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Review Article

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COLLAGEN BASED DRUG DELIVERY SYSTEM

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ABSTRACT

Collagen, a biopolymer finds its application in the preparation of pharmaceutical products that are used in wound management, ophthalmic, orthopaedic and oral surgeries. This wide applicability is due its special properties such as biodegradability, biocompatibility, easy availability and high versatility. Collagen is isolated from various sources such as bovine skin, fish skin, chicken skin, skin waste of marine organisms, solid wastes of leather industry, short tendons of slaughtered cattle and bone. The functional groups such as amino and carboxylic acid present in collagen helps in its modification that suits for various end uses which include wound healing, ophthalmic defects, drug delivery and tissue engineering applications. These beneficial properties impart uniqueness to collagen molecule among the available bio molecules. Due to its biodegradability, biocompatibility, weak antigenicity it is very useful carrier for the delivery of various kinds of drugs and agents like growth factors, collagen possess some very unique properties as compare to other drug carriers that's why a numerous number of researches are in pipeline on this biomaterial. However, fundamental awareness regarding collagen biochemistry and the manufacturing knowledge in combination with understanding of the physico-chemical properties is essential for fruitful application of collagen for drug delivery systems. The purpose of this review article is to summarize information available on collagen dosage forms for drug delivery as well as to communicate an outline regarding current preparation of collagen available in market includes - collagen sponges for burns/wounds, mini-pellets and tablets, gel preparations in combination with liposomes for sustained delivery of drug.

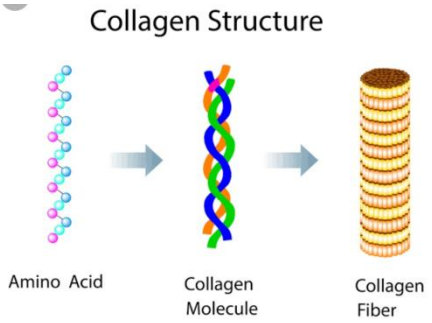
KEYWORDS: Biopolymer; Collagen; Drug delivery system; Biomaterial; Opthalmology.

INTRODUCTION

Collagen is the most abundant and ubiquitous protein in animal origin, As the main component of connective tissue, it is the most abundant protein in mammals. Making up from 25% to 35% of the whole-body protein content. Collagen consists of amino acids bound together to form a triple helix of elongated fibril known as a collagen helix. It is mostly found in connective tissue such as cartilage, bones, tendons, ligaments, and skin.[1] Collagen is mainly presents in all connective tissues, bone, cartilage, tendon and blood vessels.[2] It is widely used in medical field. Collagen plays a significant role in the formation of organs and tissue. Collagen as a biomaterial are different from those of synthetic polymers.[3] Biocompatibility, biodegradability[4] of collagen is the charactersitic feature. In the body as compared with other natural polymers like gelatin and albumin, collagen possesses good ability to penetrate a lipid-free interface. Collagen in biomedical application is useful as collagen can form fibers with extra strength and stability . In most of drug delivery systems made of collagen, in vivo absorption of collagen is controlled by the use of crosslinking agents.[5] The use of collagen for drug delivery system is increasing day by day.

STRUCTURE OF COLLAGEN

The structure of a collagen was shown in the following figure -1.



Basically collagen possesses a triple helix structure, which generally made up of two homologous chains (α-1) and one supplementary chain that varies slightly in its chemical composition (α-2). These chains are polypeptide in nature and coiled around one another in a cable form. Each has a distinct turn in the reverse direction, these chains are connected together chiefly by hydrogen bonds between nearby CO and NH groups.[6] The weight of collagen molecule is 300 k da.

It is approximately 300 nm long and 1.5 nm in diameter, and it is made up of three polypeptide strands (called alpha peptides) each of which has the conformation of a left-handed helix – this should not be confused with the right-handed alpha helix. These three left-handed helices are twisted together into a right-handed triple helix or "super helix", a cooperative quaternary structure stabilized by many hydrogen bonds. With type I collagen and possibly all fibrillar collagens[1], if not all collagens, each triple-helix associates into a right-handed super-super-coil referred to as the collagen microfibril. Each microfibril is interdigitated with its neighboring microfibrils to a degree that they are individually unstable, although within collagen fibrils, they are so well ordered as to be crystalline. The sequence of amino acids follows the pattern glycine-proline-X or glycine-X-hydroxyproline where X is the amino acid other than glycine, proline or hydroxyproline; glycines constitute about 1/3 of total sequence and proline. This whole structure is joined with the help of hydrogen bonds and linking peptide bonds.[7]

CHARACTERISTICS POSSESSED BY COLLAGEN

a. Strength: collagen fibrils provide the mechanical support that enabled large multicellular animals to evolve on earth[8],[9] Collagen has great tensile strength, and is the main component of fascia, cartilage, ligaments, tendons, bone and skin.[10]

b. Biocompatibility: Collagen is a biocompatible and bioactive non specific polymer. The use of collagen based biomaterials has been in wound healing and in reconstructive surgery.[11] These three amino acid monomers are strongly fused and they look like single monomer. Several hydrogen bonds are present in collagen, on applying stress they can be wrecked and re-joined after removal of pressure.

c. Collagen show good absorption in-vivo, it possesses weak antigenicity[12] it is biodegradable.[13]

d. Collagen is one of the long, fibrous structural proteins whose functions are quite different from those of globular proteins, such as enzymes. Tough bundles of collagen called collagen fibers are a major component of the extracellular matrix that supports most tissues and gives cells structure from the outside, but collagen is also found inside certain cells. Collagen has great tensile strength, and is the main component of fascia, cartilage, ligaments, tendons, bone and skin. Along with elastin and soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging. It strengthens blood vessels and plays a role in tissue development. It is present in the cornea and lens of the eye in crystalline form.

