen including the collagen film, which we have developed as a matrix system for evaluation of tissue calcification and for the embedding of a single cell suspension for tumorigenic study. The advantages and disadvantages of each system are also discussed.

Keywords: Collagen; Biomaterial; Drug/protein/gene delivery; Tissue engineering

1. Introduction

Collagen, a well-known protein, has been widely used in medical applications. Many natural polymers and their synthetic analogues are used as biomaterials, but the characteristics of collagen as a biomaterial are distinct from those of synthetic polymers mainly in its mode of interaction in the body (McPherson et al., 1986). Collagen plays an important role in the formation of tissues and organs, and is involved in various functional expressions of cells. Collagen is a good surface-active agent and demonstrates its ability to penetrate a lipid-free interface (Fonseca et al., 1996). Collagen exhibits biodegradability, weak antigenecity (Maeda et al., 1999) and superior biocompatibility compared with other natural polymers, such as albumin and gelatin.

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The primary reason for the usefulness of collagen in biomedical application is that collagen can form fibers with extra strength and stability through its self-aggregation and cross-linking. In most of drug delivery systems made of collagen, in vivo absorption of collagen is controlled by the use of crosslinking agents, such as glutaraldehyde (Barbani et al., 1995), chromium tanning (Bradley and Wilkes, 1977), formaldehyde (Ruderman et al., 1973), polyepoxy compounds (Tu et al., 1993), acyl azide (Petite et al., 1990), carbodiimides (Nimni et al., 1988), and hexamethylenediisocyanate (Chvapil et al., 1993). Physical treatment, such as ultra-violet/gamma-ray irradiation and dehydrothermal treatment, have been efficiently used for the introduction of crosslinks to collagen matrix (Harkness 1966; Stenzel et al., 1969; Miyata et al., 1971; Gorham et al., 1992). The thiolation of denatured collagen, which allows precise amounts of SH groups to be attached onto the protein backbone, was also investigated (Nicholas and Gagnieu, 1997). It was reported that, under optimized conditions, oxidized and denatured thiolated collagen films are more resistant and rigid than glutaraldehyde treated ones.

The use of collagen as a drug delivery system is very comprehensive and diverse. Collagen can be extracted into an aqueous solution and molded into various forms of delivery systems. The main applications of collagen as drug delivery systems are collagen shields in ophthalmology (Kaufman et al., 1994), sponges for burns/wounds (Rao, 1995), mini-pellets and tablets for protein delivery (Lucas et al., 1989), gel formulation in combination with liposomes for sustained drug delivery (Fonseca et al., 1996), as controlling material for transdermal delivery (Thacharodi and Rao, 1996), and nanoparticles for gene delivery (Rossler et al., 1995). In addition, its uses as surgical suture (Miller et al., 1964), hemostatic agents (Cameron, 1978; Browder and Litwin, 1986), and tissue engineering including basic matrices for cell culture systems (Kemp, 2000) and replacement/substitutes for artificial blood vessels and valves (Chvapil et al., 1993; Auger et al., 1998; Huynh et al., 1999) were reported earlier.

Due to its excellent biocompatibility and safety, the use of collagen in biomedical application has been rapidly growing and widely expanding to bioengineering areas. However, some disadvantages of collagen-based systems arose from the difficulty of assuring adequate supplies, their poor mechanical strength, and ineffectiveness in the management of infected sites (Friess, 1998). Improvement of the physical, chemical and biological properties will be necessary to address some of drawbacks in collagen based applications. The better collagen delivery systems having an accurate release control can be achieved by adjusting the structure of the collagen matrix or adding other proteins, such as elastin, fibronectin or glycosaminoglycans (Doillon and Silver, 1986; Lefebvre et al., 1992, 1996). A combination of collagen with other polymers, such as collagen/liposome (Kaufman et al., 1994; Fonseca et al., 1996) and collagen/silicone (Suzuki et al., 2000), has been proposed to achieve the stability of a system and the controlled release profiles of incorporated compounds.

The purpose of this article is to review biomedical applications of collagen including the collagen film, which we have developed as a matrix system for evaluation of tissue calcification and controlled delivery of cardiovascular drugs. This collagen matrix system further serves as the substrate for the embedding of a single cell suspension or a small tumor specimen in the evaluation process of cancer therapy. The advantages and disadvantages of each system are also discussed.

2. Characterization of collagen as a biomaterial

Collagen is the primary structural material of vertebrates and is the most abundant mammalian protein accounting for about 20–30% of total body proteins (Harkness, 1961). It is present in tissues of primarily mechanical function. About one half of the total body collagen is in the skin and about 70% of the material other than water present in dermis of skin and tendon is collagen. Collagen made its appearance at the early stage of evolution in such primitive animals as jellyfish, coral and sea anemones (Bergeon, 1967). Collagen is synthesized by fibroblasts, which usually originate from pluripotential adventitial cells or reticulum cells.

The molecular structure of collagen has been firmly established on the evidence from earlier studies, such as amino acid composition analysis, X-ray diffraction analysis, electron microscopy and physicochemical examination of solutions (Gross, 1963; Ramachandran and Sasisekharan, 1965; Burge, 1964). Collagen has a unique structure, size and amino acid sequence (Miyata et al., 1992; Rao 1995). The collagen molecule consists of three polypeptide chains twined around one another as in a three-stranded rope. Each chain has an individual twist in the opposite directions. The principal feature that affects a helix formation is a high content of glycine and amino acid residues (Piez, 1984). The strands are held together primarily by hydrogen bonds between adjacent –CO and –NH groups, but also by covalent bonds (Harkness, 1966). The basic collagen molecule is rod-shaped with a length and a width of about 3000 and 15 Å, respectively, and has an approximate molecular weight of 300 kDa (Traub and Piez, 1971; Nimni and Harkness, 1988).

At least 19 types of collagen have been reported. Types I, II and III collagen as well as types V and XI are built up of three chains and all are composed of the continuous triple-helical structure. Types I, II, III, and V are called as fibril forming collagens and have large sections of homologous sequences independent of species (Timpl, 1984). In type IV collagen (basement membrane), the regions with the triple-helical conformation are interrupted with large non-helical domains as well as with the short non-helical peptide interruption. Fibril associated collagens (Type IX, XI, XII and XIV) have small chains, which contain some non-helical domains. Type VI is microfibrilla collagen and type VII is anchoring fibril collagens (Samuel et al., 1998).

An investigation on the native collagen has led to a better understanding of a structure function relationship between target drugs and collagen. A three-dimensional (3-D) model of fibril-forming human type II collagen was proposed for the development of synthetic collagen tissues and the study of the structural and functional aspects of collagen (Chen et al., 1995). This system also allows the studies of the stereochemistry of all the side chain groups and specific atomic interactions, and further evaluation of its therapeutic effects on collagen related diseases. The orderly arrangement of triple helix tropocollagen molecules results in a formation of fibrils having a distinct periodicity. Nonhelical telopeptides are attached to both ends of the molecule and serve as the major source of antigenecity. Atelocollagen, which is produced by elimination of the telopeptide moieties using pepsin, has demonstrated its potential as a drug carrier, especially for gene delivery (Kohmura et al., 1999; Ochiya et al., 1999).

Table 1 summarizes the major characteristics of collagen, which are suitable for medical applications (Jerome and Ramshaw, 1992; Rao, 1995; Friess, 1998; Fujioka et al., 1998; Maeda et al.,

Table 1
Advantages and disadvantages of collagen as a biomaterial[a]

Advantages
Available in abundance and easily purified from living organisms (constitutes more than 30% of vertebrate tissues);
Non-antigenic;
Biodegradable and bioreabsorbable;
Non-toxic and biocompatible;
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;
Biodegradability can be regulated by cross-linking;
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 (e.g. crosslink density, fiber size, trace impurities, etc.);
Hydrophilicity which 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

[a] Sources; Jerome and Ramshaw, 1992; Rao, 1995; Friess, 1998; Fujioka et al., 1998.

1999). It is easily absorbable in the body and has very low antigenicity. It has high tensile strength and high affinity with water. Moreover, it is non-toxic, biocompatible and biodegradable (Friess, 1998; Maeda et al., 1999). It can be prepared in a number of different forms including strips, sheets, sponges and beads. Collagen can be solubilized into an aqueous solution, particularly in acidic aqueous media, and can be engineered to exhibit tailor-made properties. Collagen is relatively stable due to its function as the primary structural protein in the body, but it is still liable to collagenolytic degradation by enzymes, such as collagenase and telopeptide-cleaving enzymes (Woolley, 1984). Collagenase binds tightly to triple helices, and degrades collagen starting from the surface. The melting profile of the pepsin-solubilized collagen showed a biphasic transition, indicating that age-related decrease in thermal stability has implications for the mechanical strength and turnover of the bone collagen (Danielsen, 1990).

Reports of adverse reactions to collagen have been restricted to localized redness and swelling following plastic surgery using collagen implants and wound breakdown with the use of catgut suture material (Webster et al., 1984; Carrol, 1989). Clinical reactions to collagen were rare, but two cases of allergic (IgE-mediated) reactions to bovine collagen were reported (Mullins et al., 1996). Patients in both cases developed conjunctive edema in response to the topical application of highly purified bovine collagen to eye during ophthalmic surgery. When irritant effects and cytotoxicity of various products developed from collagen were evaluated, cell response to exogenous collagen starts shortly after the material is kept in contact with tissues, evoking a local and fast inflammatory response, whose intensity depends on the pharmaceutical formulation in use (Trasciatti et al., 1998). Even though, unknown reverse effects may be discovered in the future application of collagen for gene delivery or tissue engineering, since collagen can help to avoid side effects originated from incorporated drugs, therapeutic peptides and proteins, the need for the continuous effort in development and evaluation of collagen based systems is manifest.

3. Collagen-based drug delivery systems

3.1. Film/sheet/disc

The main application of collagen films is as barrier membrane. Films with the thickness of 0.01–0.5 mm and made of biodegradable materials, such as prepared from telopeptide-free reconstituted collagen, demonstrated a slow release profile of incorporated drugs (Rubin et al., 1973). The drugs can be loaded into collagen membranes by hydrogen bonding, covalent bonding or simple entrapment. They can be sterilized and become pliable upon hydrolyzation, while retaining adequate strength to resist manipulation. When collagen film was applied to eye, it was completely hydrolyzed after 5–6 h (Bloomfield et al., 1978). This finding adds to evidence that collagen-based systems are suitable for resembling current liquid and ointment vehicles.

Collagen film/sheet/disc have been used for the treatment of tissue infection, such as infected corneal tissue or liver cancer. Soluble ophthalmic insert in the form of a wafer or a film was introduced as a drug delivery system for the treatment of infected corneal tissue using a high dose of antibiotic agents, such as gentamicin (Bloomfield et al., 1978) and tetracycline (Minabe et al., 1989a). The wafer route of administration gave the highest tissue concentration of incorporated drugs. The duration of therapeutic effect after administration of the collagen film containing tetracycline demonstrated that tetracycline could be detected in the plasma for more than 7 days after implantation into rabbits (Minabe et al., 1989a,b). The microfibrous collagen sheets as a local delivery carrier for the treatment of cancer was evaluated (Sato et al., 1996). The local application of collagen sheets loaded with anticancer agent, ectopocide (VP-16), resulted in a relatively long maintenance of drug concentrations at the target site, which, in this case, is the liver.

Some modifications on collagen film/sheet/disc have been made to control the release rate of incorporated drugs from delivery systems. Collagen films crosslinked by chromium tanning, formaldehyde or a combination of both have been successfully used as implantable delivery systems

in achieving the sustained release of medroxyprogesterone acetate (Bradley and Wilkes, 1977). A modification by attaching another membrane to collagen based film/sheet/disc was attempted to achieve the controlled release rate of incorporated drugs. A transdermal delivery device containing nifedipine, whose release rate was well controlled by an attached membrane made of chitosan, showed the highest therapeutic efficacy in the treatment of tissue infection (Thacharodi and Rao, 1996).

