International Journal of Pharmacy Teaching & Practices 2013, Vol.4, Issue 4, 811-820.

A Review on Collagen Based Drug Delivery Systems

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Review Article

Please cite this paper as: Vikash Chak*, Dharmendra Kumar, Sharad Visht. A Review on Collagen Based Drug Delivery Systems. IJPTP, 2013, 4(4), 811-820.

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Abstract

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 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. The main application of collagen is collagen shields which are used in opthalmology. 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, formulations for transdermal drug delivery, and nanospheres for gene delivery, collagen matrices for cell culture.

Keywords: *Collagen; Drug delivery system; Biomaterial; Opthalmology;*

Introduction

In the beginning of 1970s and 1980s the research on collagen was initiated by interested scientists and commercial research laboratories expanding medical applications of biomaterials and connective tissue research. In present years biotechnology has given a support to research the collagen material useful for drug delivery. Basically Collagen is a protein which is widely used in medical field. Collagen plays a significant role in the formation of organs and tissues, and is involved in different functional expressions of cells. Many natural polymers and their synthetic analogues are used as a biomaterial, but the characteristics of collagen as a biomaterial are different from those of synthetic polymers mainly in its mode of interaction inside the body [1].The important features of collagen are its biocompatibility, biodegradability and weak antigenicity **[**Maeda et al., 1999**]**.In the body as compared with other natural polymers like gelatin and albumin. Collagen possesses good abilityto penetrate a lipid-free interface. 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.

1. Collagen:-

Basically collagen is a naturally existing protein present in the animal body, fibrous in nature, and especially found in the connective tissue and flesh of mammals. Approximately 25%-35% of total body protein is comprised of collagen, in the form of elongated fibrils; collagen is abundantly present in fibrous tissue like bone, cartilage, tendons, blood vessels, ligament, skin, cornea, inter-vertebral disc and the gut. The synthesis of collagen in the body is made by fibroblast cells. Collagens possess good tensile strength, and found both outside and inside the body cells. In combination with elastic, collagen provides support to body tissues and organs, basically collagen offers firmness and strength and elastic provides flexibility to body tissues. In fact gelatin which is used in food and pharmaceutical industries is collagen that has been hydrolyzed irreversibly.

2. Structure of collagen:-

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 [20]. The weight of collagen molecule is 300 k da [21, 22] and its structure is rope shaped and having a length of 300 NM and a width of 1.5 NM. The major content of glycine and amino acid residue is affecting the helix formation [22]; in each of three chains of collagen molecule the amino acids are regularly arranged. 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 or hydroxyproline accounting for the 1/6 of the sequence. This whole structure is joined with the help of hydrogen bonds and linking peptide bonds.

3. Characteristics possessed by collagen:-

- Stretch-ability under stress condition collagen stretch rather than break;
- Strength;
- Biochemical compatibility;
- 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 canbe wrecked and re-joined after removal of pressure;
- Collagen is biodegradable;
- Collagen show good absorption in-vivo;
- Collagen possesses weak antigenicity;

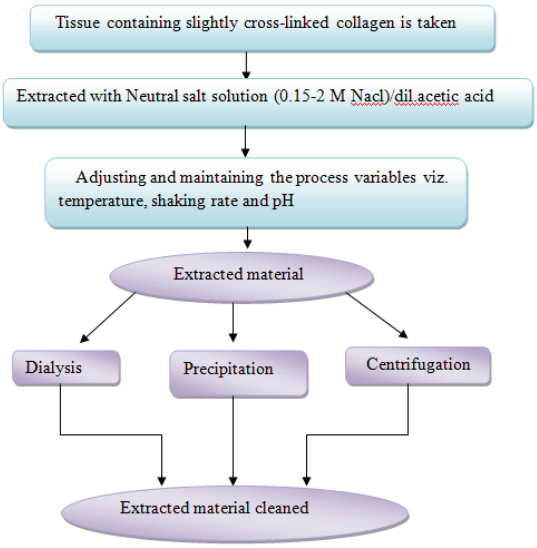
4. Isolation and Purification of Collagen:-

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 [23], 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 [24]. 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. 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.

