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COLLAGEN AS A POTENTIAL BIOMATERIAL IN BIOMEDICAL APPLICATIONS

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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 isolated collagen from biological wastes is found to be cost effective due to the adaptation of simple methods for its isolation when compared with other commercially available biological macromolecules. 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.

1. INTRODUCTION

Collagen is a type of biological macromolecule which forms a highly organized, three-dimensional architecture and can carry any component due to its network-like structural nature. Collagen is used as a biomaterial because of its wide applicability in various fields. Its versatile role is due to its immense properties such as biodegradability, biocompatibility and easy availability [1-3]. The molecular weight of an acid soluble collagen is found to be approximately of 300 kDa and this is distributed in the electrophoretic pattern as two α chains and a β chain. The pattern arrangement also details that the α chains are of two types such as α1 and α2 which posses a molecular weight below 200 kDa and a dimer β chain with molecular weight above 200 kDa [4,5]. Different types and forms of collagen which are commercially available are distributed in various tissues such as bone, skin, tendons, ligaments, cartilage, blood vessels, vitreous humour in the eye and intervertebrate disc. The complex scenario of collagen characteristics, types, fibril arrangement and collagen structure-related functions are also addressed in detail in this article so that the structural information can be understood clearly which may aid to functionalize the material for biomedical purposes [3,6]. The survey of fundamental property of collagen forms a basis to understand the biomedical role of collagen in fields such as ophthalmology, tissue engineering, wound healing and as delivery vehicle [7].

Ophthalmic preparations are used to treat eye disorders locally. The drug delivery system such as soluble ocular drug insert (SODI), ocusert, collagen shields, ocufit, minidisc and new ophthalmic deliv-

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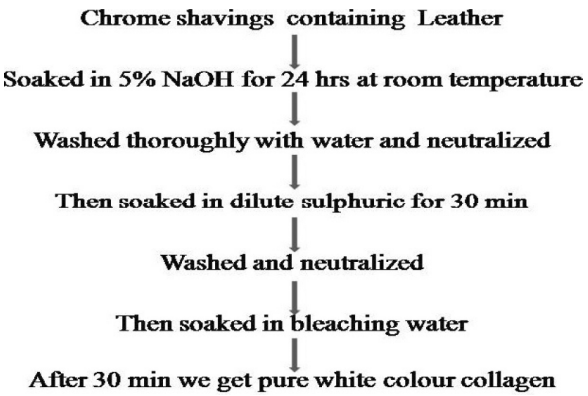


Scheme 1. Isolation of collagen from chromium containing leather waste.

ery system (NODS) provides an improved treatment when compared with that of conventional therapy. These ophthalmic preparations are prepared using polymers such as collagen which plays a vital role in drug delivery applications [8]. The controlled release and targeted drug delivery in ophthalmics can be achieved by using formulations such as niosomes and liposomes. The niosomes are drug delivery vesicles prepared using detergents such as Tween 60, Tween 80 or Brij 35 and helps in loading water soluble antibiotics such as Gentamicin. Liposomes are formulations containing phosphatidylcholine and other constituents such as cholesterol and lipid-conjugated hydrophilic polymers which provide an improved applicability in delivering topical agents due to its enhanced bio adhesiveness and penetrability properties [9,10].

Tissue engineering is one of the new and emerging technique which helps in the development of new tissues as well as organs. Usually cells undergo proliferation, differentiation and attachment on the scaffolds for their development. In order to achieve better results, collagen based scaffolds with good pore size; porosity and pore distribution are prepared to overcome hindrances of the conventional biomaterials [11]. Sustained release of therapeutic substances is one of the important aspects of drug delivery system and this aspect in tissue engineering can be achieved by formulating collagen based biomaterials using solid freeform fabrication techniques [12].