ISOLATION AND PURIFICATION OF COLLAGEN

Even though the mammalian body retains a plenty amount of collagen, those tissues rich in fibrous possess collagen such as skin and tendons, are commonly used as preliminary materials to produce collagen for use in transplants, wound dressings, or drug delivery systems. In addition procaine, bovine and sheep collagen varieties derived from many different sources including marine sources, human placenta[14], and recombinant human collagen from transgenic animals must be labelled. Autologous collagen material deals additional gut alternative mucosa which is consumed in the building of surgical sutures.[15] Collagen is insoluble in organic solvents. Water-soluble collagen denotes only a minor fraction of total collagen and the quantity depends on the age of the animal and kind of tissue extracted.[16] In certain tissues, especially the skin of young animals, cross linking is sufficiently little to extract a few percent in suitable conditions. Still, collagen molecules present inside fibril masses can be separated and brought into aqueous solution. Though, the nature of the crosslink dominant in different tissues decides the particular solvent to be used and the resulting yields.

4.1. Process to Isolate Neutral Salt Soluble Collagen

1)Tissue containing slightly crossed link collagen are taken
2) Extracted with neutral salt solution(0.15-2mlNacl) dil acetic acid
3) Adjusting and maintaining the process variables viz temperature, ph and shaking
4) Extracted material can be obtained by dialysis, precipitation, Centrifugation.
5) Extracted material is cleared
Note- Most tissues have minute or no saltextractable collagen. In demand to increase the yield for research purposes animals can be put on the diet contain b-aminopropionitrile, an inhibitor of peptidyllysyl oxidase, yet this method is in adequate for larger commercial scale

4.2. Process to Isolate Acid Soluble Collagen

1) Tissues containing higher percentage of keto amine bonds, bone, cartilage,Or material from older animals is taken
2) Tissue is ground in colda washed with neutral saline to remove solube proteins and polysaccarides
3) Low ionic strength acid solution (0.5Macetic acid., citrate buffer)
4) Collagen extracted can be divided as soluble collagen (3%) and insoluble collagen (97%)
5) Soluble collagen when adjusted with PH and temperatures we get reconsitution into large fibrils with similar properties as native fibrils
6) Insoluble collagen are further as
a) strong alkali or enzymes
b) suspend or dissolve at first acid -insoluble structures
7) Which are disintegrated without major damaging to the triple helical structure

Collagen tissues are rich in fibrous possess they are commonly used as preliminary materials to produce collagen for use in transplants, wound dressings, or drug

delivery systems. Autologous collagen material deals gut alternative mucosa which is consumed in the building of surgical sutures. Collagen is insoluble in organic solvents, water-soluble collagen denotes only a minor fraction of total collagen and the quantity depends on the age of the animal and kind of tissue extracted(16).

Basically 4 types of collagen can be isolated and purified for the implementation in pharmaceutical industries for the delivery of drugs are following.

1. Natural salt soluble collagen
2. Alkali and enzyme treated collagen
3. Acid soluble collagen
4. Insoluble collagen.

The in vivo absorption of collagen is controlled by the use of cross-linking agents, such as:-

- Gluteraldehyde,
- Chromium tanning,
- Formaldehyde,
- Poly epoxycompounds,
- Acylazide

Physical treatment, such as ultra-violet/gammaray irradiation and dehydrothermal treatments have been efficiently used for the introduction of cross links to the collagen matrix . The use of collagen as a drug delivery system is very comprehensive and diverse. It can be extracted into an aqueous solution and moulded into various forms of delivery systems.

SOME SPECIFIC TYPE OF DRUG DELIVERY SYSTEMS CONSTRUCTED ON COLLAGEN NANOPARTICLES/NANOSPHERES/MICROSPHERES

Nano delivery systems are a relatively new but rapidly developing science where materials in the nanoscale range are employed to serve as means of diagnostic tools or to deliver therapeutic agents to specific targeted sites in a controlled manner. Nanotechnology offers multiple benefits in treating chronic human diseases by site-specific, and target-oriented delivery of precise medicines.

In the collagen fold configuration, the crystallites suspended in the gel aggregates, this property is used to formulate aggregates as colloidal drug delivery carriers. The construction of nanosphereis determined by a mixture of electronic and electrostatic forces with sodium sulphate engaged as a liquefying reagent to facilitate greater charge-charge relations among plasmid DNA and collagen.The stability of the produced collagen nanoparticles is depended upon following factors such as.