Collagen film and matrix were used as gene delivery carriers for promoting bone formation. A composite of recombinant human bone morphogenetic protein 2 (rhBMP-2) and collagen was developed to monitor bone development and absorbent change of carrier collagen (Murata et al., 1999, 2000). The rhBMP-2/collagen onlay implant resulted in active bone formation, whereas the collagen alone resulted in no bone formation. Collagen provides an anchorage for cell differentiation and remains as an artificial matrix in woven bone. In a similar study, collagen matrix loaded with BMP and placed in a close contact with osteogenic cells achieved direct osteoinduction without causing a cartilage formation (Nakagawa and Tagawa, 2000). These results indicate that a collagen based gene delivery system is very efficient as a biological onlay implant.

Collagen film and disc as gene delivery systems have many advantages. Systems that isolate transplanted cells from the host immune system might be beneficial and economically attractive, because they would allow the use of allogenic or even xenogenic cells in many patients (Liu et al., 1993; Al-Hendy et al., 1996; Tani et al., 1989; Brauker et al., 1995). The use of genetically modified cells for a long-term delivery of a therapeutic transgene product has been an attractive option for the treatment of monogenetic hereditary as well as many multifactorial nongenetic diseases (Barr and Leiden, 1991; Scharfmann, et al., 1991; Heartlein, et al., 1994). Biodegradable collagen films or matrices have served as scaffolds for a survival of transfected fibroblasts (Rosenthal and Kohler, 1997).

While these methods seem to allow an adequate cell survival, the concerns about long-term biocompatibility of non-degradable materials also arose. In several animal models, a long-term expression of a foreign gene after implantation of transfected cells has not been achieved (Aebischer et al., 1996). A combination of collagen and other polymers, such as atelocollagen matrix added on the surface of polyurethane films, enhanced attachment and proliferation of fibroblasts and supported growth of cells (Park et al., 2000). Transplantation of cells embedded in a lattice of polytetrafluoroethylene fibers coated with rat collagen followed by a mixing with matrigel as well as basic fibroblast growth factor showed that a long-term expression of human β-glucuronidase by retroviral-transduced murine or canine fibroblasts was achievable using a collagen-based matrix system. (Moullier et al., 1993a,b, 1995). Therefore, collagen-based film/disc systems seem to need to contain extra matrices that improve conditions for a long-term cell survival.

3.2. Collagen film as a calcifiable matrix system

One of the problems with implantable biomaterials is their calcification, which is influenced by structure of the implantable system, and determines its in vivo therapeutic efficiency and clinical fate (Schoen et al., 1992). Calcification of tissue or systems depends on chemical factors that operate at the cellular level around various tissues or biomaterials (Wada et al., 1999). Both collagen and elastin are major components of connective tissues, which possess a structure that compromises collagen fibers intimately associated with a remarkably stable elastin network. The suitability of collagen and elastin in many potential medical applications in reconstructive and plastic surgery including controlled delivery of bone morphogenetic protein has been reported (Vardaxis et al., 1996).

In our laboratory, the matrix films, which are composed of various combinations of collagen and elastin, have been developed and evaluated for its uses in tissue calcification and as a controlled delivery device for cardiovascular drugs. Collagen films, which we have developed, were used to simulate the calcification process of implantable biomaterials, such as bioprosthetic heart

valve (BHV). In BHV, the aortic wall and leaflet have mainly served as calcifiable matrix. Recently, the calcification of the bioprosthetic aortic wall has been extensively studied due to increasing use of the stentless BHV. The stentless BHV has a relatively large segment of exposed aortic wall comparing to the stented valve, in which virtually no aortic wall is exposed outside the stent. Thus, bioprosthetic aortic wall calcification has a potential to cause the clinical failure of the stentless BHV. Aortic wall is composed of 90% collagen and 10% of elastin, while leaflet is mainly composed of collagen (99%). Due to their differences in composition, the calcification rates of aortic wall and leaflet and their response to anti-calcification agents are different. For example, ethanol pretreatment completely inhibited calcification of porcine leaflet, while only partially (about 50%) inhibited calcification of porcine aortic wall (Lee et al., 1998). This may indicate that elastin has a more critical role in tissue calcification than collagen. This result has led to investigation of the elastin concentration effects on the calcification rate of implantable biomaterials.

Collagen films made of various combinations of collagen and elastin were evaluated for their suitability as drug delivery systems. Biomaterials should possess mechanical properties capable of withstanding the forces and motions experienced by the normal tissues and have sufficient fatigue strength to ensure a long life of the implant in vivo (Meaney, 1995). Tensile strength can be used for evaluation of mechanical strength, resilient activity, endurance and biocompatibility of the systems. Collagen films, we have developed, has a size of 6×10 mm and a thickness of 1 mm. Its tensile strength was determined using the following equation,

$$\text{Tensile strength } (\sigma) = \frac{\text{Force or load } (F)}{\text{MA}}$$

where F is the maximum load (in Newton) and MA is the minimum cross-sectional area of the film specimen (in square meters) (Fell and Newton, 1970). Chatillon DFM-10 Gauge equipped with LTC Manual Test Stand and GF-1 Grips (Ametek Test & Calibration Instruments Division, Largo, FL) was used. The tensile strength was almost constant irrespective of a loading ratio of collagen and elastin and within a durable range as shown in Table 2. There was no sign of swelling of the system, which is a sensitive marker of collagen deterioration (Bank et al., 2000). This system was well endurable after subcutaneous implantation in the rat for up to three weeks and no side effect or infection was detected. As shown in Fig. 1, as the concentration of elastin in a system increased from 10 to 90%, the total amount of calcium accumulation in a system implanted in the rat subcutaneous model also increased. This result indicates that elastin has a more critical role in tissue calcification than collagen and that this system can be used as a calcifiable matrix simulating calcification process of implantable tissues, such as BHV. This system can be further applicable to local and systemic delivery of various anti-calcification agents, cardiovascular drugs and antibiotics for the treatment of heart diseases.

Table 2
The tensile strength of films containing various combinations of collagen and elastin[a]

FILM Composition (Collagen:Elastin)	Tensile Strength (MPa $\pm$ S.D.)
90:10	2.9 ± 0.2
50:50	3.0 ± 0.3
20:80	3.1 ± 0.4

[a] $N = 6$ each. Values are not significantly different.

The collagen-based matrix, we have developed, can be used as a lattice for tissue culture systems. In the collagen based culture system, a film prepared from collagen fiber provides the substrate for the embedding of a single cell suspension or a small tumor specimen. Three-dimensional aggregates can be kept viable and proliferating for weeks, demonstrating very good degrees of resemblance compared with the in vivo tumors from which they were derived. The matrix remained stable in shape and size during the cell culture. The three-dimensional models show more resistance to treatment with anticancer drugs than cells growing as monolayers. They can serve as better models for the biological and biochemical characteristics of human solid tumors than monolayer subconfluent cell cultures. Collagen-based

matrix cultures offer unique opportunities for the study of the mechanisms behind human tumor progress.

3.3. Collagen shields

The collagen shield was originally designed for bandage contact lenses, which are gradually dissolved in cornea (Wedge and Rootman, 1992). The idea of using a shield or a hydrogel lens as a delivery device has led to the development of various drug delivery systems for ophthalmic applications. One of the merits of the collagen-based drug delivery systems is the ease with which the formulation can be applied to the ocular surface and its potential for self administration (Friedberg et al., 1991; Lee, 1990). The collagen corneal shield is fabricated from porcine sclera tissue that closely resembles collagen molecules of the human eye (Harrison, 1989). The mechanical properties of the shield protect the healing corneal epithelium from the blinking action of the eyelids (Mondino, 1991). The collagen corneal shield would promote epithelial healing after corneal transplantation and radial keratomy (Robin et al., 1990; Shaker et al., 1989; Marmer 1988; Poland and Kaufman, 1988; Waltman and Kaufman, 1970; Unterman et al., 1988).

Drug delivery by collagen shields depends on loading and a subsequent release of medication by the shield (Leaders et al., 1973). The collagen matrix acts as a reservoir and the drugs are entrapped in the interstices of the collagen matrix in a solution for water-soluble drugs or incorporated into the shield for water-insoluble drugs. As tears flush through the shield and the shield dissolves, it provides a layer of biologically compatible collagen solution that seems to lubricate the surface of the eye, minimize rubbing of the lids on the cornea, increase the contact time between the drug and the cornea, and foster epithelial healing (Kaufman, 1988; Kaufman et al., 1994). A bolus release of drug from the lenses was attributable to the enhanced drug effect (Waltman and Kaufman, 1970; Podos et al., 1972). Therefore, this system allows the higher corneal concentrations of drug, and the more sustained drug delivery into the cornea and the aqueous humor.

As shown in the Table 3, collagen shields were used as delivery devices for the treatment of various local infections and their therapeutic effects were compared with the conventional formula-

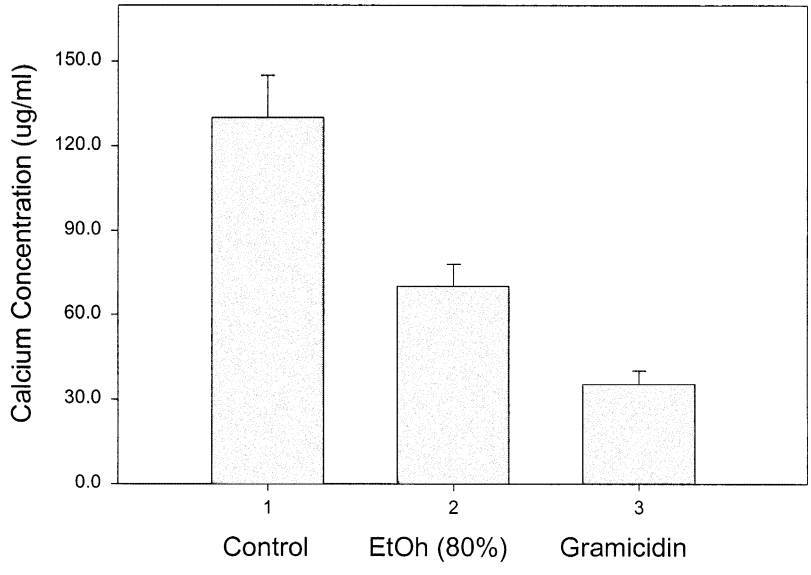

Fig. 1. The Effect of ethanol or gramicidin on the calcification rates of porcine aortic wall implanted in rat subcutaneous models ($N = 6$).

Table 3
The application of collagen shields for various topical agents

Drugs/Agents	Reference
Antibiotics	
Gentamicin	Bloomfield et al., 1978; Baziuk et al., 1992; Liang et al., 1992; Milan et al., 1993
Vancomycin	Phinney et al., 1988
Tobramycin	O'Brien et al., 1988; Poland and Kaufman, 1988; Unterman et al., 1988; Aquavella et al. 1988; Hobden et al., 1988; Sawusch et al., 1988a,b; Assil et al., 1992
Netilimycin	Dorigo et al., 1995
Polymyxin B Sulfate	Palmer and McDonald, 1995
Trimethoprim	Palmer and McDonald, 1995
AmphotericinB	Schwartz et al., 1990; Mendicute et al., 1995
Trifluorothymidine	Gussler et al., 1990
Acyclovir	Willey et al., 1991
Ofloxacin	Taravella et al., 1999
Steroids	
	Aquavella et al., 1988; Sawusch et al., 1988a,b; Hwang et al., 1989; Milan et al., 1993; Palmer and McDonald, 1995
Cholinergic:	
Pilocarpine	Aquavella et al., 1988
Antineoplastic:	
5-fluorouracil	Finkelstein et al., 1991
Anticoagulant:	
Heparin	Murray et al., 1990
Immunodepressant	
Cyclosporin	Chen et al., 1990; Reidy et al., 1990; Sato et al., 1996; Gebhardt and Kaufman, 1995
Gene therapy	
Plasmid DNA	Angella et al., 2000

tions. The use of antibiotic collagen shields supplemented by frequent topical applications has been clinically useful for preoperative and postoperative antibiotic prophylaxis, the initial treatment of bacterial keratitis, and the treatment of corneal abrasions (Poland and Kaufman, 1988; Friedberg et al., 1991). Most of these studies showed that shields provided equal or better drug delivery of fluorescein (Reidy et al., 1990), prednisolone acetate (Sawusch et al., 1988a,b), cyclosporine (Reidy et al., 1990), and ofloxacin (Taravella et al., 1999) to the anterior segment. Delivery of drugs through the impregnated collagen shield was more comfortable and reliable than frequent application of other conventional treatments, such as drops, ointment or daily subconjunctive injection (Friedberg et al., 1991).