Wound healing is the process of repairing the damaged soft tissues of the body to a normal state by shrinking the wound surface. Such skin disorders can be treated using collagen based membranous materials available in different shapes and forms of formulations such as gel, film and sponge. Collagen sponges containing live cells such as keratinocyte and fibroblast are capable of proliferating cytokines and growth factors. These biological molecules are capable of providing skin regeneration and wound healing activity in burn patients [13-15]. By considering the specific characteristics and wide applicability of collagen isolated from various biological waste materials, we have discussed its role in selected biomedical applications.

2. ISOLATION OF COLLAGEN FROM VARIOUS SOURCES FOR ITS POTENTIAL BIO MEDICAL APPLICATION.

Collagen can be isolated from various wastes products such as bovine limed split wastes [16], fish skin, bone and fin [17], the outer skin of cuttlefish [18], skins of Baltic cod [19], chicken skin [20], skins of young and adult Nile perch [21], skin and bone of bigeye snapper[22], solid wastes of leather industry [23], skin of deep-sea redfish [24], skin of threadfin bream [25], short tendons of slaughtered cattle [4], mangrove archeaogastropod [26], swim bladder of catfish [5], natural marine source jellyfish species etc. [27]. The isolated collagen from

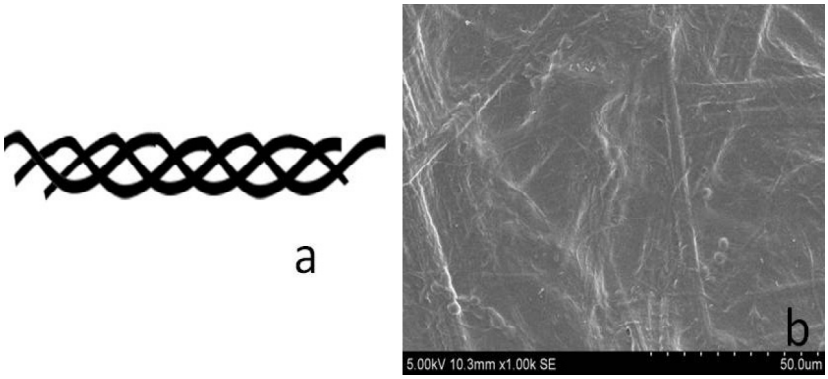


Fig. 1. (a) Schematic diagram of triple helical structure of collagen and (b) SEM image of isolated collagen from CCLW.

various sources seeks its useful application in various fields such as biomaterials, functional additives, cosmetics and pharmaceutical industries [25]. Collagen can also be isolated from chromium containing leather wastes (CCLW) which may be useful for biomedical applications. The schematic layout for the isolation of collagen from CCLW is given below (Scheme 1)

Collagen usually exhibits a triple helical strand (Fig. 1a) which is rich in aminoacids such as glycine and proline/hydroxyproline. The fibrous nature of the isolated collagen can be understood by visualising the surface morphological structure using scanning electron microscopic images (Fig. 1b) [28].

2. DIFFERENT TYPES OF COLLAGENS AND ITS APPLICATION

Collagen based biomaterials are widely used as scaffolds for bone formation, tissue substitutes, gene delivery and as biomarkers in osteoarthritis. Various types of collagen, their tissue distribution [3,29-34] and functions are given below in Table 1.

2.1. Applications of collagen

Collagen, a versatile biomaterial finds its application in various fields and has its wide applicability in

Table 1. Different types of collagens and its application.