1)molecular weight of collagen
2)temperature
3) PH

The nanoparticles and nanospheres based on biodegradable collagen; are enabled and enhanced uptake of exogenous compounds such as antiHIV in a number of cells, especially macrophages, that is an advantage of collagen based nanoparticles as a systemic delivery carrier as they are thermally stable and easily sterilized. Cytotoxic drugs like Campthocin can be easily delivered in systemic circulation with the help of collagen nanoparticles. Collagen based nanoparticles can be readily used in sustained and delayed release formulation for steroids and antibiotics because

- Large surface area;
- Smaller size;
- Great absorptive capability;
- Capacity to diffusing in water to form a colloidal solution; For example- Dermal delivery of retinol enhanced in collagen nanoparticles.

FABRICATION METHODS FOR COLLAGEN NANOPARTICLES

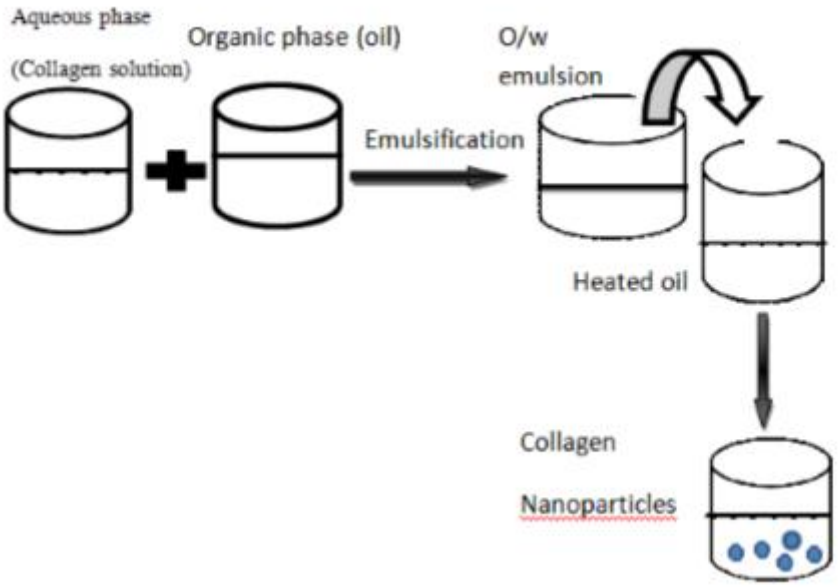
There are basically four type of methods for the manufacturing the protein based nanoparticles namely.

- Emulsification
- Desolvation
- Coacervation
- Spray drying

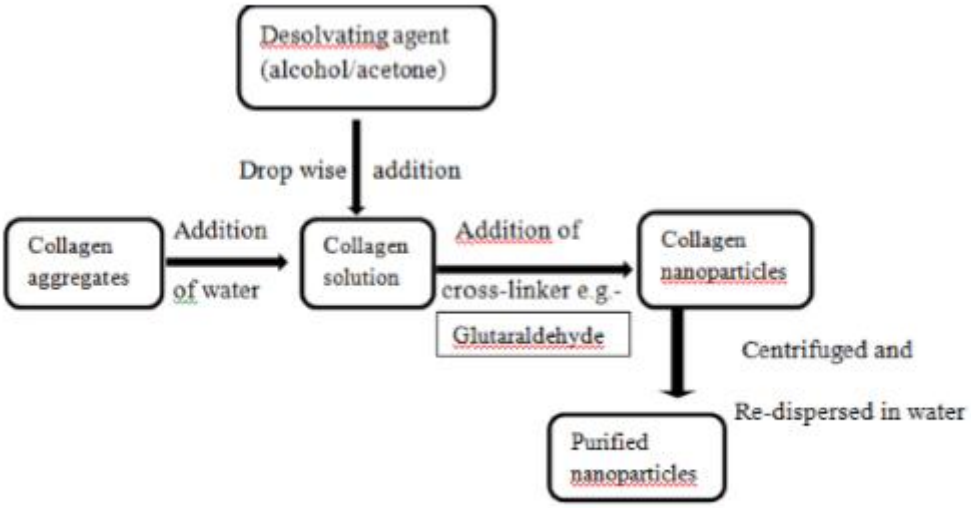
Some additional methods are, jet milling technique, fluidization and solvent precipitation method, Interfacial polymerization etc.

Processes involved

1) **Emulsification**- In this process, A collagen aqueous phase containing a hydrophilic surfactant and water, and an organic phase containing a lipophilic surfactant, oil and water miscible solvent is mixed with rapid agitation by a mechanical homogenizer at room temperature to form a homogeneous emulsion. Then the above emulsion will be mixed in preheated oil drop by drop resulting formation of collagen nanoparticles. This method was developed by F Scheffel and coworkers in direction to prepare albumin sphere nanoparticles and then it was improved by Gao and his Coworkers. Emulsification method for nanoparticles manufacturing was shown in the following figure -2.



