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Exploiting the Potential of Collagen as a Natural Biomaterial in Drug Delivery

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Abstract: *Collagen is the most important structural protein that connects and supports bodily tissues like skin, bone, tendons, muscles cartilage and all internal organs. Collagen is one of the most abundant proteins found in mammals accounting for about 30 percent of the total amount of proteins in the body. There are more than 25 types of collagens that naturally occur in the body. Collagen has immense potential as a biomaterial. Collagen is biodegradable, posses weak antigenecity or low immunogenicity and has superior biocompatibility compared with other natural polymers, due to which it is considered as one of the best biomaterial for use in medical practice. Additionally collagen can be processed into a number of forms such as sheets, tubes, sponges, powders, fleeces, injectable solutions and dispersions, all of which have found use in medical practice. Use of collagen for administration of antibacterial, anticancer drugs has shown it as one of the efficient drug delivery system in the field ophthalmology, wound and burn dressing, tumour treatment, etc. Currently its potential in tissue engineering is also being explored vigorously.*

I INTRODUCTION

Among the natural polymers and their synthetic analogues used as biomaterials, the characteristics of collagen are distinct mainly in its mode of interaction in the body (McPherson et al., 1986). Collagen plays an important role in the formation of tissues/organs and is involved in various functional expressions of cells. It exhibits biodegradability, weak antigenecity and superior biocompatibility compared with other natural polymers, such as albumin and gelatin (Maeda et al., 1999). During 1970s and 1980s expanding medical applications of biomaterials and connective tissue research made laboratories to focus their studies on collagen (Nimni et al., 1987; Yannas and Burke, 1980; Yannas et al., 1980). And medical-grade collagen became easier to obtain, the processing technology improved and new collagen products were successfully placed on the market (Pachence et al., 1887). The use of collagen as a drug delivery system is diverse. 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 is still needed to address some of drawbacks in collagen based applications.

II TYPES OF COLLAGEN

Collagen constitute approximately 30% of all vertebrate body protein. More than 90% of the extra cellular matrix protein in the tendon, bone and more than 50% in the skin consist of collagen (Piez, 1985). This collagenous spectrum is not only present in the scaffolding in mammals, but it also marks its presence from achilles tendons to the cornea. Collagen has a unique triple-helix configuration of three polypeptide subunits known as α-chains in common. Currently at least 13 types have been isolated which vary in the length of the helix and the nature, size of the non-helical portions (Table 1) (Kucharz, 1992). Type I collagen is predominant in higher order animals especially in the skin, tendon and bone, where extreme forces are transmitted. Type II collagen is essentially unique to hyaline cartilage. Type III is found in limited quantities (approximately 10%) in association with type I. In addition, blood vessels predominantly contain type III. Type IV is a highly specialized form found only as a loose fibrillar network in the basement membrane (Kucharz, 1992).

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Table 1 : Chain Composition and Body Distribution of Different Collagen Types (Kucharz, 1992).

Collagen typ	*Chain composition*	*Tissue distribution*
I	(a1(I))2a2(I), trimer (a1(I))3	Skin, tendon, bone, cornea, dentin, fibrocartilage, large vessels, intestine, uterus, dentin, dermis, tendon
II	(a1(II)) 3	Hyaline cartilage, vitreous, nucleus pulposus, notochord
III	(a1(III))3	Large vessels, uterine wall, dermis, intestine, heart valve, gingiva (usually coexists with type I except in bone, tendon, cornea)
IV	(a1(IV))2a2(IV)	Basement membranes
V	a1(V)a2(V)(3(V) or (a1(V))2a2(V) or (a1(V))3	Cornea, placental membranes, bone, large vessels, hyaline cartilage, gingiva
VI	a1(VI)a2(VI)a3(VI)	Descemet's membrane, skin, nucleus pulposus, heart muscle
VII	(a1(VII)) 3	Skin, placenta, lung, cartilage, cornea
VIII	a1(VIII) a2(VIII) chain organization of helix unknown	Produced by endothelial cells, Descemet's membrane
IX	a1(IX)a2(IX)a3(IX)	Cartilage
X	(a1(X))3	Hypertrophic and mineralizing cartilage
XI	1a2a3a1 or a1(XI)a2(XI)a3(XI)	Cartilage, intervertebral disc, vitreous humour
XII	(a1(XII))3	Chicken embryo tendon, bovine periodontal ligament
XIII	Unknown	Cetal skin, bone, intestinal mucosa