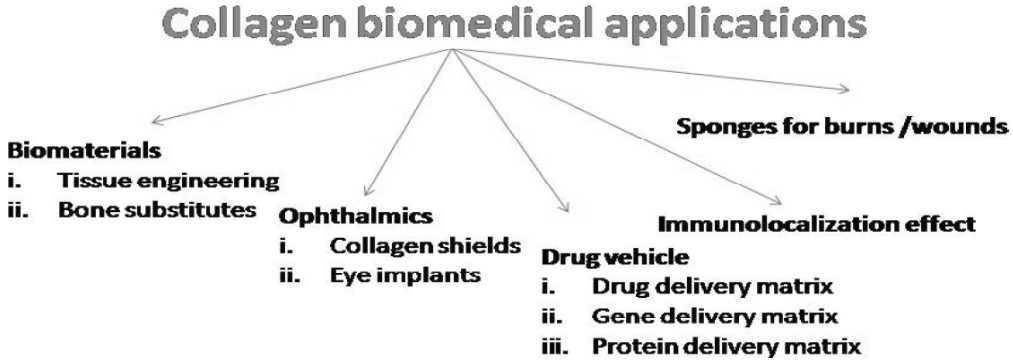
S.No	Types	Collagen type	Tissue Distribution	Functions
1	Fibril-forming collagens	I	Skin, tendon, bone, cornea, dentin, fibrocartilage, large vessels, intestine, uterus, dermis and tendon	Tissue repair and replacement [35]
2		II	Hyaline cartilage, vitreous, nucleus pulposus, notochord and intervertebrate disc	Provides biomarkers for osteoarthritis [34]
3		III	Large vessels, uterine wall, dermis, intestine, heart valve and gingiva	Fibrillogenesis of collagen I and for normal cardiovascular development [36]
4	Basement membrane collagens	IV	Basement membranes	Supports matrix organization, platelet adhesion and aggregate formation [37]
5	Fibril-forming collagens	V	Cornea, placental membranes, bone, large vessels, hyaline cartilage and gingival	Regulates collagen fibrillogenesis and matrix assembly [38]
6	Microfibrillar collagen	VI	Descemet's membrane, skin, nucleus pulposus and heart muscle	Platelet adhesion and aggregation in vascular enthothelial regions [39]
7	Anchoring fibrils	VII	Skin, placenta, lung, cartilage and cornea	Anchoring fibrils at the dermal-epidermal junction [40]
8	Hexagonal network-forming collagens	VIII	Endothelial cells and descemet's membrane	Facilitate movement of endothelial cells in angiogenesis, smooth muscle cells in intimal invasion and myofibroblasts in fibrotic conditions [41]
9	FACIT collagens	IX	Cartilage	Regulates fibre diameter in articular cartilage [42]
10	Hexagonal network-forming collagens	X	Hypertrophic cartilage	Helps in mineralization of cartilage [43]

11	Fibril-forming collagens	XI	Cartilage, intervertebral disc and vitreous humour	Involved in organization of the pericellular matrix [44]
12	FACIT collagens	XII	Chicken embryo tendon and bovine periodontal ligament	Regulates osteoblast differentiation and bone matrix formation [45]
13	Transmembrane collagens	XIII	Foetal skin, bone and intestinal mucosa	Promotes cellular adhesion [46]
14	FACIT collagens	XIV	Dermis, tendon, vessel wall, placenta, lungs and liver	Mediate a controlled lineargrowth phase during tendon fibrillogenesis [47]
15	Multiplexins	XV	Fibroblasts, smooth muscle cells, kidney and pancreas	Stabilize skeletal muscle cells and micro vessels [48]
16		XVI	Fibroblasts, amnion and keratinocytes	Contribute structural integrity to various tissues [49]
17	Transmembrane collagens	XVII	Dermal-epidermal junctions	Epithelial-basement membrane interactions [50]
18	Multiplexins	XVIII	Lungs and liver	Anchoring vitreal collagen fibrils to the inner limiting membrane [51]
19	FACIT collagens	XIX	Human rhabdomyosarcoma	Formation of specialized basement membrane zones [52]
20		XX	Corneal epithelium, embryonic skin, sternal cartilage and tendon	Links matrix molecules to the surface of collagen [53]
21		XXI	Blood vessel wall	Links matrix molecules to the surface of collagen [53]

medical field which is being revealed in detail in this review. Since it exists in various forms it plays a vital role in biomedical field. Various formulations and their respective applications are detailed as follows:

i. Shields in ophthalmology,
ii. Sponges for burns and wounds,
iii. Mini-pellets for protein delivery and drug delivery,
iv. Controlling material for transdermal delivery,
v. Nanoparticles for gene delivery,
vi. Used in the form of film, sheet and disc for collagen based drug delivery formulations for tissue infections,
vii. 3D scaffold for cell culture based biological studies such as evaluation of tissue calcification and for embedding of single cell suspension for tumorogenic study,
vii. An effective biomaterial which forms organoids or neo-organs in gene therapy.