2) Desolvation : - The process of desolvation includes the addition of alcohol or natural salt as desolvation factor to the collagen solution, which alters the tertiary structure of collagen, when the critical level of desolvation attained the formation of collagen mass, starts lastly glutaraldehyde will be added as a cross-linking material, and then nanoparticles is formed. This process was firstly employed by Marty and coworkers. A new two-step desolvation method for manufacturing gelatin nanoparticles was developed. After the first desolvation step, the low molecular gelatin fractions present in the supernatant were removed by decanting. The high molecular fractions present in the sediment were redesolved and then desolvated again at pH 2.5 in the second step. The resulting particles can then be easily purified by centrifugation and redispersion. The different fractions obtained during the process were analysed by gel permeation chromatography (GPC). Based on these results, it can be concluded that the molecular weight of gelatin has a decisive influence on the stability of the manufactured gelatin nanoparticles. In addition, two fluorescent dyes were coupled to the nanoparticles for cell uptake studies. The fluorescent nanoparticles showed a high uptake into monocytes/macrophages. Desolvation method for nanoparticles manufacturing was shown the following figure -3.



3**) Coacervation**:- This method is similar to desolvation method, the difference is only in various parameters like-temperature, molar ratio of organic solvent and protein, rate of solvent addition, concentration of cross-linker used, pH, speed of homogenizer etc.[17] The method was shown in the figure -4.

Eg- Complex coacervation of collagen hydrolysate extracted from leather solid wastes and chitosan for controlled release of lavender oils solid collagen-based protein hydrolysate was isolated from chromium-tanned leather wastes and its chemical properties were determined. Microcapsules of collagen hydrolysate (CH) – chitosan (C) crosslinked with glutaraldehyde (GA) containing Lavender oil (LO) were prepared by complex coacervation method.

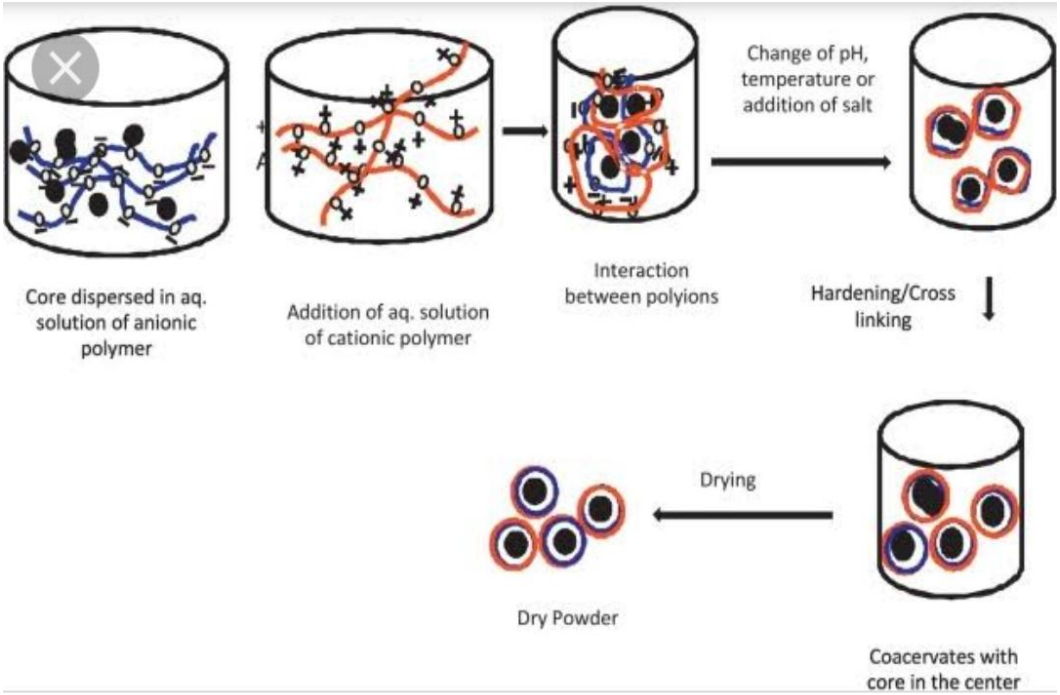


Figure -4 : Coacervation method for collagen

4) **Spray drying**:- Basically spherical collagen nanoparticles is fabricated by this process. This process include the spraying of dilute solution of collagen leads to the formation of hollow spheres using elevated temperature, increased temperature can lead denaturation of collagen triple –helical structure.[17] So collagen solution is sprayed into liquid nitrogen to prevent denaturation. After that the fabricated nanospheres are successively frozen, tempered, lyophilized, cross-linked, and sterilized.[18] Fabrication of solid collagen nanoparticles using electrospray deposition. Collagen is a promising biomaterial for drug delivery due to advantages including high biocompatibility and biodegradable property. However, transforming collagen into solid nanoparticles is difficult, although the solid dosage form is advantageous for some administration routes including pulmonary and oral drug delivery. In this study, collagen solid nanoparticles are prepared in one-step using electrospray deposition under ambient temperature and pressure conditions. Although collagen molecules formed micron-sized aggregates in acetic acid solutions spontaneously, electrospraying the collagen solutions resulted in formation of nanofibers. Solid nanoparticles were obtained by increasing conductivity of the solution and/or inducing structural perturbation of the collagen molecules using salts. The ability of solid collagen particles as a drug carrier was demonstrated by incorporating theophylline as a model drug using a coaxial spray technique. Release of theophylline was controlled by cross-linking collagen molecules. Electrospray deposition was proved to be a powerful method for producing solid collagen nanoparticles for drug delivery.