Structure

The first correct model for the structure of collagen was put forward by Ramachandran and Kartha and was popularly known as the *Madras Model* (Sasisekharan and Yathindra, 1999). The triple helix of collagen molecule is basically composed of three polypeptide α-chains, each consisting of more than 1000 amino acids with the amino acid glycine making the smallest side group and its repetition at every third position on the sequence allow close packaging of the chains into a helix which leaves little space for residues in the core. About 35% of the non-glycine positions in the repeating unit Gly-X-Y are occupied by proline, which is found almost exclusively in the X-position, and 4-hydroxyproline, predominantly in the Y-position. Hydroxyproline is derived from proline by post-translational hydroxylation mediated by prolylhydroxylase (Kucharz, 1992). It comprises approximately 10% of the amino acid composition of collagen and offers ways to quantify collagen or its degradation products in the presence of other proteins (Woessner, 1961). Collagen also contains the unusual amino acid hydroxylysine which is formed from lysine in the endoplasmic reticulum via enzymatic hydroxylation by lysyl hydroxylase. Formation of hydroxylysyl residues allows the attachment of sugar components for the formation of the triple-helical structure of the collagen molecule (Piez, 1984). Due to their alicyclic nature, imino acids (approximately 23% of the residues) stabilize the triple helix and they stiffen the α-chain and form hydrogen-bonds limiting rotation (Piez, 1984). There are only minor differences between the collagen of different vertebrate species (Timpl, 1984).

III ISOLATION AND PURIFICATION OF COLLAGEN

Collagen is insoluble in organic solvents. Water-soluble collagen represents only a small fraction of total collagen and the amount depends on the age of the animal and type of tissue extracted. But collagen molecules present within fibrillar aggregates can be dissociated and brought into aqueous solution. The major hurdle for dissolution of collagen from tissue is the presence of covalent crosslinks between molecules. In some tissues, notably skin from young animals, crosslinking is sufficiently low to extract a few percent under appropriate conditions. However, the nature of the crosslinks prevalent in different tissues determines the particular solvent to be used and the corresponding yields.

IV NEUTRAL SALT SOLUBLE COLLAGEN

Neutral salt solution (0.15-2 M NaCl) or dilute acetic acid are some of the most commonly used solvents for extraction of collagen (Fielding, 1976). Freshly synthesized and negligibly crosslinked collagen can easily be extracted with neutral salt solution (Fielding, 1976). Modifications in temperature, shaking rate, and volume of extractant to tissue ratio will inevitably alter the composition of the purified collagen (Fielding, 1976). The extracted material is purified by dialysis, precipitation, and centrifugation. Most tissues have little or no salt-extractable collagen.

V ACID SOLUBLE COLLAGEN

Dilute acidic solvents, e.g. 0.5 M acetic acid, citrate buffer, or hydrochloric acid pH 2–3 are more efficient than neutral salt solutions as because the intermolecular crosslinks of the aldimine type are dissociated by the dilute acids and the repulsive repelling charges on the triple-helices lead to swelling of fibrillar structures (Trelstad, 1982). The first step in acid extraction of collagen involves grinding of tissues in cold, followed by washing with neutral saline to remove soluble proteins and polysaccharides and finally extraction with a low ionic strength, acidic solution (Bazin, 1976).

It is possible to solubilize approximately 2% of the tissue collagen with dilute salt or acid solutions. The remaining 98% is referred to as insoluble collagen although this dominant collagen material is not absolutely insoluble and can be further disintegrated without major damage to the triple-helical structures. The two most common approaches are the use of strong alkali or enzymes to cleave additional crosslinks and suspend or dissolve at first acid-insoluble structures.

VI ALKALI-AND ENZYME-TREATED COLLAGEN

Additional collagen material can be solubilized by treating connective tissue with an aqueous solution comprised of alkali hydroxide and alkali sulfate, e.g. approximately 10% sodium hydroxide and 10% sodium sulfate for approximately 48 h (Roreger, 1995). The size and molecular weight of the resulting collagen material depend on the time of treatment and alkali concentration (Roreger, 1995).

Much higher yields compared with acidic extraction can be achieved by taking advantage of the fact that the collagen triple-helix is relatively resistant to proteases below approximately 20°C (Piez, 1984). Pepsin at a 1:10 weight ratio of enzyme to dry weight tissue in dilute organic acid (0.5 M acetic acid) provides a propitious medium in which collagen can be swollen and solubilized (Piez, 1985).