An application of collagen shield was extended to gene delivery area. Delivery of plasmid DNA into the bleb through a collagen shield increased chloramphenicol acetyltransferase, the reporter gene, 30-fold over injection of plasmid DNA through saline vehicle (Angella et al., 2000). Gene therapy using naked plasmid DNA and a simple collagen shield delivery was very useful for regulating wound healing after glaucoma surgery.

Modifications of collagen were made to simplify the application, to meet the highest compliance, to reduce blurring of vision, and to enhance the drug concentration and bioavailability of drugs in the cornea and aqueous humor (Kuwano et al., 1997). The cross-linked collagen using glutaraldehyde or chromium tanning can serve as a drug reservoir and provide more desirable drug delivery than non cross-linked collagen shields by increasing the contact time between the drug and the cornea. Collasomes, in the form of collagen pieces, were developed by adding long hydrocarbon side chains to the collagen (Kaufman et al., 1994). This modification increases not only the hydrophobicity of the collagen but also the total surface area, which effectively decreases the diffusion rate of hydrophilic drug molecules from the collagen matrix. Collasomes can be formulated with various constituents and chemically alternated with the addition of lipid (called Lacrisomes) for the treatment of dry eyes (Kaufman et al., 1994). In this work, a delivery system in which collagen pieces were suspended in a viscous vehicle was instilled into the lower forniceal space. Collasomes hydrated in a solution of sodium fluorescein and suspended in a methylcellulose vehicle as a model for delivery of water-soluble drugs produced fluorescein concentrations much higher in the cornea and aqueous humor, comparing with fluorescein-containing vehicle alone.

Collagen shields as a drug carrier for topical agents have many advantages. Experimental and clinical studies showed that the speed of epithelial healing is faster and more complete with the use of the collagen shield than conventional formulations (Marmer, 1988). There was less stromal edema at the wound sites in collagen-treated corneas. The collagen shield seemed to protect keratocytes adjacent to the wound sites and diminish inflammatory reaction of keratocyte. Moreover, the surface epithelial bonding appeared to be normal with the use of the collagen shield. However, the application of collagen shields for drug delivery is limited by several disadvantages, such as reducing visual activity, causing slight discomfort, and a short duration at the inserted site (Friess, 1998). Some side effects of collagen shields were also reported. Shields were implanted into rabbit and guinea pig eyes and a possible toxic response was tested to determine whether collagen shields produce histological evidence of inflammation when implanted subconjunctively (Finkelstein et al., 1991). The inflammatory response in rabbit was noticeable after 7-day implantation and was much severe than in guinea pig. This study may indicate that the inflammatory response in rabbits is species specific and that collagen shields are useful as a drug delivery system for antifibroblast drugs in other species.

3.4. Collagen sponges

Collagen sponges have been very useful in the treatment of severe burns and as a dressing for many types of wounds, such as pressure sores, donor sites, leg ulcers and decubitus ulcers as well as for in vitro test systems (Geesin et al., 1996). Human collagen membrane has been a major resource of collagen sponge used as a biological dressing since 1930s (Rao, 1995). Collagen sponges have the ability to easily absorb large quantities of tissue exudate, smooth adherence to the wet wound bed with preservation of low moist climate as well as its shielding against mechanical harm and secondary bacterial infection (Pachence et al., 1987; Yannas, 1990). Experiments using sponge implantation demonstrated a rapid recovery of skin from burn wounds by an intense infiltration of neutrophils into the sponge (Boyce et al., 1988). Coating of a collagen sponge with growth factor further facilitates dermal and epidermal wound healing (Marks et al., 1991; Royce et al., 1995).

The sponges made from pure collagen isolated from bovine skin, were swollen at pH 3.0 and stabilized into the physical form of a sponge layer. In order to achieve highly resilient activity and fluid building capacity, collagen sponges have been combined with other materials like elastin, fibronectin or glycosaminoglycans (Lefebvre et al., 1992, 1996; Doillon and Silver, 1986). The starting material can be crosslinked with glutaraldehyde and subsequently graft-copolymerized with other polymers, such as polyhydroxyethyl methacrylate (PHEMA) (Sastry and Rao, 1991). The grafted PHEMA chains, which are hydrophilic, keep the membranes wet and increase their tensile strength. This further affects the efficiency in the management of infected wounds and burns. The importance of matrix compliance to mechanical response was also reported (Prajapati et al., 2000). The physiological loading of fibroblasts in three-dimensional collagen lattices elicited complex and substantial changes in protease activities, which were subjected to matrix modification.

Collagen sponges were found suitable for short-term delivery (3–7 days) of antibiotics, such as gentamicin (Wachol-Drewek et al., 1996). Gentamicin containing collagen sponges placed on a septic focus in the abdomen reduce local infection (Vaneerdeweg et al., 1998, 2000). This therapy achieved high concentration of gentamicin at the local site, while low concentration in serum. Collagen sponges containing antibiotics did not show any side effects and the collagen is reabsorbed after a few days (Stemberger et al., 1997).

Like collagen film, which was used as gene delivery carriers for promoting bone formation, collagen sponges were also used for osteoinduction. An absorbable collagen sponge containing bone morphogenetic protein 2 (rhBMP-2) was tested in the rat model for the evaluation of the efficacy of rhBMP-2 produced in *Escherichia coli* on promoting bone healing (Kimura et al., 2000).

Bacterially expressed rhBMP-2 loaded in collagen sponge was osteogenic in vivo. An absorbable collagen sponge containing bone morphogenetic protein 2 (rhBMP-2) stabilize endosseous dental implants in bony areas and normal bone formation was restored without complication (Cochran et al., 2000).

Collagen sponges were also used for delivery of steroids through topical applications, such as intravaginal delivery of lipophilic compounds including retinoic acid (Dorr et al., 1982; Chvapil et al., 1985). Collagen-based sponge was inserted into a cervical cap made of hydrogel hypan, which, when in contact with wet tissue surfaces, adheres to them by the force of differential osmotic pressure (Peng et al., 1986). This novel system is associated with high local concentrations of drugs without producing any systemic symptoms, and has been very useful for local drug delivery.

Collagen sponge as a drug carrier or a vaginal contraceptive barrier showed many advantages, such as the controlled release of spermicidal agents and reducing the tissue-irritation activity, over the rubber diaphragm. The main drawbacks of sponges appear to be the difficulty of assuring adequate supplies and their preservation. Other problems arose from their poor mechanical strength and ineffectiveness in the management of infected wounds and burns.

3.5. Gel, hydrogel, liposomes-collagen

Hydrogels have been widely used as a drug carrier due to its ease in manufacturing and self-application. The production of a large and constant surface area is one of the major merits for them to be widely used for clinical and fundamental applications. Various combinations of polymers were made into hydrogel formulations to investigate their potential as a drug delivery system. The combination of natural and synthetic polymers may provide mechanical stability and biological acceptability, acquiring from synergistic properties of both materials.

An attempt of combining collagen and polyhydroxyethyl methacrylate (PHEMA) into hydrogels was made to develop a delivery system for anti-cancer drugs, such as 5-FU (Jeyanthi and Rao, 1990). The hydrogels were found stable and resilient and did not show any adverse effects, such as calcification, after 6 month of subcutaneous implantation in rats. This system was also very efficient in therapeutic application, which, in this case, was anticancer therapy (Jeyanthi et al., 1991). Hybrid copolymers of collagen with polyethylene glycol-6000 and polyvinyl pyrrolidone were prepared for the controlled delivery of contraceptive steroids (Shantha and Rao, 1993). Two synthetic polymers, poly(vinyl alcohol) (PVA) and poly(acrylic acid) (PAA), were blended with two biological polymers, collagen and hyaluronic acid (HA), to enhance the mechanical strength of natural polymers and to overcome the biological drawbacks of synthetic polymers. These blends were formulated into hydrogels, films and sponges, and subsequently loaded with growth hormone (GH) (Cascone et al., 1995). The results of this work showed that growth hormone could be released from collagen-PVA hydrogels in a controlled release pattern and the rate and quantity of growth hormone released were mainly dependent on collagen content in the system.

One of the successful applications of collagen for the controlled delivery systems is collagen-based gel as an injectable aqueous formulation. An injectable gel formulation in a combination of collagen and epinephrine for delivery of 5-FU was developed for cancer treatment (Sahai et al., 1995). The disappearance of 5-FU by diffusion after intratumoral injection in mice was sustained and its therapeutic effect was improved. The subcutaneous injection of soluble collagen efficiently demonstrated its potential in the repair of dermatological defects, such as vocal fold immobility (Remacle et al., 1995) and urinary incontinence (Shortliffe et al., 1989).

Collagen hydrogel was also used as gene delivery carriers. Plasmids noncovalently bound to the Fab fragment of antibody can be efficiently introduced into cells that express the polymeric immunoglobin receptor (pIgR). Human tracheal epithelial cells grown on plastic, a condition that down regulates the expression of the receptor, failed to express the reporter gene, whereas cells

from the same trachea maintained on collagen gels were transfected (Ferkol, et al., 1993). G-CSF transfected clonal murine fibroblast lines can survive with grafts and continue to synthesize the transgene as well as collagen in vivo (Rosenthal and Kohler, 1997). Due to their low antigenecity, the bovine and equine collagen matrices were well tolerated in clinical applications. Gel made of atelocollagen, which is produced by elimination of the telopeptide moieties using pepsin, has been used as a carrier for chondrocytes to repair cartilage defects (Uchio et al., 2000; Katsube et al., 2000). Chondrocytes embedded in the atelocollagen gel gradually proliferated and maintain the chondrocyte phenotype. The grafted type I atelocollagen provided a favorable matrix for cell migration in relation with collagenase expression and cell behavior was modulated by graft collagen (Suh et al., 2001).

To achieve both formulation stability and controlled release rates of entrapped materials from collagen based hydrogels, a system consisted of liposome and collagen has been developed (Rao and Alamelu, 1992). Liposomes are widely used as a drug carrier due to their biodegradability and removable versatility in terms of composition and size. It was found that a coupling of liposomes to the gel-matrices enhanced the stability of the system due to the antioxidant effect of collagen molecules when they were immobilized (Weiner et al., 1985; Pajean and Herbage, 1993). Cross-linking the functional liposomes to a collagen gel matrix further sustained the release rate of the entrapped marker (Rao, 1995). Thus, the process of cross-linking of the liposomes to the gel-matrix can significantly manipulate the release rates of entrapped drugs.

A novel drug delivery system comprising liposomes sequestered in a collagen gel has demonstrated controlled release profiles of insulin and growth hormone into the circulation (Weiner et al., 1985). Moreover, since coated vesicles were more stable than control liposomes, the permeation rates of incorporated drugs from small unilamellar liposomes coated by collagen into systemic circulation were much greater (Fonseca et al., 1996). Blood elimination and liver uptake of collagen containing vesicles were about 2-fold faster and 1.5–2-fold higher than those of control liposomes, respectively. Collagen-based systems soaked in liposomes were found to deliver significantly higher levels of immunosuppressive agent cyclosporin A to the cornea, anterior sclera, aqueous humor and vitreous in rabbit eyes than collagen shields without liposomes (Pleyer et al., 1994). Inclusion of antimicrobials or cell growth factors within the liposomes could facilitate enhanced cell growth and prevention of infection, while a base collagen provided a substrate for cell attachment and proliferation (Weiner et al., 1985).