Drug delivery is the method or process of administering pharmaceutical compound to achieve a therapeutic effect in humans or animals. Collagen gels are one of the first natural polymers to be used as a promising matrix for drug delivery.

COLLAGEN SHIELD

The collagen shield was designed for bandage contact lenses, which are gradually dissolved in cornea. The use 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. The mechanical properties of the shield protect the healing corneal epithelium from the blinking action of the eyelids. Drug delivery by collagen shields depends on loading and a subsequent release of medication by the shield. 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 increase the epithelial healing A bolus release of drug from the lenses leads to the enhanced drug effect. Therefore, this system allows the higher corneal concentrations of drug, and the more sustained drug delivery into the cornea and the aqueous humor.[19] These are contact lens-shaped, dissolvable protein matrixes placed on the cornea, primarily indicated for the promotion of epithelial wound healing. The shield can also serve to deliver ocular medications if soaked in a water-soluble antibiotic and/or steroid solution prior to their placement. Collagen shields have distinct advantages over other precorneal drug reservoirs, such as intensive topical therapy, which inconveniences both the patients and caregivers, and subconjunctival injections, which risks hemorrhaging and perforation. It has also been suggested that collagen shields can be used to augment ocular tissue concentrations of various medications over topical administration alone. It is also known as collagen corneal shield, they are newly developed, potentially versatile ophthalmic lens, which is made up of collagen, since collagen is a natural, commonly available protein involved in the support and protection of vital structures, many researchers have tried to use peripheral collagen to protect the surface of the eye in a variety of diseased states, like traumatic and non-traumatic states after surgery[20]; after corneal transplantation, radial keratomy. Generally collagen shields are manufactured from bovine or procaine collagen, there are three kind of collagen shield available in market having dissolving time of 12, 24, 72 hours. Some other marketed preparations are (proshieldO, MediLenso, Fort Worth, Chiron, TX, Irvine). These shields are able to enhance the penetration of corticosteroid, subconjunctival antibiotics in eye. They are act as a short term bandage and allow sufficient oxygen transmission for essential metabolism occurring in eye cornea For the corneal surface lubrication these shields dissolve in collagen solution that minimize lids rubbing. Mainly water soluble antibiotics and steroids are used in combination with collagen shields for example- Vancomycin, Trimethoprim, Amphotericin-B, Gentamycin, Polymyxin-B sulfate, Tobramycin, steroids, pilocarpine. The applications of collagen shields on cornea is demonstrated in figure 5 and 6.

Figure – 5 : Corneal collagen shield.

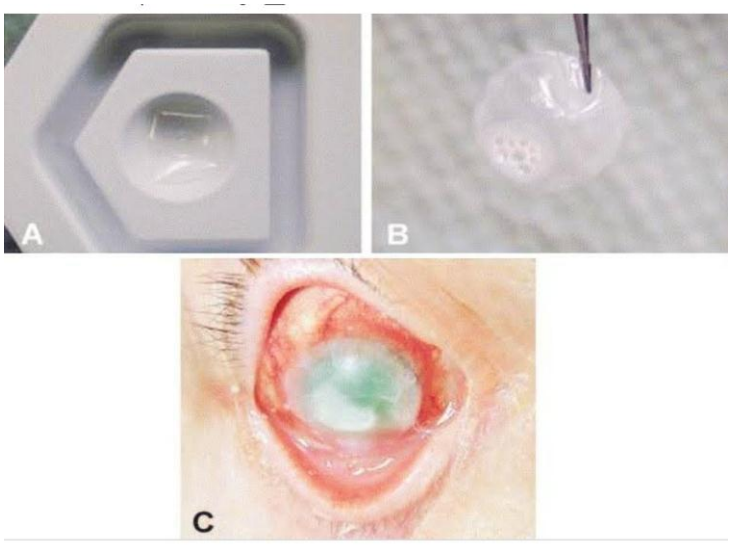


Figure -6: Corneal Collagen shield.

MARKETED PREPARATION OF COLLAGEN SHIELDS

- Biocora®
- ProshieldO®
- MediLenso®
- Irvine®
- Chiron®

COLLAGEN FILMS

Collagen films have also been used as a drug carrier for antibiotics after implantation into rabbits, The duration of therapeutic effect of tetracycline is increased after administration of the collagen film containing tetracycline and it could be detected in the plasma for more than 7 days after implantation into rabbits. Biodegradable collagen films or matrices have served as scaffolds for a survival of transfected fibroblasts. 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.[12] Porous collagen films were fabricated by ion leaching technique. The films shown in the following figure -7 have suitable mechanical properties and optical performance. Human corneal epithelial cells studies confirmed the biocompatibility of the films.