Apart from this, it also finds its application in tissue engineering including skin replacement, bone substitutes, and as artificial blood vessels and valves[7,52-54]. The major biomedical field applications are shown as a schematic layout (Scheme 2) and their detailed descriptions are covered in the review as follows.



Scheme 2. Schematic presentation of collagen for biomedical applications.

2.2. Tissue engineering application

A wide variety of substances such as metals (titanium), ceramics (alumina), synthetic polymers (polyurethanes, silicones, polyglycolic acid (PGA), polylactic acid (PLA), copolymers of lactic and glycolic acids (PLGA), polyanhydrides (polyorthoesters) and natural polymers (chitosan, glycosaminoglycans, collagen) are used in the production of biomaterials for biomedical applications. Among these substances collagen-based biomaterials find their potential application in tissue engineering because it is distinct from other synthetic polymers [55]. Generally biomaterials such as collagen enhance repair of tissues such as bone, tendon, ligament, skin, vascular and connective tissues. Due to the coherency with the biological property with that of native collagen that already exists in our body system it may also function as cell scaffold for tissue engineering applications. This biocompatibility with the native collagen has provoked its potential role in tissue engineering and in the design of biomaterials which can regulate and define most of the tissues which depend on the collagen based biomaterials [2]. The applicability of it in tissue engineering applications has an intensive growth for the past new decades [3]. The fabrication of dense and porous collagen membranes for wound dressing and tissue engineering were carried out using techniques such as air-drying and freeze-drying [14]. The use of collagen and fibrin in hydrogel scaffolds plays an important role in promoting cell differentiation on adipose derived stem cells (ASC's) [56].

2.3. Synthesis of biomimetic hydroxyapatite (HA) nanoparticles

The problems associated with the synthesis of biomimetic monodisperse particles with uniform morphology and narrow size distribution for HA synthesis prevails as a challenge for the scientists working on materials and this may be overcome using nanoparticle synthesis [57]. Collagen composite scaffolds formed by 70 wt.% HA particles dispersed in it provide a chemical environment characteristic of bone and are intended for tissue engineering applications [11]. Though bio mimetic HA is prepared using different technologies the synthetic HA prepared using direct nucleation technique mimicked the natural bone [58]. Collagen-fibroin/ HA composite prepared using a combination of Type I collagen and silk fibroin were used to fabricate bio mimetic bone substitute materials [59]. Certain studies have proved the safety and potential clinical benefit of Type I collagen used to prepare HA nanoparticles apart from its biomimetic property. Such biomaterial prepared using the Type I collagen as a scaffold is used to regenerate cartilage and subchondral bone [60]. Though collagen based porous HA biomaterial is widely used as a scaffold for bone tissue engineering its use is limited due to its high cost and low initial strength. This problem can be overcome by using porous 3D collagen and incorporating nanosized HA particles [61].

2.4. As bone substitutes

HA powder coated with collagen is used as an injectable bone substitute [62]. Platelet-derived growth factor-BB when incorporated into an organic bovine bone-collagen matrix stimulates osteoblastic cell proliferation and also acts as a mineral-collagen bone substitute (MCBS) [63]. MCBS when combined with recombinant human platelet-derived growth factor-BB also finds its application in soft tissue healing such as buccal wall extraction defects [64]. SiO_2-based calcium phosphate - collagen is used as a biocompatible biomaterial bone substitute granules to assist rapid vascularization and to promote cell growth [65]. Collagen and HA composites incorporated with growth factors helps in repairing hard tissues such as bones [66]. Bone composite materials containing collagen/chitosan microgranules were prepared and found to have high bone forming efficacy. This property may be due to the interconnected pores formed between the microgranules which inturn allowed new bone ingrowth and vascularisation [67].