The formulation, in which liposome was sequestered in collagen gel, appears to have several advantages over other liposome formulations or gel formulations. This technology seems to have a potential for topical application in the treatment of surgical or nonsurgical wounds and burns. Since the release kinetics of hydrophilic and lipophilic substance encapsulated in liposome were similar to those of a non-encapsulated drug, the combination of liposomes with collagen is very useful for drugs which do not penetrate the ocular surface as well as systems in need of prolonged corneal contact time (Grammer et al., 1996). Liposome encapsulated formulations did not show any toxicity associated with a variety of potentially beneficial drugs.

3.6. Pellet/tablet

Minipellets made of collagen have been developed for various candidate compounds (Takenaka et al., 1986; Yamahira et al., 1991; Matsuoka et al., 1988; Fujioka et al., 1995; Maeda et al., 1999). A rod with a diameter and a length of 1 mm and 1 cm, respectively, is a useful shape as a drug delivery device, because this rod (minipellet) is small enough to be injected into the subcutaneous space through a syringe needle and still spacious enough to contain large molecular weight protein drugs, such as interferon (Miyata et al., 1992) and interleukin-2 (Matsuoka et al., 1988). A single subcutaneous injection of a mini pellet caused a prolonged retention of interleukin-2 and decreased its maximal concentration in the serum. This pellet-type carrier was used for local delivery of minocycline and lysozyme for the

treatment of periodontitis symptoms. An attempt to produce a pellet type controlled-release delivery vehicle made of purified type I collagen for water-soluble osteogenic proteins was described (Lucas et al., 1989). Water-soluble proteins from a collagen-based system induced cartilage and bone with a success rate of 76%, demonstrating the feasibility to formulate controlled-release delivery systems for soluble bioactive factors, which interact with local responsive cells.

Collagen-based pellet as a gene delivery carrier has been extensively studied. The effect of atecollagen-based mini-pellet on the mRNA expression and functional status of facial nerve in the rat model was investigated (Kohmura et al., 1999). The facial nerve transaction and immediate repair was accelerated by this system and the facial nerve regeneration was ultimately achieved. A minipellet with a cylindrical shape (0.6 mm in diameter and 10 mm in length) containing 50 μg of plasmid DNA and human HST-1/FGF-4 cDNA was evaluated as a controlled delivery system for plasmid DNA (Ochiya et al., 1999). This gene transfer method allowed a sustained release and expression of plasmid DNA in normal adult animals through the use of atelocollagen, a biocompatible polymer, as a carrier.

The solid nature of atelocollagen in vivo seems to have a great potential for site- or tissue-specific transportation of target genes. The controlled gene transfer using atelocollagen in a form of pellet has allowed a prolonged systemic circulation of target products and has facilitated a long-term use of naked plasmid vectors for somatic gene therapy (Ochiya et al., 1999). The mechanism of direct bone formation by BMPs–collagen complex was ultrastructurally investigated (Nakagawa and Tagawa, 2000). This study proved that direct bone formation is ectopically induced by bone morphogenetic proteins (BMPs) without cartilage formation when atelocollagen type I collagen pellet is used as a carrier. Further studies are needed to assess the applicability of atelocollage-based pellet systems for the augmentation of the bioavailability of low molecular weight materials, such as antisense oligonucleotides and biologically active oligopeptides, or virus vectors.

3.7. Nanoparticles/nanospheres

A property, in which the crystallites in the gel aggregates appear as multiple chain segments in the collagen-fold configuration, has been used to prepare aggregates as colloidal drug delivery carriers (Muller et al., 2000). Nanosphere formation is driven by a combination of electrostatic and electropic forces with sodium sulfate employed as a dissolving reagent to facilitate greater charge–charge interactions between plasmid DNA and collagen (Marty et al., 1978). The molecular weight of collagen or gelatin has a decisive influence on the stability of the manufactured gelatin nanoparticles (Coester et al., 2000). The molecular weight profile of the collagen solution was affected by pH and temperature, both of which further influenced the noncovalent interactions responsible for the molecular structure of collagen (Farrugia and Groves, 1999). The relationship between electropic forces and gene factors was also evaluated. Polyion complexation between basic fibroblast growth factor and gelatin was studied by turbidity change of a mixed solution and isoelectric electrophoresis (Muniruzzaman et al., 1998). It was found that an electrostatic interaction was the main driving force for the complexation between acidic gelatin and basic fibroblast growth factor.

The biodegradable collagen based nanoparticles or nanospheres are thermally stable, readily achieving their sterilization (Rossler et al., 1995). Moreover, nanoparticles can be taken up by the reticuloendothelial system (Marty et al., 1978), and enable an enhanced uptake of exogenous compounds, such as anti-HIV drugs, into a number of cells, especially macrophages (Bender et al., 1996), which may be an additional advantage of collagen based nanoparticles as a systemic delivery carrier. Thus, nanoparticles were used as a parenteral carrier for cytoxic agents and other therapeutic compounds, such as campthocin (Yang et al., 1999) and hydrocortisone (Berthold et al., 1998). Gelatin microspheres were used as a drug carrier for parenteral delivery of cancer drugs, such as methotrexate (Narayani and Rao, 1994). The microspheres showed zero order kinetics in the release profiles of incorporated drugs,

demonstrating prolonged action against rat fibrosarcoma and improved antitumor activity.

Due to a small size, a large surface area, high adsorptive capacity, and ability to disperse in water to form a clear colloidal solution, collagen-based nanoparticles have demonstrated their potential to be used as a sustained release formulation for anti-microbial agents or steroids (El-Samaligy and Rohdewald, 1983). Delivery of hydrocortisone, one of lipophilic steroids, was not affected by the pH of the receptor medium or its binding affinity to the particles. Collagen nanoparticles were used to enhance dermal delivery of retinol (Rossler et al., 1994). The retinol in the system was very stable and facilitated a faster and higher transportation of the incorporated drug through the skin than the freshly precipitated drug.

4. Collagen-based systems for tissue engineering

4.1. Collagen as skin replacement

Collagen based implants have been widely used as vehicles for transportation of cultured skin cells or drug carriers for skin replacement and burn wounds (Bell et al., 1983; Leipziger et al., 1985; McPherson et al., 1986; Deatherage and Miller, 1987; Harriger et al., 1997; Boyce, 1998). Since sponge implant was originally developed for recovery of skin and was very efficient in that purpose, various types of artificial skin were developed as a form of sponge. Cultured skin substitutes developed on collagen lattice were also used for skin replacement and skin wounds. Reconstituted type I collagen is suitable for skin replacement and burn wounds due to their mechanical strength and biocompatibility (Rao, 1995). Chronic wounds resulting from diabetes have been successfully cured with allogenic cultured skin substitutes prepared from cryopreserved skin cells (Boyce, 1998). In the cultured skin substitutes, the contracted collagen lattice was used as a support for epithelial growth and differentiation to replace pathological skin (Yannas et al., 1989). Allogenic cultured dermal substitute prepared by plating fibroblasts on to a collagen sponge matrix and subsequently freeze dried from a 1% aqueous solution of atelocollagen provided a good environment for epithelialization (Yamada et al., 1999). The effectiveness of collagen sponges as a substrate for human corneal cells was demonstrated and corneal cells exhibited normal cell phenotype when cultured individually on an engineered collagen sponge matrix (Orwin and Hubel, 2000). Addition of selected antimicrobial drugs like amikacin to the bovine skin implantable collagen managed to control microbial contamination and increased healing of skin wounds (Boyce et al., 1993).

The modified sponge for artificial skin was developed by combining fibrillar collagen with gelatin (Koide et al., 1993). Dehydrothermal crosslinks were used to stabilize collagen-based sponge physically and metabolically. Sponges made of gelatin by itself in a resorbable gelfoam were also used as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy (Ponticiello et al., 2000). When this gelatin sponge was implanted in an osteochondral defect in the rabbit femoral condyle, gelfoam cylinders were observed to be very biocompatible, with no evidence of immune response or lymphatic infiltration at the site.

Some limitations inherent to cultured skin substitutes, such as deficient barrier function in vitro and delayed keratinization after grafting in comparison to native skin autografts, were reported (Supp et al., 1999). To address those limitations, modifications of collagen-based systems by the combination of collagen with other proteins, such as glycosaminoglycan, fibrin, and biotin, were proposed. The role of glycosaminoglycan and difference in its concentrations between pathological and normal tissues were reported earlier (Sobolewski et al., 1995). Dermal skin substitutes (membranes) made of collagen and glycosaminoglycan were found to be suitable substrates for the culture of human epidermal keratinocytes (Boyce et al., 1988). Cultured skin substitutes consisted of human keratocytes and fibroblasts attached to collagen-glycosaminoglycan substrates, which were subsequently crosslinked, decreased the rate of biodegradation and further reduced the engraftment of skin substitutes (Boyce et al., 1995;

Harriger et al., 1997). Restoration of functional epidermis by cultured skin substitutes developed from collagen was stimulated by incubation in reduced humidity in vitro (Supp et al., 1999). In the case of collagen and fibrin combination, cultured cells were best grafted directly onto the wound bed or in combination with either a thin layer of collagen or fibrin but not both (Lam et al., 1999). Biotinylation of bovine skin collagen by covalent addition of biotin has been used to attach peptide growth factors with avidin as a bridge. This technique retained the activity of peptide growth factors and demonstrated a potential to be used as a modulator of the response in wound treatment (Boyce et al., 1992; Stompro et al., 1989).

Sponges in a combination of silicone and collagen were also used to address the limitations inherent to cultured skin substitutes. Acellular bilayer artificial skin composed of outer silicone layer and inner collagen sponge was used for a thin split thickness skin graft and achieved good performance in the long term postoperative appearance of the split thickness skin graft site (Suzuki et al., 2000).

4.2. Collagen as bone substitutes

Among the many tissues in the human body, bone has been considered as a powerful marker for regeneration and its formation serves as a prototype model for tissue engineering based on morphogenesis. Collagen has been used as implantable carriers for bone inducing proteins, such as bone morphogenetic protein 2 (rhBMP-2) (Reddi, 2000). Recently, collagen itself was used as bone substitutes due to its osteoinductive activity (Murata et al., 1999). The uses of collagen film as gene delivery carriers for osteoinduction and collagen sponge for bone related protein carriers were described earlier in Sections 3.1 and 3.4, respectively, and this section mainly focused on its uses as a bone substitute and a bone marker.

Type I collagen crosslinked *N*-telopeptide was used as a marker of bone resorption and clinically used as a marker of bone metastasis of prostate cancer and breast cancer (Kobayashi et al., 2000; Ulrich et al., 2001). The polymorphisms of collagen type Ialpha1 and vitamin D receptor as genetic markers for osteoporotic fracture in women was also reported (Uitterlinden et al., 2001). This result added to evidence that interlocus interaction is an important component of osteoporotic fracture risk.

Collagen in combination with other polymers or chemicals was also used for orthopaedic defects. Demineralized bone collagen was used as a bone graft material for the treatment of acquired and congenital orthopaedic defects either by itself or in combination with hydroxyapatite (Takaoka et al., 1988). The result of this study showed that grafted demineralized bone collagen in combination with hydroxyapatite was an excellent osteoinductive material and could be used as a bone substitute. A recent study showed that addition of 500 IU of retinoic acid to collagen at a site of a bone defect enhanced regeneration of new bone, achieving union across the defect and leading to its complete repair (Sela et al., 2000).

4.3. Collagen as bioengineered tissues

Collagen gel as human skin substitutes have demonstrated its usefulness in tissue engineering and led to the development of bioengineered tissues, such as blood vessels, heart valves and ligaments (Auger et al., 1998). Collagen shows hemostatic properties that promote blood coagulation and play an important role in tissue repair process. Collagen sponge or gel initiates adhesion and aggregation of platelets that lead to a thrombus formation (Miyata et al., 1992). Monomeric collagen does not activate platelet aggregation, while polymeric collagen having a regular arrangement of the molecules with a length of around 1 μm does activate it. Arginine side chains of collagen seemed to be responsible for its interaction with platelets (Wang et al., 1978).