Soluble collagen is purified mainly by precipitation after pH, salt concentration or temperature adjustment (Li, 1995). Soluble collagen can be stored frozen or lyophilized. In the course of drying, denaturation or non-specific crosslinking can occur and the degree of association upon reconstitution can change (Lee, 1983).

VII APPLICATIONS

Collagen is considered as one of the ideal biomaterial for use in medical practice mainly because human body recognizes collagen as a normal constituent of the body making it one of the most suitable biomaterial having low immunogenicity (Piez, 1984). Collagen can be processed into a number of forms such as sheets, tubes, sponges, powders, fleeces, injectable solutions and dispersions, all of which have use in medical practice (Chvapil et al., 1973; Gorham, 1991; Fu Lu and Thies, 1991 Ruszczak and Friess (2003).) Ongoing and future studies will no doubt show collagen as one of the efficient drug delivery system in the field of ophthalmology, wound and burn dressing, tumour treatment, and tissue engineering.

VII COLLAGEN-BASED DRUG DELIVERY SYSTEMS

Film

One of the most suitable applications of collagen films is as barrier membrane. Biodegradable films of thickness 0.5mm, e.g.- telopeptide-free reconstituted collagen , showed a slow release of incorporated drugs (Rubin et al., 1973). Hydrogen bonding, covalent bonding or simple entrapment is some of the most preferred ways by which drugs are loaded to collagen membrane. They can be sterilized and become pliable upon hydroxylation, 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). 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).

Collagen shields

Drug delivery by collagen shields depends on loading and a subsequent release of medication by the shield (Leaders et al., 1973). The collagen matrix serves as a reservoir and the drugs are entrapped in the interstices of the matrix in a solution for water-soluble drugs or incorporated into the shield for water-insoluble drugs. Biologically compatible collagen solution can be obtained by vigorous flushing through the shields resulting in solubilization.This, biologically compatible collagen solution acts as a lubricant for the surface of the eye , causes minimal rubbing of the lids on the cornea and increases the time of contact with the drug and thus facilitate epithelial healing (Kaufman, 1988, 1994).Collagen shield was found to be more efficient in plasmid DNA delivery that increased chloramphenicol acetyltransferase, the reporter gene, 30-fold over injection of plasmid DNA through saline vehicle (Angella et al., 2000). Wound healing after glaucoma

surgery was better achieved in gene therapy that used naked plasmid DNA together with simple collagen shield.

Collagen Sponges

Collagen sponges have a wide range of application in the field of surgery involving 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).Collagen sponge is mainly prepared from human collagen membrane that was used as a biological dressing since 1930s (Rao, 1995). Collagen sponges have the ability to easily absorb large quantities of tissue exudates, 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 (Yannas, 1990).Recovery from dermal and epidermal wounds can be better achieved with growth factors coated with collagen sponge. (Marks et al., 1991; Royce et al., 1995). Collagen sponges were found suitable for short-term delivery (3–7 days) of antibiotics, such as gentamicin (Wachol-Drewek et al., 1996).

Gel, Hydrogel, Liposomes-collagen

Another important use of collagen is as hydrogels. Various combinations of polymers can be made into hydrogel formulations to investigate their potential as a drug delivery system. One of the major merits for the growing importance of hydrogel in clinical and fundamental applications is the production of large and constant surface area and the ease of manufacturing. Collagen based gel are widely used as 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). Collagen hydrogel was also used as gene delivery carriers.

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). 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). Collagen-based pellet as a gene delivery carrier has been extensively studied. The effect of collagen- based mini-pellet on the mRNA expression and functional status of facial nerve in the rat model was investigated (Kohmura et al., 1999).

Nanoparticles/nanospheres

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 gelatine nanoparticles (Coester et al., 2000). The biodegradable collagen based nanoparticles or nanospheres are thermally stable and thus sterilization can easily be carried out (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). 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.

VIII CONCLUDING REMARKS

Collagen is one of the best biomaterial that has a wide range of application as carrier systems for delivery of drug, protein and gene. Though literature on wide application of collagen as drug vehicle is available, only a few collagen-based drug delivery products have so far been able to attract the market demand. Collagen have been successfully implemented as a drug vehicle in ophthalmology, as injectible dispersions in cancer treatment, as sponges carrying antibiotics and as implantable minipellets loaded with protein drugs. Pathological diseases can better be interpreted with a

much wider knowledge of native collagen for drug delivery systems and tissue engineering. The concepts of high binding affinity and specificity play a critical role intargetted delivery drug. 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. Collagen-based biomaterials are expected to become a useful matrix substance for various biomedical applications in the future.

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