2.5. Ophthalmic applications

The newer ophthalmic forms such as polymeric gels, colloidal systems, cyclodextrins and collagen shields provide an improved ocular drug bioavailability when compared to the conventional dosage. This increases the drug availability, retentivity, sustainability, penetrability and solubility at the site of action [68, 69]. Apart from this, the advanced formulation differs from conventional dosage in many aspects such as applicability, acceptability and utility which can be easily understood [70]. So, the recent developments in ophthalmic research will pay much attention to develop a non-invasive sustained drug release and as a promising approach with an improved ophthalmic therapy for the treatment of vision-threatening disorders [71]. An improved ophthalmic method of treatment remains as a good platform for the treatment of eye disorders

due to the improvement in property of drug delivery systems [72].

3.6. Ophthalmic formulations

Ophthalmic formulations can be broadly categorised into two major forms which include ophthalmic drug inserts and advanced drug delivery formulations. The ophthalmic drug inserts includes formulations such as soluble ocular drug insert (SODI), bioadhesive ophthalmic drug inserts (BODI), ocusert or ocular films, eye implants, insitu gel, collagen shields and ocufit. The advanced drug delivery formulations broadly include formulations such as nanosuspension, nanoparticles, liposomes, niosomes, iontophorosis, minidisc and new ophthalmic delivery system (NODS). Both types of formulations show a high potential application when compared to the previously existing dosage forms. Among these formulations collagen finds its application mainly in preparation of collagen shields for ophthalmic application and in other formulations it is being used as an additional substance due to its biocompatibility and easy availability [8,73].

2.7.Collagen shields

Collagen shields prepared using scleral collagen obtained from porcine or bovine possess biodegradability and cross-linking property which improves the dissolving capacity of shield when placed under the eye for a maximium period of 72 hrs. Apart from this, it extends its biomedical utility as drug delivery vehicle and as a protective and lubricating agent. Collagen shields when placed under the eye accelerate epithelialisation of the ophthalmic membrane and this property makes them particularly to be useful in ophthalmic surgical procedures. It also serves as a valuable therapeutic tool since it is useful to deliver medications such as antiviral, antifungal, and immunosuppressive agents [74,75]. Keratomycosis is a type of fungal infection which is developed after cataract surgery and this can be prevented at its early stage of infection by using collagen shields which has been already soaked in an antifungal agent such as amphotericin before application in the eye for the post operative infections [76]. Scleral collagen obtained from porcine or bovine is also available as a corneal bandage lens for the application as protecting agent in surgery and in traumatic and non-traumatic corneal conditions. It acts as an efficient preparation for eye infections when it is pre-soaked in a pharmacological agent. Pre-soaked collagen shields used for drug delivery applications are also available with varying dissolution times of 12, 24, and 72 hours [77]. Similarly, pre-soaked collagen shields prepared with water-soluble antibiotic or in steroid solution are used for delivering and sustaining higher levels of ocular activity in the targeted site to produce a higher therapeutic activity [78]. Recently, a study has proved that a major reduction in corneal ulcer is being obtained when a collagen shield is placed bilaterally in the cornea of a pet rabbit [79].