Basically four types of collagen can be isolated and purified for the implementation in pharmaceutical industries for the delivery of drugs are following (fig. 1) -

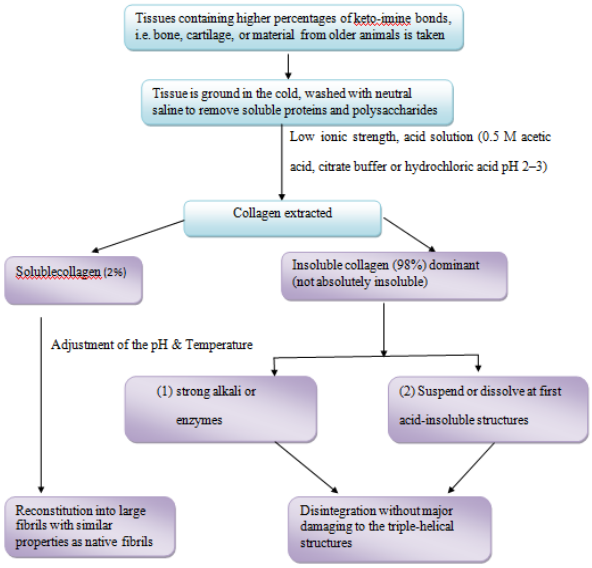
- Natural salt soluble collagen;
- Alkali and enzyme treated collagen;
- acid soluble collagen;
- Insoluble collagen;

4.1. Process to Isolate Neutral Salt Soluble Collagen:-

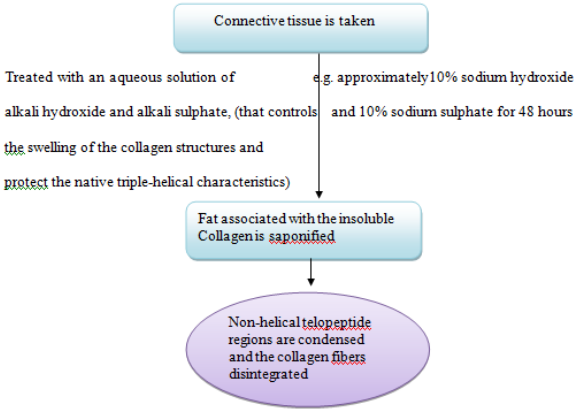


Note- Most tissues have minute or no salt-extractable collagen. In demand to increase the yield for research purposes animals can be put on the diet contain b-aminopropionitrile, an inhibitor of peptidyllysyl oxidase, yet this method is in adequate for larger commercial scale

4.2. Process to Isolate Acid Soluble Collagen:-



4.3. Process to isolate alkali- and enzyme-treated collagen



4.4. Process to Isolate Insoluble Collagen

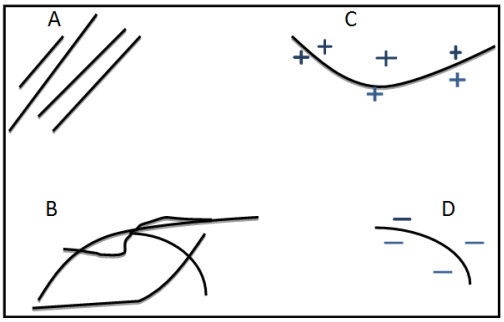


Fig. 5
Collagen types A- Collagen fibril in neutral buffer. B- Collagen molecule in neutral solution. C- methylated collagen in neutral pH D- succinylated collagen in neutral pH

5. Why Collagen can be used as a biomaterial for drug delivery [25]:-

- Collagen is biodegradable and simply absorbed in the body;
- Collagen is a part of the body that's why it is non-antigenic;
- Collagen is a non- toxic biopolymer;
- Collagen shows better biocompatibility;
- Collagen can be framed in a number of different forms;
- Shows synergism with other bioactive compounds;
- Haemostatic in nature and encourage blood coagulation;
- Compatiblewith synthetic polymers;
- By utilizing its functional group collagen can be easily modified to produce desired materials;
- Biodegradability of collagen can be controlled by cross-linking;
- Existing in plenty and simply purified from living organisms (constitutes more than 32% of vertebrate tissues);
- Biological plastic due to great ductile strength and negligible expressibility.

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

- Glutaraldehyde**[3]**,
- Chromium tanning**[4]**,
- Formaldehyde**[5]**,
- Poly epoxycompounds**[6]**,
- Acylazide**[7]**,
- Carbodiimides**[8]**
- Hexamethylenediisocyanate**[9]**.

Physical treatment, such as ultra-violet/gamma-ray irradiation and dehydrothermal treatments have been efficiently used for the introduction of cross links to the collagen matrix **[10]**. 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.