A provisional extracellular support was developed using type I collagen lattice to organize the cells into a three-dimensional structure in vitro (Kemp, 2000). A small-diameter (4 mm) graft constructed from type I bovine collagen was earlier used to integrate into the host tissue and provide a scaffold for remodeling into a functional blood vessel (Huynh et al., 1999). Three-di-

mensional collagen scaffolds, that are biodegradable in vivo and have a large surface area for cell attachment, can support vascularization processes and can be used as artificial blood vessels, heart valves or cell transplant devices (Kuzuya and Kinsell, 1994; Chevallay and Herbage, 2000). The use of collagen as a coated material for permeation filters made of culture endothelial cell monolayers was also tested to evaluate in vitro vascular permeability of a drug to contrast media (Matin-Chouly et al., 1999).

A method for generating a cellular layer of intestinal collagen from the porcine submucosa without compromising the native collagen structure further facilitated the use of collagen in tissue engineering (Abraham et al., 2000). Biological tissue grafts in the form of collagen-based matrix have been derived from bladder, ureter or small intestine (Clarke et al., 1996; Desgrandchamps, 2000). These collagen constructs were designed to be similar to synthetic polymer prostheses in terms of their ability to persist. The structure-mechanical behavior relationship of biomaterials acquired from intestine submucosa demonstrated mechanical anisotropy and stiffer direction preferred in biomaterials (Gloeckner et al., 2000). Using a phenomenological constitutive model, it was demonstrated that glycan increased the tensile stiffness and ultimate tensile strength of collagen-based matrix and further increased their resistance to collagen degradation (Girton et al., 2000).

Natural collagenous materials were used for surgical repair and abdominal wall repair by taking advantage of their inherent low antigenecity and their ability to integrate with surrounding tissues (Van der Laan et al., 1991). Moreover, new generations of collagen-based biological tissue are practical and remodelable due to its simple membranous configuration, relative uniformity and abundant availability. These characteristics are employed in a new type of surgical adhesive made from porcine collagen and polyglutamic acid, developing for sealing air leaking from the lung, which takes a relatively long period for recovery (Sekine et al., 2001). The absorption rate of collagen-based adhesive can be controlled by collagen concentration in the system. Recent progress in tissue engineering may lead to well-characterized and reproducible biomaterials from natural collagenous materials.

5. Concluding remarks

Collagen has various advantages as a biomaterial and is widely used as carrier systems for delivery of drug, protein and gene. The examples described in this paper represent selected applications of collagen in the biomedical field. The successful demonstration of usefulness of human skin substitutes made of collagen has lead to the development of bioengineering tissues, such as blood vessels and ligaments. Autologous tissue engineering provides an alternative for allogenic tissue transplantation.

The study of native collagen for drug delivery systems and tissue engineering may lead to a better understanding of pathological diseases. The concepts of high binding affinity and specificity play a critical role in targeting delivery of drugs. By understanding the nature of drug delivery systems and their durability in the body, the essential parameters for designing effective ligands, which can interact with the systems, can be identified. It can further provide a new guide for tissue growth and organization and leading to bioactive signals for tissue-specific gene expression. Collagen-based biomaterials are expected to become a useful matrix substance for various biomedical applications in the future.

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References

Abraham, G.A., Murray, J., Billiar, K., Sullivan, S.J., 2000. Evaluation of the porcine intestinal collagen layer as a biomaterial. J. Biomed. Mater. Res. 51, 442–452.

Aebischer, P., Schleup, M., Deglon, N., Joseph, J.M., Hirt, L., Heyd, B., Goddard, M., Hammang, J.P., Zurn, A.D., Kato, A.C., Regli, F., Baetge, F.E., 1996. Intrathecal delivery of CNTF using encapsulated genetically modified

xenogenic cells in amyotrophic lateral sclerosis. Nat. Med. 2, 696–699.

Al-Hendy, A., Hortelano, G., Tannenbaum, G.S., Chang, P.L., 1996. Correction of the growth defect in dwarf mice with nonautologous microencapsulated myoblasts — an alternative approach to somatic gene therapy. Hum. Gene Ther. 6, 165–175.

Angella, G.J., Sherwood, M.B., Balasubramanian, L., Doyle, J.W., Smith, M.F., van Setten, G., Goldstein, M., Schultz, G.S., 2000. Enhanced short-term plasmid transfection of filtration surgery tissues. Invest. Ophthalmol. Vis. Sci. 41 (13), 4158–4162.

Aquavella, J.V., Ruffini, J.J., LoCascio, J.A., 1988. Use of collagen shields as a surgical adjunct. J. Cataract Refractive Surg. 14, 492–495.

Assil, K.K., Zarnegar, S.R., Fouraker, S.R., Schanzlin, D.J., 1992. Efficacy of tobramycin — soaked collagen shield vs. tobramycin eyedrop loaden dose for sustained experimental *Pseudomonas aeruginosa* induced keratitis in rabbits. Am. J. Ophthalmol. 113, 418–423.

Auger, F.A., Rouabhia, M., Goulet, F., Berthod, F., Moulin, V., Germain, L., 1998. Tissue-engineered human skin substitutes developed from collagen populated hydrated gels: clinical and fundamental applications. Med. Biol. Eng. Comput. 36, 801–812.

Bank, R.A., Soudry, M., Maroudas, A., Mizrahi, J., Tekoppele, J.M., 2000. The increased swelling and instantaneous deformation of osteoarthritic cartilage is highly correlated with collagen degradation. Arthritis Rheum. 43, 2202–2210.

Barbani, N., Giusti, P., Lazzeri, L., Polacco, G., Pizzirani, G., 1995. Bioartificial materials based on collagen: 1. Collagen cross-linking with gaseous glutaraldehyde. J. Biomater. Sci. Polym. Ed. 7, 461–469.

Barr, E., Leiden, J.M., 1991. Systemic delivery of recombinant proteins by genetically modified myoblasts. Science 254, 1507–1509.

Baziuk, N., Gremillion, C.M. Jr, Peyman, G.A., Cho, H., 1992. Collagen shields and intraocular drug delivery: concentration of gentamicin in the aqueous and vitreous of a rabbit eye after lensectomy and vitrectomy. Int. Ophthalmol. 16, 101–107.

Bell, E., Sher, S., Hull, B., Merril, C., Rosen, S., Chamson, A., Asselineau, D., Dubetret, L., Coulomb, B., Lapiere, C., Nusgens, B., Neveux, K., 1983. The reconstitution of living skin. J. Invest. Dermatol. 81 (1), 2–10.

Bender, A., von Briesen, H., Kreuter, J., Duncan, I.B., Rubsamen-Waigmann, H., 1996. Efficiency of nanoparticles as a carrier system for antiviral agents in human monocytes/macrophages in vitro. Antimicrob. Agents Chemother. 40, 1467–1471.

Bergeon, M.T., 1967. Collagen: a review. J. Okla State Med. Assoc. 60 (6), 330–332.

Berthold, A., Cremer, K., Kreuter, J., 1998. Collagen microparticles: carriers for glucocorticosteroids. Eur. J. Pharm. Biopharm. 45, 23–29.

Bloomfield, S.E., Miyata, T., Dunn, M.W., Bueser, N., Stenzel, K.H., Rubin, A.L., 1978. Soluble gentamycin ophthalmic inserts as a drug delivery system. Arch. Ophthalmol. 96, 885–887.

Boyce, S.T., 1998. Skin substitutes from cultured cells and collagen-GAG polymers. Med. Biol. Eng. Comput. 36, 791–800.

Boyce, S.T., Christanson, D., Hansbrough, J.F., 1988. Structure of a collagen-GAG dermal skin substitute optimized for cultured human epidermal keratinocytes. J. Biomed. Mater. Res. 22, 939–957.

Boyce, S.T., Stompro, B.E., Hansbrough, J.F., 1992. Biotinylation of implantable collagen for drug delivery. J. Biomed. Mater. Res. 26, 547–553.

Boyce, S.T., Supp, A.P., Warden, G.D., Holder, I.A., 1993. Attachment of an aminoglycoside, amikacin, to implantable collagen for local delivery in wounds. Antimicrob. Agents Chemother. 37, 1890–1895.

Boyce, S.T., Goretsky, M.J., Greenhalgh, D.G., Kagan, R.J., Rioeman, M.T., Warden, G.D., 1995. Comparative assessment of cultured skin substitutes and native skin autograft for treatment of full-thickness burns. Ann. Surg. 222, 743–752.

Bradley, W.G., Wilkes, G.L., 1977. Some mechanical property considerations of reconstituted collagen for drug release supports. Biomater. Med. Dev. Artif. Organs 5, 159–175.

Brauker, J., Carr-Brendel, S., Neunenfeldt, S., Clarke, D., Hodgett, D., Vergoth, C., Stone, W., Dwarki, V., Chen, R., Nijjar, T., Loudovaris, T., Martison, L., Young, S., Jacobs, S., Geller, R., Maryanov, D., Levon, S., Johnston, W., Johnson, R.C., 1995. Immunoisolation in somatic cell gene therapy. J. Cell. Biochem. 21B, D1–015 Abstract.

Browder, I.W., Litwin, M.S., 1986. Use of absorbable collagen for hemostasis in general surgical patients. Am. Surg. 52, 492–494.

Burge, R.E., 1964. The structure and function of connective and skeletal tissue. In: Proceedings of NATO Advanced Study Group. Butterworth, St. Andrews, pp. 2–7.

Cameron, W.J., 1978. A new topical hemostatic agent in gynecological surgery. Obstet. Gynecol. 51, 118–122.

Carrol, R.E., 1989. Surgical Catgut: the myth of allergy. J. Hand Surg. 14B, 218–220.

Cascone, M.G., Sim, B., Downes, S., 1995. Blends of synthetic and natural polymers as drug delivery systems for growth hormone. Biomaterials 16, 569–574.

Chen, Y.F., Gebhardt, B.M., Reidy, J.J., Kaufman, H.E., 1990. Cyclosporin-containing collagen shields suppress corneal allograft rejection. Am. J. Ophtalmol. 109, 132–137.

Chen, J.M., Sheldon, A., Pincus, M.R., 1995. Three dimensional energy-minimized model of human type II smith collagen microfibrill. J. Biomol. Struct. Dyn. 12, 1129–1156.

Chevallay, B., Herbage, D., 2000. Collagen-based biomaterials as 3D scaffold for cell cultures: applications for tissue engineering and gene therapy. Med. Biol. Eng. Comput. 38 (2), 211–218.

Chvapil, M., Droegemuller, W., Heine, M.W., MacGregor, J.C., Dotters, D., 1985. Collagen sponge as vaginal contraceptive barrier: critical summary of seven years of research. Am. J. Obstet. Gynecol. 151, 325–329.

Chvapil, M., Speer, D.P., Holubec, H., Chvapil, T.A., King, D.H., 1993. Collagen fibers as a temporary scaffold for replacement of ACL in goats. J. Biomed. Mater. Res. 27 (3), 313–325.

Clarke, K.M., Lantz, G.C., Salisbury, S.K., badylak, S.F., Hiles, M.C., Voytik, S.L., 1996. Intestine submucosa and polypropylene mesh for abdominal wall repair in dogs. J. Surg. Res. 60, 107–114.

Cochran, D.L., Jones, A.A., Lilly, L.C., Fiorellini, J.P., Howell, H., 2000. Evaluation of recombinant human bone morphogenetic protein-2 in oral applications including the use of endosseous implants: 3-year results of a pilot study in humans. J. Periodontol. 71 (8), 1241–1257.

Coester, C.J., Langer, K., van Briesen, H., Kreuter, J., 2000. Gelatin nanoparticles by two step desolvation, a new preparation method, surface modifications and cell uptake. J. Microencapsulation 17, 187–193.

Danielsen, C.C., 1990. Age-related thermal stability and susceptibility to proteolysis of rat bone collagen. Biochem. J. 272, 697–701.

Deatherage, J.R., Miller, E.J., 1987. Packaging and delivery of bone induction factors in a collagenous implant. Collagen Rel. Res. 7, 225–231.

Desgrandchamps, F., 2000. Biomaterials in functional reconstruction. Curr. Opin. Urol. 10 (3), 201–206.

Doillon, C.J., Silver, F.H., 1986. Collagen — based wound dressing effects of hyaluronic acid and fibronectin on wound healing. Biomaterials 7, 3–8.