2.8. Eye implants

Eye implant is a form of sub retinal drug delivery system that has been developed in order to overcome the limitations of current treatments for retinal diseases [80]. The eye implant also plays a vital role in treating diseases such as glaucoma and other vitroretinal disorders. Apart from this, the implants are synthesized based on the electronic principle. One of such implant synthesized is microelectromechnical implant which is used to stimulate the retinal structures so that the lost in vision may be restored with the stimulatory function of retinal membranes. Similarly a chronic microelectronic retinal prosthesis is synthesized and implanted permanently in the eye of the blind in connection with electronic components and digital camera in order to detect the light, movement and recognition of shapes [81-85]. Generally biocompatible and biodegradable eye implants are preferred for the treatment of ophthalmic disorders. Such type of collagen based implant preparation has shown considerable applicability because; it provides stable and reasonable control over the post-operative complications such as intraocular pressure [86, 87]. Preparation of collagen based matrices finds their use as corneal transplant and as temporary patches to repair perforations in case of emergency situations [88] .

2.9. Controlled ocular drug delivery systems

Controlled and sustained release of drugs has shown its importance over conventional ophthalmic preparations due to its reduced side effects, sustained and controlled drug delivery, increased ocular bioavailability and targeted drug delivery. This improved and controlled way of drug delivery effects provides an efficient therapeutic activity and better sustainability of drug within the body when compared to the other forms of dosages since the frequency of drug administration is reduced when a controlled drug delivery is maintained [69].

2.10. Delivery vehicle

2.10.1. 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. 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 [12]. 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 [89].

2.10.2. 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 [90]. Gene modified bone marrow stromal cells when treated with collagen containing therapeutic protein such as erythropoietin serves as an implant for systemic delivery. This study has been experimentally proved *in vivo* using C57Bl/6 mice which impart an idea that any plasma-soluble therapeutic protein can be easily delivered using collagen as drug and gene delivery matrix [91]. An effective way of obtaining sustained gene delivery with preparations such as lipo-polyplexes containing collagen sponges provides good stability and biocompatibility for both *in vitro* and *in vivo* gene delivery when compared to the naked DNA-loaded sponges and vectors which are widely used [92]. Collagen - glycosaminoglycan scaffold incorporated with growth factors such as insulin-like growth factor-1 provides an effective non-viral mode of gene delivery along with sustained and enhanced cartilage regeneration [30]. Thus an improved mode of gene delivery can achieve by replacing the conventional system using the advanced methods which has emerged recently.

2.10.3. 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 [93]. Collagen-binding growth factor consisting of epidermal growth factor and the fibronectin collagen-binding domain are biologically active fusion protein which binds stably to collagen materials, and exert its growth factor activity even after collagen binding. Injection of such a fusion protein into the hind limbs revealed that the delivery system was effective for direct administration to muscular tissue [94]. 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 [95].

2.11. Immuno localization effect

The cell adhesion assay, mediated through membrane receptors was carried out using collagen isolated from jelly fish. The molecular determinant present in the isolated collagen was involved in human cell adhesion through integrins and heparin-sulfate receptors existing on the surface of the human cells. This cell adhesion property was recently highlighted using plating technique. The *in vitro* study results suggested that after *in vivo* implantation, the jelly fish collagen may promote cell adhesion, proliferation or migration of the human cells. The biocompatibility and cell adhesion assay results suggest that the collagen isolated from the marine source may act as an ideal substitute for bovine or human collagen in particular biomedical applications [27].

2.12. 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 [96-98]. 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 [99]. Succinylated collagen prepared as a bilayer dressing material when loaded with chemotherapeutic agent such as ciprofloxacin is used for the control-

Table 2. Various phases and cellular events that occur during wound healing.

S.No	Type of phase	Phase	Cellular processing events	End product formation
1.	Inflammatory phase (few hours)	Hemostasis	Vascular constriction, platelet aggregation, degranulation, and fibrin formation	Clot formation
2.	Inflammatory phase (0~2 days)	Inflammation	Neutrophil infiltration, monocyte infiltration and differentiation to macrophage, lymphocyte infiltration	Brings inflammatory cells to the injured area
3.	Proliferative phase (2 days ~3 weeks)	Cell proliferation	Re-epithelialization, angiogenesis, collagen synthesis, extra cellular matrix formation	Re-growth of epithelial tissue and matrix formation over the wound surface
4.	Maturation phase (3 weeks ~ 2 years)	Remodeling	Collagen remodelling, vascular maturation and regression	Collagen deposit and contraction of wound

led release of drugs to attain sustained release activity [100]. Collagen sponge when loaded with therapeutic substances such as cytokines and growth factors helps in proliferation of keratinocyte and fibroblast donor cells formation [15].