7. The applications of collagen as drug delivery systems are:-

- Used in the formation of microspheres for drug delivery **[11]**;
- In formulation of nanoparticles for gene delivery**[12]**;
- Collagen is used in the manufacturing of collagen Sponges for burns/wounds;
- Development of tablets and pellets for the delivery of proteins;
- Collagen is used for gel formulation and combined with liposomes for sustained delivery of drugs;
- In the treatment of cancer collagen is used as aqueous injection **[13]**;
- Collagen is used in ophthalmology as collagen shields **[14]**;
- Collagen is used as controlling material for the delivery of drugs in transdermal patches **[15]**;
- As films for the delivery of human growth hormone, immune-stimulants, tetracycline, growth factors **[16]**;
- For the delivery of glucocorticosteroids, microparticles of collagen are used.

Collagen delivery systems having a smooth release control can be attained by balancing the configuration of the collagen matrix or attaching other proteins, such as Austin, fibronectin**[17, 18]**or by fusion of collagen with other polymers, such as collagen/liposome and collagen/silicone **[19]**.

8. Some specific type of drug delivery systems constructed on Collagen base:-

8.1. Nanoparticles/Nanospheres/Microspheres:-

In the collagen fold configuration, the crystallites suspended in the gel aggregates seem as a multiple chain system; this property is used to formulate aggregates as colloidal drug delivery carriers **[25]**. 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**[26]**.The stability of the produced collagen nanoparticles is depending on the molecular weight of collagen **[27]**, and temperature and pH is greatly affecting the molecular weight profile of the collagen solution , and these are also further affect the non-covalent interactions liable for the molecular structure of collagen**[28]**. The nanoparticles and nanospheres based on biodegradable collagen; are enabled and enhanced uptake of exogenous compounds such as anti-HIV in a number of cells, especially macrophages **[29]**, that is an advantage of collagen based nanoparticles as a systemic delivery carriers; and they are also thermally stable and easily sterilized **[30]**. Some other drugs like steroids **[31]**, cytotoxic drugs like Campthocin**[32]** 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 **[33]** because of their: –

- 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. Retinol in collagen nanoparticle was stable shows a quicker transportation of incorporated drug through the skin **[34]**.

8.1.1. 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

  And some additional methods are, Jet milling technique, fluidization and solvent precipitation method, Interfacial polymerization etc.

Processes involved: -

1) Emulsification (fig. 2):-

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 (120) drop by drop resulting formation of collagen nanoparticles.

This method was developed by F Scheffel and coworkers (1972) in direction to prepare albumin sphere nanoparticles and then it was improved by Gao and his Coworkers (1995)

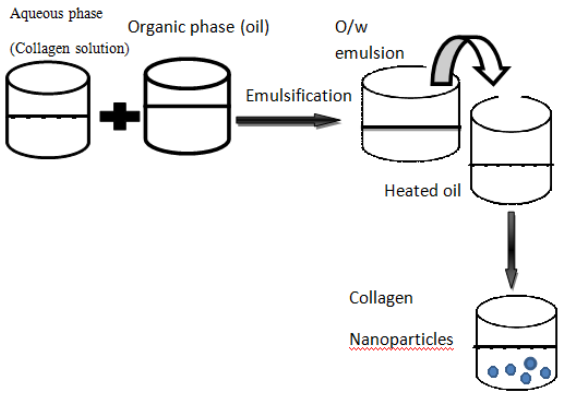


Fig.2. Emulsification method for nanoparticles manufacturing

2) Desolvation (fig.3): -

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 co-workers.

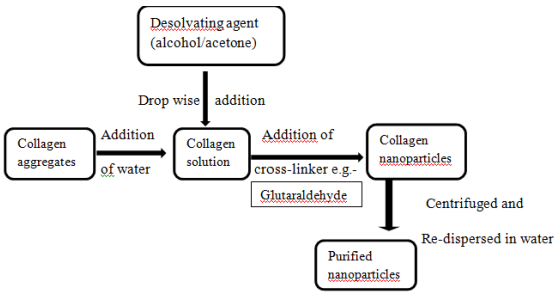


Fig3. desolvation method for nanoparticles manufacturing

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.

4) Spray drying:-

Basically spherical collagen nanoparticle 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 **[35]**. So collagen solution is sprayed into liquid nitrogen to prevent denaturation **[36]**. After that the fabricated nanospheres are successively frozen, tempered, lyophilized, cross-linked, and sterilized.

8.2. Collagen based 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 **[37]**. These pellet are also proven to be effective in vivo for the delivery of interleukin-2 (half-life =360 min for subcutaneously injected IL-2 mini-pellets as compare to, 15 min and 8 min respectively for subcutaneous and intravenous injections of an aqueous preparation) **[38]**. It is also proved that single subcutaneous injection of a mini-pellet cause a prolonged retention of IL-2 and maximal concentration in the serum. The similar result was also observed for interferon **[39, 40]**. In 1989 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.