Dorigo, M.T., De Natale, R., Miglioli, P.A., 1995. Collagen shields delivery of netilmicin: a study of ocular pharmacokinetics. Chemotherapy 41, 1–4.

Dorr, R.T., Surwit, E.A., Droegemueller, W., Alberts, D.S., Meyskens, F.L., Chvapil, M., 1982. In vitro retinoid binding and release from a collagen sponge material in a simulated intravaginal environment. J. Biomed. Mater. Res. 16, 839–850.

El-Samaligy, M.S., Rohdewald, P., 1983. Reconstituted collagen nanoparticles, a novel drug carrier delivery system. J. Pharm. Pharmacol. 35, 537–539.

Farrugia, C.A., Groves, M.J., 1999. Gelatin behavior in dilute aqueous solution: designing a nanoparticulate formulation. J. Pharm. Pharmacol. 51, 643–649.

Fell, J.T., Newton, J.M., 1970. Determination of tablet strength by diametrical compression test. J. Pharm. Sci. 69, 688–691.

Ferkol, T., Kaetzel, C.S., Davis, P.B., 1993. Gene transfer into respiratory epithelial cells by targetting the polymeric immunoglobulin receptor. J. Clin. Invest. 92, 2394–2400.

Finkelstein, I., Trope, G.E., Heathcote, J.G., Rootman, D.S., Spero, L., Menon, I.A., 1991. Further evaluation of collagen shields as a delivery sytem for 5-fluoruracil: histopathological observations. Can. J. Ophthalmol. 26, 129–132.

Fonseca, M.J., Alsina, M.A., Reig, F., 1996. Coating liposomes with collagen (Mr 50000) increases uptake into liver. Biochim. Biophys. Acta 1279 (2), 259–265.

Friedberg, M.L., Pleyer, U., Mondino, B.J., 1991. Device drug delivery to the eye. Ophthalmology 98, 725–732.

Friess, W., 1998. Collagen-biomaterial for drug delivery. Eur. J. Pharm. Biopharm. 45, 113–136.

Fujioka, K., Takada, Y., Sato, S., Miyata, T., 1995. Novel delivery system for proteins using collagen as a carrier material: the minipellet. J. Contr. Release 33, 307–315.

Fujioka, K., Maeda, M., Hojo, T., Sano, A., 1998. Protein release from collagen matrices. Adv. Drug. Del. Rev. 31, 247–266.

Gebhardt, B.M., Kaufman, H.E., 1995. Collagen as a delivery system for hydrophobic drugs: studies with cyclosporinee. J. Ocul. Pharmacol. Ther. 11, 319–327.

Geesin, J.C., Brown, L.J., Liu, Z., Berg, R.A., 1996. Development of a skin model based on insoluble fibrillar collagen. J. Biomed. Mater. Res. 33, 1–8.

Girton, T.S., Oegema, T.R., Grassl, E.D., Isenberg, B.C., Tranquillo, R.T., 2000. Mechanisms of stiffening and strengthening in media equivalents fabricated using glycation. J. Biomech. Eng. 122, 216–223.

Gloeckner, D.C., Sacks, M.S., Billiar, K.L., Bachrach, N., 2000. Mechanical evaluation and design of a multilayered collagenous repair. Biomaterial 52, 365–373.

Gorham, S.D., Light, N.D., Diamond, A.M., Willins, M.J., Bailey, A.J., Wess, T.J., Leslie, M.J., 1992. Effect of chemical modifications on the susceptibility of collagen to proteolysis. II. Dehydrothermal crosslinking. Int. J. Biol. Macromol. 14, 129–138.

Grammer, J.B., Kortum, F.A., Wolburg, H., Ludtke, R., Schmidt, K.H., Thiel, H.J., Pleyer, U., 1996. Impregnation of collagen corneal shields with liposomes: uptake and release of hydrophilic and lipophilic marker substances. Curr. Eye Res. 15 (8), 815–823.

Gross, J., 1963. Comparative Biochemistry, vol. 5. Academic Press, New York, pp. 307–345.

Gussler, J.R., Ashton, P., Vanmeter, W.S., Smith, T.J., 1990. Collagen shield delivery of trifluorothymidine. J. Cataract Refractive Surg. 16, 719–722.

Harkness, R.D., 1961. Biological functions of collagen. Biol. Rev. 36, 399–463.

Harkness, R.D., 1966. Collagen. Sci. Prog. Oxf. 54, 257–274.

Harriger, M.D., Supp, A.P., Warden, G.D., Boyce, S.T., 1997. Glutaraldehyde crosslinking of collagen substrates inhibits degradation in skin substitutes grafted to athymic mice. J. Biomed. Mater. Res. 35, 137–145.

Harrison, K.W., 1989. Collagen corneal shields — an important therapeutic modality. J. Ophthal. Nurs. Technol. 8 (3), 97–98.

Heartlein, M.W., Roman, V.A., Jiang, J.-L., Sellers, J.W., Zuliani, A.M., Treco, D.A., Selden, R., 1994. Long-term production and delivery of human growth hormone in vivo. Proc. Natl. Acad. Sci. USA 91, 10967–10971.

Hobden, J.A., Reidy, J.J., O'Callaghan, R.J., Hill, J.M., Insler, M.S., Rootman, D.S., 1988. Treatment of experimen-

tal pseudomonas keratitis using collagen shields containing tobramycin. Arch. Ophthalmol. 106, 1605–1607.

Huynh, T., Abraham, G., Murray, J., Brockbank, K., Hagen, P.-O., Sullivan, S., 1999. Remodeling of an acellular collagen graft into a physiologically responsive neovessel. Nat. Biotechnol. 17, 1083–1086.

Hwang, D.G., Stern, W.H., Hwang, P.H., MacGowan-Smith, L.A., 1989. Collagen shield enhancement of topical dexamethasone penetration. Arch. Ophthalmol. 107, 1375–1380.

Jerome, A., Ramshaw, J.A., 1992. Editorial: collagen-based. Biomaterials 9, 137–138.

Jeyanthi, R., Rao, K.P., 1990. In vivo biocompatibility of collagen-poly(hydroxyethyl methacrylate) hydrogels. Biomaterials 11 (4), 238–243.

Jeyanthi, R., Nagarajan, B., Rao, K.P., 1991. Solid tumour chemotherapy using implantable collagen-poly (HEMA) hydrogel containing 5-fluorouracil. J. Pharm. Pharmacol. 43 (1), 60–62.

Katsube, K., Ochi, M., Uchio, Y., Maniwa, S., Matsusaki, M., Tobita, M., Iwasa, J., 2000. Repair of articular cartilage defects with cultured chondrocytes in Atelocollagen gel. Comparison with cultured chondrocytes in suspension. Arch. Orthop. Trauma Surg. 120 (3–4), 121–127.

Kaufman, H.E., 1988. Collagen shield symposium. J. Cataract Refractive Surg. 14 (5), 487–488.

Kaufman, H.E., Steinemann, T.L., Lehman, E., Thompson, H.W., Varnell, E.D., Jacob-La Barre, J.T., Gerhardt, B.M., 1994. Collagen based drug delivery and artificial tears. J. Ocul. Pharmacol. 10 (1), 17–27.

Kemp, P.D., 2000. Tissue engineering and cell populated collagen matrices. In: Streuli, C., Grant, M. (Eds.), Methods in Molecular Biology, vol. 139, pp. 287–293.

Kimura, M., Zhao, M., Zellin, G., Linde, A., 2000. Bone-inductive efficacy of recombinant human bone morphogenetic protein-2 expressed in *Escherichia coli*: an experimental study in rat mandibular defects. Scand. J. Plast. Reconstr. Surg. Hand Surg. 34 (4), 289–299.

Kobayashi, Y., Ochi, M., Tokue, A., 2000. Clinical usefulness of crosslinked *N*-telopeptide of type I collagen as a bone metastatic marker in patients with prostate cancer: comparison with serum PICP, PINP and ICTP. Hinyokika 46, 869–872.

Kohmura, E., Yuguchi, T., Yoshimine, T., Fujinaka, T., Koseki, N., Sano, A., Kishino, A., Nakayama, C., Sakaki, T., Nonaka, M., Takemoto, O., Hayakawa, T., 1999. BNDF atelocollagen mini-pellet accelerates facial nerve regeneration. Brain Res. 849, 235–238.

Koide, M., Osaki, K., Konishi, J., Oyamada, K., Katakura, T., Takahashi, A., Yoshizato, K., 1993. A new type of biomaterial for artificial skin: dehydrothermally crosslinked composites of fibrillar and denatured collagens. J. Biomed. Mater. Res. 27, 79–87.

Kuwano, M., Horibe, Y., kawashima, Y., 1997. Effect of collagen cross-linking in collagen corneal shields on ocular drug delivery. J. Pharmacol. Ther. 13, 31–40.

Kuzuya, M., Kinsell, J.L., 1994. Induction of endothelial cell differentiation in vitro by fibroblast-derived soluble factors. Exp. Cell Res. 215, 310–318.

Lam, P.K., Chan, E.S., Liew, C.T., Lau, C.H., Yen, S.C., King, W.W., 1999. The efficacy of collagen dermis membrane and fibrin on cultured epidermal graft using an athymic mouse model. Ann. Plast. Surg. 43, 523–528.

Leaders, F.E., Hecht, G., VanHoose, M., 1973. New polymers in drug delivery. Ann. Ophthalmol. 5, 513–522.

Lee, V.H., 1990. New directions in the optimization of ocular drug delivery. J. Ocul. Pharmacol. 6, 157–164.

Lee, C.H., Vyavahare, N., Zand, R., Kruth, H., Schoen, F.J., Bianco, R., Levy, R.J., 1998. Inhibition of aortic wall calcification in bioprosthetic heart valves by ethanol pretreatment: biochemical and biophysical mechanisms. J. Biomed. Mater. Res. 42 (1), 30–37.

Lefebvre, F., Gorecki, S., Bareilli, R., Amedee, J., Bordenave, L., Rabaud, M., 1992. New artificial connective matrix-like structure made of elastin solubilized peptides and collagens: elaboration, biochemical and structural properties. Biomaterials 13, 28–33.

Lefebvre, F., Pilet, P., Bonzon, N., Daculsi, G., Rabaud, M., 1996. New preparation and microstructure of the Endo-Patch elastin–collagen containing glycosaminoglycans. Biomaterials 17, 1813–1818.

Leipziger, L.S., Glushko, V., Dibernado, B., Shafaie, F., Noble, J., Nichols, J., Alvarez, O.M., 1985. Dermal wound repair: role of collagen matrix implants and synthetic polymer dressings. J. Am. Acad. Dermatol. 12, 409–419.

Liang, F.G., Viola, R.S., del Cerro, M., Aquavella, J.V., 1992. Noncross-linked collagen discs and cross-linked collagen shields in the delivery of gentamicin to rabbit eyes. Invest. Ophthalmol. Vis. Sci. 33, 2194–2198.

Liu, H.-W., Ofosu, F.A., Chang, P.L., 1993. Expression of human factor IX by microencapsulated recombinant fibroblasts. Hum. Gene Ther. 4, 291–301.

Lucas, P.A., syftestad, G.T., Goldberg, V.M., Caplan, A.I., 1989. Ectopic induction of cartilage and bone by water soluble proteins from bovine bone using a collagenous delivery vehicle. J. Biomed. Mater. Res. 23, 23–39.

Maeda, M., Tani, S., Sano, A., Fujioka, K., 1999. Microstructure and release characteristics of the minipellet, a collagen based drug delivery system for controlled release of protein drugs. J. Controlled. Rel. 62, 313–324.

Marks, M.G., Doillon, C., Silver, F.H., 1991. Effects of fibroblasts and basic fibroblast growth factor on facilitation of dermal wound healing by type I collagen matrices. J. Biomed. Mater. Res. 25, 683–696.

Marmer, R.H., 1988. Therapeutic and protective properties of the corneal shield. J. Cataract Refractive Surg. 14, 496–499.