Wound healing, a multistage biological process occurs within the injured soft tissues of the body. The mechanism of wound healing mainly includes phases such as inflammatory, proliferative and maturation. The sequential phases and their cellular events are given below (Table 2).

The mechanism of wound healing using a collagen sheet can be easily understood with diagrammatic representation given below (Fig. 2).The rate of wound healing process depends on factors such as oxygenation, age, gender, secretion of sex hormones, stress, obesity, nutrition, alcoholism and smoking, medications, immunocompromised conditions and in certain diseased states. Thus, collagen sheet can be used for treating the skin disorders [101-104].

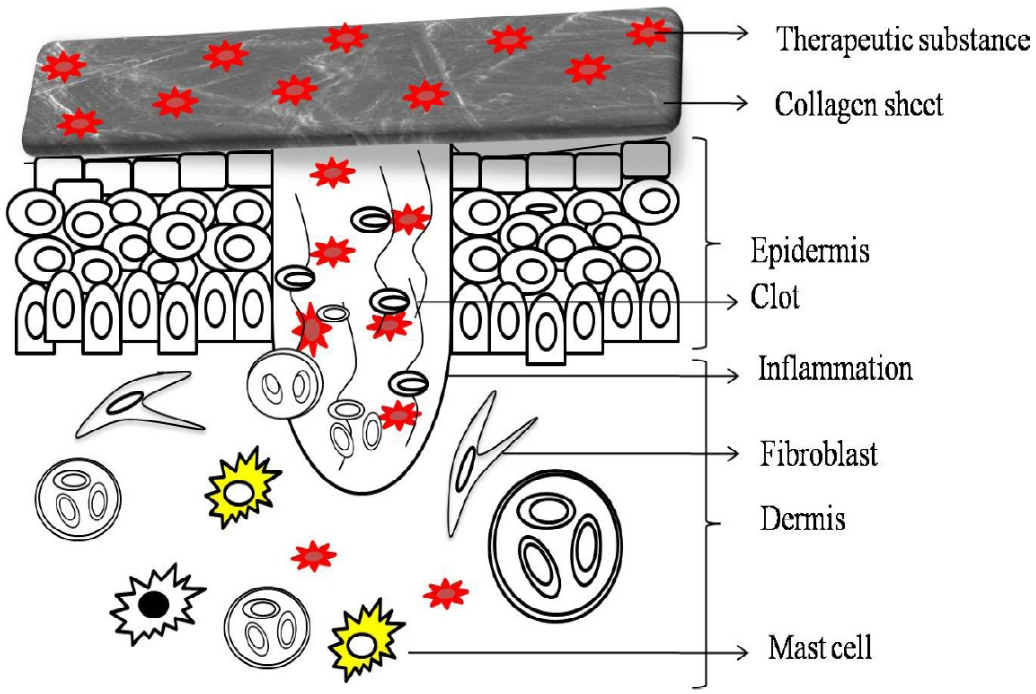


Fig. 2. Schematic diagram depicting the release of therapeutic substance from a collagen sheet to aid wound healing.

3. CONCLUSION

In conclusion, collagen, a biological macromolecule can be isolated using simple methods from the biological connective tissue wastes. The isolated collagen from different sources finds its versatile role in pharmaceutical and biomedical applications. Collagen finds its potential applications in fields such as ophthalmic, orthopaedics, burn/wound management and tissue engineering applications. Apart from this it plays a major role in delivering therapeutic substances such as drugs, proteins and genes at the targeted site. Thus, this review highlights the benefits of collagen in various biomedical fields and its isolation from bio wastes which helps in reducing the environmental pollution.

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