8.3. 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.

Bradley used collagen films cross-linked by chromium tanning, formaldehyde to sustain the release of medroxyprogesterone acetate. In this investigation cross-linking was done on films already incorporated with drugs, endangering the Pharmacokinetic and pharmacodynamic activity of the drug. This threat can be overcome with collagen materials which are cross-linked with glutaraldehyde prior to protein incorporation **[42]**. Collagen films also can be used as a drug carrier for antibiotics **[43-45]**. E.g. when collagen films containing tetracycline were implanted in rabbits suffering from periodontal disease; tetracycline was remained in plasma for more than seven days and its activity was sustained for more than ten days. After four and seven weeks the clinical symptoms of disease were decreased significantly.

A European patent application was filed which contain the research information regarding sustained release delivery of platelet derived growth factor; was improved by multiple and single layer collagen films and it was found that this specific preparation had got a release factor up to 100 h and thereby it leads to an enhanced wound healing in vivo **[46]**.

In the case of liver cancer or tissue infection collagen film implants are very useful. When collagen film applied to eye it was totally hydrolysed within 5-6 hr **[47]** in the case of corneal tissue infection. Collagen sheets of micro-fibrous collagen was used as local delivery carrier in the treatment of cancer, locally implanted collagen sheets which incorporated with anticancer agents such as methotrexate and ectopocide needs low plasma concentration management **[48]**. The formation of bone tissues was enhanced when a collagen matrix and film were used as a carrier for gene delivery. This shows that collagen based gene delivery system is very efficient as implant.

8.4. Collagen shields:-

Collagen shields 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; after corneal transplantation, radial keratomy **[48, 49, 50, 51]**. 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.

Bausch & Lomb Pharmaceuticals, a division of Bausch & Lomb, Inc. acquired the rights to develop and market these collagen contact lenses, now known as Bio Cora collagen shields. After much research, Bausch & Lomb has been able to produce the shields in a reproducible manner and in a variety of shapes and thickness. Some other marketed preparations are (proshieldO, MediLenso, Fort Worth, Chiron, TX, Irvine). These shields are able to enhance the penetration of corticosteroid, sub-conjunctival antibiotics in eye. They are act as a short term bandage and allow sufficient oxygen transmission for essential metabolism occurring in eye cornea **[52]**.For the corneal surface lubrication these shields dissolve in collagen solution that minimize lids rubbing **[53, 54]**. Mainly water soluble antibiotics and steroids are used in combination with collagen shields for example- Vancomycin**[55]**, Trimethoprim **[56, 57]**, Amphotericin-B**[58, 59]**,Gentamycin **[60, 61]**Polymyxin-B sulfate**[62]**, Tobramycin **[63-68]**, steroids, pilocarpine**[67]**, Application of collagen shields on cornea is demonstrated in figure-4.

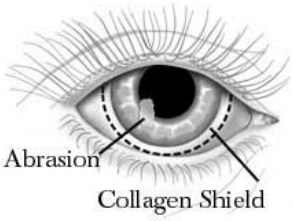


Figure.4- application of collagen shield on cornea

8.4.1. Marketed preparation of collagen shields:-

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

8.5. Collagen sponge:-

Collagen sponge 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 glycosaminoglycans 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.

Collagen sponges were basically developed as 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 (table 1)

Table .1 The application of collagen sponges for various topical agents

Drugs/ agents	Uses	References
Gentamycin	In septic focus in abdomen	WacholDrewek et al.1996
Growth factor (rhBMP-2)	In bone formation and wound healing	Stemberger et al., 1997.Kimura et al., 2000.Cochran et al., 2000.

Collagen sponges are found very useful in dressing for leg ulcers, decubitus ulcer **[69]**, 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 **[70, 71]**, in addition collagen sponges promote inflammatory cells activity to porous scaffolds and cellular growth **[72, 73]**. Thus collagen sponges can be considered asactive 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

8.6. Collagen hydrogels/gels:-

Collagen hydrogels/gels are processed by cross-linking 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 **[74]**.Collagen gels are excessively used as injectables the most common form are-

1. Non-fibrillar viscous solution in aqueous medium**[75]**
2. Fibersinjectables 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 **[76]**.Shows a great potential for sustained and controlled delivery of medicaments.

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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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.