Marty, J.J., Openheim, R.C., Speiser, P., 1978. Nanoparticles-a new colloidal drug delivery system. Pharm. Acta. Helv. 53, 17–23.

Matin-Chouly, C.A., Youmine, H., Saiag, B., Hentsch, A.M., Corot, C., Legrand, A., 1999. In vitro evaluation of vascular permeability to contrast media using cultured endothelial cell monolayers. Invest. Radiol. 34, 663–668.

Matsuoka, J., Sakagami, K., Shiozaki, S., Uchida, S., Fujiwara, T., Gohchi, A., Orita, K., 1988. Development of an

interleukin-2 slow delivery system. Trans. Am. Soc. Artif. Intern. Organs 34, 729–731.

McPherson, J.M., Sawamura, S., Amstrong, R., 1986. An examination of the biologic response to injectable, glutaraldehyde cross-linked collagen implants. J. Biomed. Mater. Res. 20, 93–107.

Meaney, D.F., 1995. Mechanical properties of implantable biomaterials. Clin. Podiatr. Med. Surg. 12 (3), 363–384.

Mendicute, J., Ondarra, A., Eder, F., Ostolaza, J.I., Salaberria, M., Lamsfus, J.M., 1995. The use of collagen shields impregnated with amphotericin B to treat aspergillus keratomycosis. CLAO J. 21, 252–255.

Milan, J.K., Verbukh, I., Pleyer, U., Sumner, H., Adamu, S.A., Hilabi, H.P., Chou, H.J., Lee, D.A., Mondino, B.J., 1993. Collagen shields impregnated with gentamicin–dexamethason as a potential drug delivery device. Am.J. Ophthalmol. 116, 622–627.

Miller, J.M., Zoll, D.R., Brown, E.O., 1964. Clinical observation on use of an extrude collagen suture. Arch. Surg. 88, 167–174.

Minabe, M., Takeuchi, K., Tamura, K., Hori, T., Umemoto, T., 1989a. Subgingival administration of tetracycline on a collagen film. J. Periodontol. 60, 552–556.

Minabe, M., Uematsu, A., Nishijima momat, K., Tomomatsu, E., Tamura, T., Hori, T., Umemoto, T., Hino, T., 1989b. Application of a local drug delivery system to periodontal therapy: I. Development of collagen preparations with immmobilized tetracycline. J. Periodontol. 60, 113–117.

Miyata, T., Sohde, T., Rubin, A.L., Stenzel, K.H., 1971. Effects of ultraviolet irradiation on native and telopeptide-poor collagen. Biochem. Biophys. Acta. 229, 672–680.

Miyata, T., Taira, T., Noishiki, Y., 1992. Collagen engineering for biomaterial use. Clin. Mater. 9 (3-4), 139–148.

Mondino, B.J., 1991. Collagen shields. Am. J. Ophthalmol. 112 (5), 587–590.

Moullier, P., Bohl, D., Heard, J.-M., Danos, O., 1993a. Correction of lysosomal storage in the liver and spleen of MPS VII mice by implantation of genetically modified skin fibroblasts. Nat. Genet. 4, 154–159.

Moullier, P., Marechal, V., Danos, O., Heard, J.M., 1993b. Continuous systemic secretion of lysosomal enzyme by genetically-modified mouse skin fibroblasts. Transplantation 56, 427–432.

Moullier, P., Bohl, D., Cardoso, J., Heard, J.-M., Danos, O., 1995. Long-term delivery of a lysosomal enzyme by genetically modified fibroblasts in dogs. Nat. Med. 1, 353–357.

Muller, R.H., Mader, K., Gohla, S., 2000. Solid lipid nanoparticles for controlled drug delivery-a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.

Mullins, R.J., Richards, C., Walker, T., 1996. Allergic reactions to oral, surgical and topical bovine collagen. Anaphylactic risk for surgeons. Austr. New Zealand J. Ophthalmol. 24 (3), 257–260.

Muniruzzaman, T., Tabata, Y., Ikada, Y., 1998. Complexation of basic fibroblast growth factor with gelatin. J. Biomater. Sci. Polym. Ed. 9, 459–473.

Murata, M., Huang, B.Z., Shibata, T., Imai, S., Nagai, N., Arisue, M., 1999. Bone augmentation by recombinant human BMP-2 and collagen on adult rat parietal bone. Int. J. Oral. Maxillofac. Surg. 28 (3), 232–237.

Murata, M., Maki, F., Sato, D., Shibata, T., Arisue, M., 2000. Bone augmentation by onlay implant using recombinant human BMP-2 and collagen on adult rat skull without periosteum. Clin. Oral Implants Res. 11 (4), 289–295.

Murray, T.G., Stern, W.H., Chin, D.H., MacGowan-Smith, L.A., 1990. Collagen shield heparin delivery for prevention of postoperative fibrin. Arch. Ophthalmol. 108 (1), 104–106.

Nakagawa, T., Tagawa, T., 2000. Ultrastructural study of direct bone formation induced by BMPs-collagen complex implanted into an ectopic site. Oral Dis. 6 (3), 172–179.

Narayani, R., Rao, K.P., 1994. Controlled release of anticancer drug methotrexate from biodegradable gelatin microspheres. J. Microencapsulation 11 (1), 69–77.

Nicholas, F.L., Gagnieu, C.H., 1997. Denatured thiolated collagen. II. Crosslinking by oxidation. Biomaterials 18, 815–821.

Nimni, M.E., Harkness, R.D., 1988. Molecular structures and functions of collagen. In: Nimni, M.E. (Ed.), Collagen-Biochemistry, vol. I. CRC Press, Boca Raton, FL, pp. 1–79.

Nimni, M.E., Cheung, D., Strates, B., Kodama, M., Sheikh, K., 1988. Bioprosthesis derived from crosslinked and chemically modified collagenous tissue. In: Nimni, M.E. (Ed.), Collagen Biotechnology, vol. III. CRC Press, Boca Raton, FL, pp. 1–38.

O'Brien, T.P., Sawusch, M.R., Dick, J.D., Hamburg, T.R., Gottsch, J.D., 1988. Use of collagen corneal shields versus soft contact lenses to enhance penetration of topical tobramycin. J. Cataract Refractive. Surg. 14, 505–507.

Ochiya, T., Takahama, Y., Naghara, S., Sumita, Y., Hisada, A., Itoh, H., Nagai, Y., Terada, M., 1999. New Delivery system for plasmid DNA in vivo using atelocollagen as a carrier material: the Minipellet. Nat. Med. 5 (6), 707–710.

Orwin, E.J., Hubel, A., 2000. In vitro culture characteristics of corneal epithelial, endothelial, and keratocyte cells in a native collagen matrix. Tissue Eng. 6 (4), 307–319.

Pachence, J.M., Berg, R.A., Silver, F.H., 1987. Collagen: its place in the medical device industry. Med. Device Diagn. Ind. 9, 49–55.

Pajean, M., Herbage, D., 1993. Effect of collagen on liposome permeability. Int. J. Pharm. 91, 209.

Palmer, R.M., McDonald, M.B., 1995. A corneal lens/shield system to promote postoperative corneal epithelial healing. J. Cataract Refractive. Surg. 21, 125–126.

Park, J.C., Hwang, Y.S., Lee, J.E., Park, K.D., Matsumura, K., Hyon, S.H., Suh, H., 2000. Type I atelocollagen grafting onto ozone-treated polyurethane films: cell attachment, proliferation, and collagen synthesis. J. Biomed. Mater. Res. 52 (4), 669–677.

Peng, Y.-M., Alberts, D.S., Graham, V., Surwit, E.A., Weiner, S., Myerskens, F.L., 1986. Cervical tissue uptake of all-trans-retinoic acid delivered via a collagen sponge-cervical cap delivery device in patients with cervical dysplasia. Invest. New Drugs 4, 245–249.

Petite, H., Rault, I., Huc, A., Menasche, P.H., Herbage, D., 1990. Use of the acyl azide method for crosslinking collagen rich tissues such as pericardium. J. Biomed. Mater. Res. 24, 179–187.

Phinney, R.B., Schwartz, S.D., Lee, A., Mondino, B.J., 1988. Collagen shield delivery of gentamicin and vancomycin. Arch. Ophthalmol. 106, 1599–1604.

Piez, K.A., 1984. Molecular and aggregate structures of the collagens. In: Piez, K.A., Reddi, A.H. (Eds.), Extracellular Matrix Biochemistry. Elsevier, New York, pp. 1–40.

Pleyer, U., Elkins, B., Ruckert, D., Lutz, S., Grammer, J., Chou, J., Schmidt, K.H., Mondino, B.J., 1994. Ocular absorption of cyclosporine A from liposomes incorporated into collagen shields. Curr. Eye Res. 13 (3), 177–181.

Podos, S.M., Becker, B., Asseff, C., 1972. Pilocarpine therapy with soft contact lenses. Am. J. Ophthalmol. 73, 336–341.

Poland, D.E., Kaufman, H.E., 1988. Clinical uses of collagen shields. J. Cataract Refractive. Surg. 14, 489–491.

Ponticiello, M.S., Schinagl, R.M., Kadiyala, S., Barry, F.P., 2000. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. J. Biomed. Mater. Res. 52 (2), 246–255.

Prajapati, R.T., Chavally-Mis, B., Herbage, D., Eastwood, M., Brown, R.A., 2000. Mechanical loading regulates protease production by fibroblasts in three dimensional collagen substrates. Wound Repair Regen. 8, 226–237.

Ramachandran, G.N., Sasisekharan, V., 1965. Refinement of the structure of collagen. Biochim. Biophys. Acta., 109 27 (1), 314–316.

Rao, K.P., 1995. Recent Developments of Collagen-based materials for medical applications and drug delivery systems. J. Biomater. Sci. 7 (7), 623–645.

Rao, K.P., Alamelu, S., 1992. Effect of crosslinking agent on the release of an aqueous marker from liposomes sequestered in collagen and chitosan gels. J. Membr. Sci. 71, 161.

Reddi, A.H., 2000. Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. Tissue Eng. 6 (4), 351–359.

Reidy, J.J., Gebhardt, B.M., Kaufman, H.E., 1990. The collagen shield: a new vehicle for delivery of cyclosporine A to the eye. Cornea 9, 196–199.

Remacle, M., Dujardin, J.M., Lawson, G., 1995. Treatment of vocal fold immobility by glutaraldehyde-crosslinked collagen injection: long term results. Ann. Otol. Rhinol. Laryngol. 104, 437–441.

Robin, J.B., Keys, C.L., Kaminski, L.A., Viana, M.A., 1990. The effect of collagen shields on rabbit corneal re-epithelization after chemical debridement. Invest. Ophthalmol. Vis. Sci. 31, 1294–1300.

Rosenthal, F.M., Kohler, G., 1997. Collagen as matrix for neo-organ formation by gene-transfected fibroblasts. Anticancer Res. 17, 1179–1186.

Rossler, B., Kreuter, J., Scherer, D., 1994. Effect of Collagen microparticles on the stability of retinol and its absorption into hairless mouse skin in vitro. Pharmazie 49, 175–179.

Rossler, B., Kreuter, J., Scherer, D., 1995. Collagen microparticles: preparation and properties. J. Microencapsulation 12, 49–57.

Royce, P.M., Kato, T., Ohsaki, K., Miura, A., 1995. The enhancement of cellular infiltration and vascularization of a collagenous dermal implant in the rat by platelet-derived growth factor BB. J. Cermatol. Sci. 10, 42–52.

Rubin, A.L., Stenzel, K.H., Miyata, T., White, M.J., Dunn, M., 1973. Collagen as a vehicle for drug delivery. J. Clin. Pharmacol. 13 (8), 309–312.

Ruderman, R.J., Wade, C.W.R., Shepard, W.D., Leonard, F., 1973. Prolonged resorption of collagen sponges: vapor-phase treatment with formaldehyde. J. Biomed. Mater. Res. 7, 263–265.

Sahai, A., Kanekal, S., Jones, R.E., Brown, D., 1995. An injectable sustained release drug delivery system markedly enhances intratumoral retention of C14-fluorouracil in murine fibro sarcomas. Pharm. Res. 12, S227.