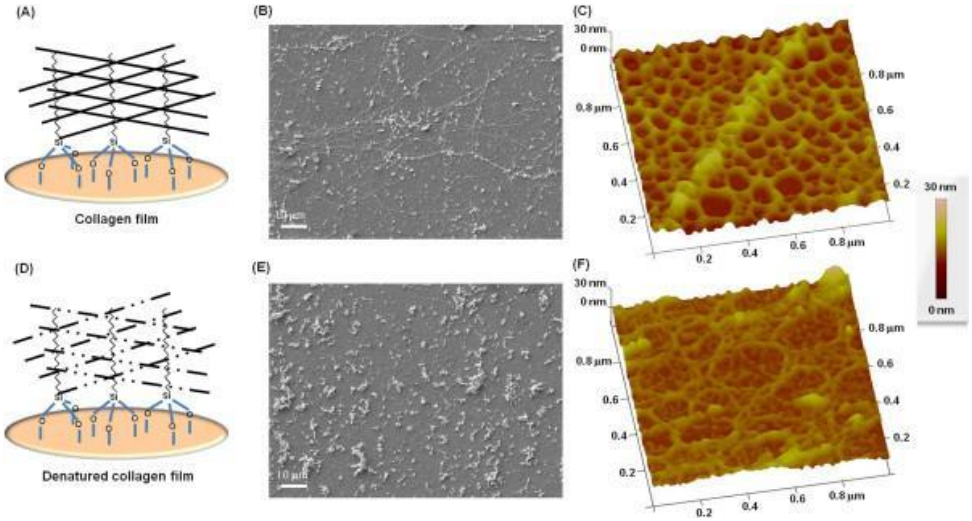


Figure -7: structure of collagen films.

Collagen films are basically sterilized films of collagen having thickness of 0.01-0.5 mm; incorporated with drugs like steroids, antibiotics, hormones (obtained from rDNA technology E.g. human growth hormone, rhBMP-2 etc.) intended for local action. They are simply manufactured by air- drying of casted collagen mainly used as a barrier membrane, the drug incorporation in collagen films done by covalent bonding, hydrogen bonding and by simple entrapment.

COLLAGEN SPONGES

Collagen sponges were found suitable for short term delivery (3–7 days) of antibiotics, such as gentamicin. Gentamicin containing collagen sponges placed on a septic focus in the abdomen reduce local infection. 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. Collagen sponges were also used for delivery of steroids through topical applications, such as intravaginal delivery of lipophilic compounds including retinoic acid. Collagen-based sponge was inserted into a cervical cap made of hydrogel hyphen, which, when in contact with wet tissue surfaces, adheres to them by the force of differential osmotic pressure. 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. These are manufactured from pure bovine collagen obtained from bovine skin, bovine collagen is firstly put into a solution having pH 3.0 and then stabilize into physical form of a sponge layer. And then this sponge layer is combined with fibronectin, elastin or glycosamino glycans to achieve a fluid building capacity and elasticity. They are also manufactured by freeze-drying of alkali or acid, swollen collagen containing 0.1- 5% dry matter content. Collagen sponges can be cross-linked with glutaraldehyde and copolymerised with other synthetic as well as natural polymers, for example collagen sponges copolymerized with PHEMA (polyhydroxyethylmethacrylate) are more hydrophilic in nature, retain wetness for longer period and also possesses more tensile strength. Used for hemostyptics and wound dressings but in present time they are also used for antibiotics, steroids and growth factor delivery for wound healing and for bone forming implants. The application of collagen sponges for delivery of topical agents.[21] These are also useful in dressing for leg ulcers, decubitus ulcer , donor sites, pressure sores. The major benefits of collagen sponges includes their ability to absorb enormous quantity of tissue exudates and smooth adherence to the wet wounds with preservation of micro climate as well as shielding against secondary bacterial

infection and mechanical harm. In addition collagen sponges promote inflammatory cells activity to porous scaffolds and cellular growth. Thus collagen sponges can be considered as active dressings, which aid in the healing process.

MARKETED PREPARATIONS OF COLLAGEN SPONGES

COLLARX®, COLLATAMP® G, COLLATAMP® EG, SULMYCIN® IMPLANT, GARAMYCIN® SCHWAMM, DURACOL®, DURACOLL®, GENTACOL®, GENTACOLL®, GARACOL®, GARACOLL®, and CRONOCOL® - Gentamicin Surgical Implants. One of the marketed collagen sponge named as SALVIN ORA PLUG was shown the following figure – 8.

Figure -8 : Marketed Collagen sponge , SALVIN ORA PLUG.

Sponges for burns/wounds

Collagen due its special properties such as porosity, meshwork and sponge like structure forming capacity, good biocompatibility and surface properties aid in finding its biomedical application. Such type of collagen is used in the treatment of skin disorders such as burns and ulcers. Epidermal growth factor (EGF) loaded in collagen sponge matrix helps in the formation of the dermal matrix and improves the wound mechanical strength by wound contraction and scar tissue development. Succinylated collagen prepared as a bilayer dressing material when loaded with chemotherapeutic agent such as ciprofloxacin is used for the control. Silk-elastin sponge can help promote wound healing for burns after tangential excision. Furthermore, silk elastin sponge containing bFGF showed the best effect on wound healing in our study. This is because silk-elastin enables the gradual release of bFGF. One of the marketed example for collagen sponge used for burns or wounds is " Syner HEAL Sponge" was shown in figure -9.

Figure – 9: Syner HEAL sponge.