Samuel, C.S., Coghlan, J.P., Bateman, J.F., 1998. Effects of relaxin, pregnancy and parturition on collagen metabolism in the rat public symphysis. J. Endocrinol. 159, 117–125.

Sastry, T.P., Rao, P.K., 1991. High performance biomaterials. In: Szycher, M. (Ed.), A Comprehensive Guide to Medical/Pharmaceutical Application. Technomic, Lancaster, PA, p. 171 Ch II.

Sato, H., kitazawa, H., Adachi, I., Horikoshi, I., 1996. Microdialysis assessment of microfibrous collagen containing a p-glycoprotein-mediated transport inhibitor, cyclosporine A, for local delivery of etopocide. Pharm. Res. 13, 1565–1569.

Sawusch, M.R., O'Brien, T.P., Dick, T.P., Gottsch, J.D., 1988a. Use of collagen corneal shields in the treatment of bacterial keratitis. Am. J. Ophthalmol. 106, 279–281.

Sawusch, M.R., O'Brien, T.P., Updegraff, S.A., 1988b. Collagen corneal shields enhance penetration of topical prednisolone acetate. J. Cataract Refractive. Surg. 14, 625–628.

Scharfmann, R., Axelord, J.H., Verma, I.M., 1991. Long-term in vivo expression of retrovirus-mediated gene transfer in mouse fibroblast implants. Proc. Natl. Acad. Sci. USA 88, 4626–4630.

Schoen, F.J., Levy, R.J., Hilbert, S.L., Bianco, R.W., 1992. Antimineralization treatments for bioprosthetic heart valves: assessment of efficacy and safety. J. Thorac. Cardiovasc. Surg. 104 (5), 1285–1288.

Schwartz, S.D., Harrison, S.A., Engstrom, R.E. Jr, Bawdon, R.E., Lee, D.A., Mondino, B.J., 1990. Collagen shield delivery of amphotericin B. Am. J. Ophthalmol. 109, 701–704.

Sekine, T., Nakamura, T., Shimizu, Y., Ueda, H., Matsumoto, K., 2001. A new type of surgical adhesive made from porcine collagen and polyglutamic acid. J. Biomed. Mater. Res. 54, 305–310.

Sela, J., Kauffman, D., Shoshan, S., Shani, J., 2000. Retinoic acid enhances the effect of collagen on bone union, following induced non-union defect in guinea pig ulna. Inflamm. Res. 49, 679–683.

Shaker, G.J., Ueda, S., LoCascio, J.A., Aquavella, J.V., 1989. Effect of collagen shield on cat corneal epithelial wound healing. Invest. Ophthalmol. Vis. Sci. 30, 1565–1568.

Shantha, K.L., Rao, K.P., 1993. J. Bioact. Compatible Polym. 8, 142.

Shortliffe, L.M.D., Freiha, F.S., Kessler, R., Stamey, T.A., Constantinou, C.E., 1989. Treatment of urinary incontinence by the periurethal implantation of glutaraldehyde crosslinked collagen. J. Urol. 141, 538–541.

Sobolewski, K., Wolanska, M., Bankowski, E., Gacko, M., Glowinski, S., 1995. Collagen, elastin and glycosaminoglycans in aortic aneurysms. Acta Biochimica. Polonica 42, 301–308.

Stemberger, A., Grimm, H., Bader, F., Rahn, H.D., Ascherl, R., 1997. Local treatment of bone and soft tissue infections with the collagen gentamicin sponge. Eur. J. Surg. 578 (Suppl 163), 17–26.

Stenzel, K.H., Dunn, M.W., Rubin, A.L., Miyata, T., 1969. Collagen gels: design for vitreous replacement. Science 164, 1282–1283.

Stompro, B.E., Hansbrough, J.F., Boyce, S.T., 1989. Attachment of peptide growth factors to implantable collagen. J. Surg. Res. 46, 413–421.

Suh, H., Hwang, Y.S., Lee, J.E., Han, C.D., Park, J.C., 2001. Behavior of osteoblasts on a type I atelocollagen grafted ozone oxidized poly L-lactic acid membrane. Biomaterials 22 (3), 219–230.

Supp, A.P., Wickett, R.R., Swope, V.B., Harriger, M.D., Hoath, S.B., Boyce, S.T., 1999. Incubation of cultured skin substitutes in reduced humidity promotes cornification in vitro and stable engraftment in athymic mice. Wound Repair Rege. 7, 226–237.

Suzuki, S., Kawai, K., Ashoori, F., Morimoto, N., Nishimura, Y., Ikada, Y., 2000. Long-term follow-up study of artificial dermis composed of outer silicone layer and inner collagen sponge. Br. J. Plast. Surg. 53 (8), 659–666.

Takaoka, K., Nakahara, H., Yoshikawa, H., Masuhara, K., Tsuda, T., Ono, K., 1988. Ectopic bone induction on and in porous hydroxyapatite combined with collagen and bone morphogenetic protein. Clin. Orthop. 234, 250–254.

Takenaka, H., Fujioka, K., Takada, Y., 1986. New Formulations of bioactive materials. Pharm. Tech. Japan 2, 1083–1091.

Tani, K., Ozawa, K., Ogura, H., Takahashi, T., Ohano, A., Watari, K., Matsudaira, T., Tajika, K., Karasuyama, H., Nagata, S., Asano, S., Takaku, F., 1989. Implantation of fibroblasts transfected with human granulocyte colony-stimulating factor cDNA into mice as a model of cytokine supplement gene therapy. Blood 74, 1274–1280.

Taravella, M.J., Balentine, J., Young, D.A., Stepp, P., 1999. Collagen shield delivery of ofloxacin to the human eye. J. Cataract Refractive. Surg. 25 (4), 562–565.

Thacharodi, D., Rao, K.P., 1996. Rate-controlling biopolymer membranes as transdermal delivery systems for nifedipine: development and in vitro evaluations. Biomaterials 17, 1307–1311.

Timpl, R., 1984. Immunology of the collagens. In: Piez, K.A., Reddi, A.H. (Eds.), Extracellular Matrix Biochemistry. Elsevier, New York, pp. 159–190.

Trasciatti, S., Podesta, A., Bonaretti, S., Mazzoncini, V., Rosini, S., 1998. In vitro effects of different formulations of bovine collagen on cultured human skin. Biomaterials 19, 897–903.

Traub, W., Piez, K.A., 1971. The chemistry and structure of collagen. In: Anfinsen, C.B., Edsalla, J.T., Richards, F.M. (Eds.), Advances in Protein Chemistry, vol. 25. Academic Press, New York, p. 245.

Tu, R., Lu, C.L., Thyagarajan, K., Wang, E., Nguyenm, H., Shen, S., Hata, C., Quijano, R.C., 1993. Kinetic study of collagen fixation with polyepoxy fixatives. J. Biomed. Mater. Res. 27, 3–9.

Uchio, Y., Ochi, M., Matsusaki, M., Kurioka, H., Katsube, K., 2000. Human chondrocyte proliferation and matrix synthesis cultured in Atelocollagen gel. J. Biomed. Mater. Res. 50 (2), 138–143.

Uitterlinden, A.G., Weel, A.E., Burger, H., Fang, Y., van Duijn, C.M., Hofman, A., van Leeuwen, J.P., Pols, H.A., 2001. Interaction between the vitamin D receptor gene and collagen type Ialpha 1 gene in susceptibility for fracture. J. Bone Miner. Res. 15, 379–385.

Ulrich, U., Rhiem, K., Schmolling, J., Flaskamp, C., Paffenholz, I., Salzer, H., Bauknecht, T., Schlebusch, H., 2001. Cross linked type I collagen C- and N-telopeptides in women with bone metastases from breast cancer. Arch. Gynecol. Obstet. 264, 186–190.

Unterman, S.R., Rootman, D.S., Hill, J.M., Parelman, J.J., Thompson, H.E., Kaufman, H.E., 1988. Collagen shield drug delivery: therapeutic concentrations of tobramycin in the rabbit cornea and aqueous humour. J. Cataract Refractive. Surg. 14, 500–504.

Van der Laan, J.S., Lopez, G.P., van Wachem, P.B., Nieuwenhuis, P., Ratner, B.D., Bleichrodt, R.P., Schakenraad, J.M., 1991. Tee-plasma polymerized dermal sheep collagen for the repair of abdominal wall defects. Int. J. Artif. Organs 14, 661–666.

Vaneerdeweg, W., Bresseleers, T., Du Jardin, P., Lauwers, P., Pauli, S., Thyssens, K., Van Marck, E., Elseviers, M., Eyskens, E., 1998. Comparison between plain and gentamicin containing collagen sponges in infected peritoneal cavity in rats. Eur. J. Surg. 164 (8), 617–621.

Vaneerdeweg, W., Hendriks, J.M., Lauwers, P.R., Ieven, M., Eyskens, E.J., 2000. Effect of gentamicin-containing sponges on the healing of colonic anastomoses in a rat model of peritonitis. Eur. J. Surg. 166 (12), 959–962.

Vardaxis, N.J., Boon, M.E., Ruijgrok, J.M., 1996. Calcification of cross-linked collagen-elastin membrane implants in vivo and their proposed use in bone regeneration. Biomaterials 17, 1489–1497.

Wachol-Drewek, Z., Zpfeiffer, M., Scholl, E., 1996. Comparative investigation of drug delivery of collagen implants saturated in antibiotic solutions and a sponge containing gentamycin. Biomaterials 17, 1733–1738.

Wada, T., Mckee, M.D., Steitz, S., Giachelli, C.M., 1999. Calcification of vascular smooth muscle cell cultures: inhibition by osteopontin. Circ. Res. 84, 166–178.

Waltman, S.R., Kaufman, H.E., 1970. Use of hydrophilic soft lenses to increase ocular penetration of topical drugs. Invest. Ophthalmol. 9, 250–255.

Wang, C.L., Miyata, T., Weksler, B., Rubin, A.L., Stenzel, K.H., 1978. Collagen-induced platelet aggregation and release. Biochim. Biophys. Acta. 544, 555–567.

Webster, R.C., Kattner, M.D., Smith, R.C., 1984. Injectable collagen for augmentation of facial areas. Arch. Otolaryngol. 110, 652–656.

Wedge, C.I., Rootman, D.S., 1992. Collagen shields: efficacy, safety and comfort in the treatment of human traumatic corneal abrasion and effect on vision in healthy eyes. Can. J. Ophthalmol. 27 (6), 295–298.

Weiner, A.L., Carpenter-Gren, S.S., Soehngen, E.C., Lenk, R.P., Popescu, M.C., 1985. Liposome-collagen gel matrix, A novel sustained drug delivery system. J. Pharm. Sci. 74, 922–925.

Willey, D.E., Williams, I., Faucett, C., Openshaw, H., 1991. Ocular acyclovir delivery by collagen discs: a mouse model to screen anti-viral agents. Curr. Eye 10, 167–169.

Woolley, D.E., 1984. Mammalian collagenases. In: Piez, K.A., Reddi, A.H. (Eds.), Extracellular matrix Biochemistry. Elsevier, New York, pp. 119–158.

Yamada, N., Uchinuma, E., Kuroyanagi, Y., 1999. Clinical evaluation of an allogeneic cultured dermal substitute composed of fibroblasts within a spongy collagen matrix. Scand. J. Plast. Reconstr. Surg. Hand Surg. 33 (2), 147–154.

Yamahira, Y., Fujioka, K., Sato, S., Yoshido, N. 1991. Sustained release injections, Eur. Patent 84112313.6.

Yang, S.C., Lu, L.F., Cai, Y., Zhu, J.B., Liang, B.W., Yang, C.Z., 1999. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J. Control. Release 59, 299–307.

Yannas, I.V., 1990. Biologically active analogues of the extracellular matrix: artificial skin and nerves. Angew Chem. Int. Ed. 29, 20–35.

Yannas, I.V., Lee, E., Orgill, D.P., Skrabut, E.M., Murphy, G.F., 1989. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. Proc. Natl. Acad. Sci. USA 86, 933–937.