COLLAGEN GELS

Collagen gels are widely used in tissue engineering, the nature of their application has changed little in more than a decade. They are visco-elastic; i.e. they are semi-solid when at rest, but can be induced to flow under stress. In addition, they exhibit good cell and tissue compatibility, and thus should not interfere with normal function at the site and systemically. Collagen gels are flowable, suggesting the possibility of an easily injectable, biocompatible drug delivery matrix. Collagen gels are primarily used for injectable systems. The most readily available forms of such injectable collagen gels are.

[a] Injectable suspensions of collagen fibers

[b] Nonfibrillar, viscous solutions in aqueous media., embedded chondrocytes in collagen gels to repair articular cartilage defects(3).

Collagen hydrogels/gels:- Collagen hydrogels/gels are processed by crosslinking of collagen with chemicals like poly epoxy compounds, carbodiimides, polyphenolic compounds, aldehydes, and acyl azide compounds which leads to the formation of bonds between molecules and fibrils. Collagen hydrogels possess a unique property of soaking and swelling on hydration with biological fluids and they are also capable to maintain their integrity after soaking. These hydrogels are very patients compliant because ease of application, high bio-adhesion and compatible with a large varieties of drugs and agents. Collagen gels are excessively used as injectables the most common form are-

1. Non-fibrillar viscous solution in aqueous medium
2. Fibers injectables suspensions For ophthalmic purpose these suspensions can be mixed with drugs and administered, these preparations are patented, when inject; initially remains in liquid state and then after some time convert into gel. Shows a great potential for sustained and controlled delivery of medicaments.

Hydrogels

Hydrogels have been widely used as a drug carrier due to its ease in manufacturing and self application. Hydrogels may be used as carriers to control delivery of the virus and resultant tissue regeneration. Hydrogels consist of a 3D polymer network, which swells in water or aqueous media. The viscoelastic nature, high hydration state, and porous form of these polymers have made them useful scaffolds for in vitro and in vivo cellular support. 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. Collagen and polyhydroxyethyl methacrylate into hydrogels was made to develop a delivery system for anticancer drugs, such as 5 FU. Hybrid copolymers of collagen with polyethylene glycol and polyvinyl pyrrolidone were prepared for the controlled delivery of contraceptive steroids. Implantable collagen hydrogels have been incorporated as agents for delivery of chemotherapeutic agents and new ocular drug delivery systems are being evaluated using collagen inserts as a controlled-release system.

Liposomes

Liposome is a spherical vesicle having at least one lipid bilayer. The liposome can be used as a drug delivery vehicle for administration of nutrients and pharmaceutical drugs, such as lipid nanoparticles in mRNA vaccines, and DNA vaccines. Liposomes can be prepared by disrupting biological membranes. The structure of a liposome was shown in the following figure -10.

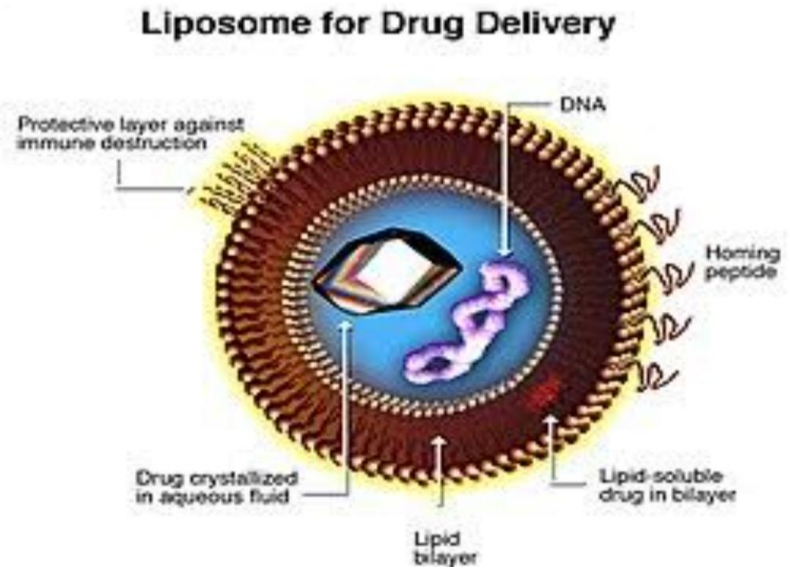


Figure -10: Structure of a liposome.

It is widely used as a drug carrier due to their biodegradability and removable versatility in terms of composition and size. The combination of liposomes and collagen-based technologies has been long achieved since the early 80s. In this case, drugs and other bioactive agents were firstly encapsulated in the liposomes and then embedded inside a depot composed of collagen-based systems, including scaffolds and gels. The combination of these two technologies [i.e., liposomes and collagen-based system] has improved storage stability, prolonged the drug release rate, and increased the therapeutic efficacy. 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. 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. 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 .

Pellets

Minipellets made of collagen have been developed for various candidate compounds. A 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 and interleukin-2. An attempt to produce a pellet type controlled-release delivery vehicle made of purified type I. Collagen for water soluble osteogenic proteins was described. The effect of collagen- based mini-pellet on the mRNA expression and functional status of facial nerve in the rat model was investigated. The controlled gene transfer using collagen 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.

pellets/tablet:- Collagen pellets are extensively used in Japan. These pellets are also known as monolithic devices .They are tiny rods of approximately 1mm in diameter and 15 mm in length manufactured from collagen by cutting, moulding, and drying. These devices are cylindrical in structure, and they can be administered via injection using a syringe with a plugar. Pellets are very suitable for the local delivery of lysozyme and minocycline in the treatment of clinical symptoms of periodontitis. Lucas and his co-workers was designed a collagen pellet type controlled release delivery vehicle which is made up of type-1 collagen for water soluble bone forming proteins(3).

NANOSPHERE

The nanosphere is the simplest type of nanoparticle with only one adjustable geometrical parameter (radius) which exhibits resonant responses under optical excitation. 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. 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.

a) Nanoparticles The molecular weight of collagen or gelatin has a decisive influence on the stability of the manufactured gelatin nanoparticles. The biodegradable collagen based nanoparticles or nanospheres are thermally stable, readily achieving their sterilization. Moreover, nanoparticles can be taken up by the reticuloendothelial system and enable an enhanced uptake of exogenous compounds, such as antiHIV drugs, into a number of cells, especially macrophages, 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 and hydrocortisone. Collagen nanoparticles were used to enhance dermal delivery of retinol .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.

DRUG DELIVERY MATRIX

Collagen in the form of gel acts as a drug delivery matrix due to its characteristic properties such as flowablility, injectablility, and biocompatibility. These essential properties help in achieving sustained release action of therapeutic molecules and to be an attractive biomaterial in tissue engineering application.[22] Usually, the non fibrillar collagen possesses lesser pore size when compared to the fibrillar collagen and this effective pore size plays a major role in tissue engineering studies since the cells has to be retained within the gel after its proliferation and differentiation. Collagen gel with suitable pore size alone can act as an effective biomaterial when compared to the other commercially available form of collagen . Collagen-synthetic polymer composites and collagen-based diffusion membranes are generally used for controlled drug delivery and prolonged drug release treatment. These forms of dosage have shown an improved prophylactic activity when loaded with suitable antibiotics to treat infections of bone and soft tissues.[23]

a. Gene delivery matrix

An efficient and highly localized gene delivery system for both in vitro and in vivo can be achieved by immobilizing plasmid DNA on collagen matrix through covalently coupled anti-DNA antibody. Gene modified bone marrow stromal cells when treated with collagen containing therapeutic protein such as erythropoietin serves as an implant for systemic delivery.[24]

b. Protein delivery matrix

Apatite coated collagen scaffold act as a carrier to deliver therapeutic proteins such as bone morphogenetic protein-2 (BMP-2) and provides a sustained release of it. Thus, the sustained release of BMP-2 provides an enhanced effect in orthopaedics to prove its efficacy in bone formation and fracture healing.[25] A novel collagen microsphere-based protein delivery system was developed by a photochemical cross linking method which aids in safe delivery of protein based products (4).

THE APPLICATIONS OF COLLAGEN AS DRUG DELIVERY SYSTEMS ARE

The recent applications of collagen as a biomaterial and in drug delivery systems.

The main applications covered include:

- collagen for burn/wound cover dressings;
- osteogenic and bone filling materials

- antithrombogenic surfaces and immobilization of therapeutic enzymes.
- , Recently collagen used as a carrier for drug delivery.

Collagen-based drug delivery systems include.
1) injectable microspheres based on gelatin implantable
2)collagen-synthetic polymer hydrogels
3) interpenetrating networks of collagen
4) synthetic polymers collagen membranes for ophthalmic delivery.
5)collagen-liposomal composites for controlled drug delivery, as well as collagen as controlling membranes for transdermal delivery.
6) Due to its biodegradability, biocompatibility, weak antigenicity and well-known safety profile, collagen becomes a very useful carrier for the delivery of various kinds of drugs. Collagen hydrolysates worked as diet supplement.
7) Collagen-based drug delivery systems include injectable microspheres based on gelatin (degraded form of collagen), implantable collagen–synthetic polymer hydro gels, interpenetrating networks of collagen and synthetic polymer collagen membranes for ophthalmic delivery.
8) Resorbable forms of collagen have been used to dress oral wounds, for closure of graft and extraction sites and to promote healing. Collagen-based membranes have also been used in periodontal and implant therapy as barriers to prevent epithelial migration and allow cells with regenerative capacity to repopulate the defect area.[26]

CONCLUSION

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 signify selected applications of collagen in the biomedical field. The effective demonstration of usefulness of human skin substitutes made of collagen has leads to the development of bioengineering tissues, such as blood vessels and ligaments. Although many applications of collagen as a drug vehicle discussed in the paper, it should be noted the information regarding collagen is very less as compare to synthetic polymers in literature because, the pure type-1 collagen is very costly, variability in different forms, complex handling processes, and risk of Bovine spongiform encephalopathy (BSE). Beside them collagen possess some very extraordinary properties which make it a very useful biomaterial for drug delivery includes its biocompatibility, absorbability on biological membranes, no antigenicity, low toxicity, synergism with other bioactive compounds etc. these advantage will carry the future development of this biomaterial.

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