

Obtaining of Collagen Biomaterials and Their Use in the Medical Field

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Abstract

Collagen is an important biomaterial in medical applications due to its special characteristics such as biodegradability and weak antigenicity. Thus collagen, as a new type of biomaterial, has been used in drug delivery systems and tissue engineering. In addition, collagen is closely linked to several diseases, such as rheumatoid arthritis and systemic sclerosis. Gelatine can be made into roll film and drug capsules and can also be used as a delivery system which is very different from the traditional capsule. Collagen hydrolysate is a polypeptide composite obtained through further hydrolysis of denatured collagen. It has been widely used in cosmetics as well as a food additive. The three major methods of collagen extraction produce neutral salt-solubilised collagen, acid-solubilised and pepsin-solubilised collagen.

Keywords: Collagen, Skin, Collagen Hydrolysate, Biomaterial

Introduction

Collagen-based biomaterial can be represented by a wide variety of molecular structures (micro- and nanostructures), such as powders, hydro-gels and injectable compounds, films, membranes, and matrices. Through non-denatured collagen extraction technology, gels containing 70-80% non-denatured collagen molecules are obtained, while gelatine and hydrolysates are obtained through denaturation extraction technology, the extracts containing approximately 90% collagen molecules represented by polypeptide chains. [1].

In order to obtain biomaterials, all types of collagen extracts can be used: collagen fibrous paste, gels or solutions, gelatine or total and partial hydrolysates.

Hydrogels obtained through conditioning of fibrous paste and collagen solutions/gels. They can also be obtained through drying or lyophilisation, methods which do not allow for collagen to be heated above 300°C, which might lead to denaturation of the triple-helical conformation. Thus, finished biomaterials can be obtained, as follows:

- Hydrogels used for the controlled release of various encompassed compounds;
- Membranes used for dialysis, enzyme or protease immobilization;

- Drug storage;
- Sponges (matrices) used as bandages for burns, varicose ulcers, haemostatics, tissue replacements, implants, support systems, and so on;
- Fibbers used to reinforce matrices and membranes;
- Haemostatic fibrous compounds'
- Surgical suture wires;
- Ointments/cremes.

All of the above mentioned collagen products can also initially conditioned as pastes, gels, liquid hydrolysates, with the use of chemical food or pharmacologic preservatives or through sterile bottling in the case of hydrolysates. Collagen hydrolysates, obtained at high temperatures, which have a tight ball conformation (spherical) compared to the loose ball conformation characteristic of gelatine, can also be dried through atomization. During atomization, the maximum temperature finely dispersed particles is 40°C, which does not influence the structural conformation of hydrolysates.

Collagen hydrolysates can also be used as ointments/cremes, in the pharmaceutical and cosmetic fields.

Gelatine can be processed through various techniques as membranes, matrices, hydrogels, capsules, microspheres, and granules.

Establishing conditioning and sterilization techniques for materials and finished products has represented an important issue, due to the fact that collagen products are an auspicious environment for microorganism growth and the use of finished products in medicine requires the need to ensure product sterility. Sterilization of dry products (membranes, wires, sponges and pulvis hydrolysates) is performed with the use of gamma radiation and ethylene oxide, efficient methods which are used on an industrial scale. The temporal stability of products dried through lyophilisation and atomization and stored in adequate conditions is virtually unlimited.

Obtaining Collagen Biomaterial

Collagen extracts are watery systems; consequently, any ulterior processing requires maintaining water solubility, especially in the case of non-denaturated collagen extracts used as biomaterials.

The technology used to dry is selected according to the nature of the extract (denaturated or non-denaturated) and the morphological structure of the bioproduct, that is, sponge, fibres or membranes. The most commonly used drying techniques are lyophilisation and natural air drying at temperatures of approximately 25°C. Neither of the two techniques leads to denaturation of the extracts.

Lyophilisation is a drying procedure relying on the rapid freeze of collagen solutions from – 25°C to – 70°C and sublimation of the ice directly in the water vapour phase (2 x 10⁻³ torri). In place of the ice crystals, pores are formed and collagen molecules are restructured into fibres and fibrils. Lyophilized collagen is represented by a sponge called matrix, with similar characteristics to the extracellular matrix.

Natural air drying of collagen extracts is performed in shelved dryers with warm air current at a temperature of 25°C and a rigorous control of humidity, so that drying can be performed slowly in 48-72 hours. Under these circumstances, between the collagen molecules of the extract intermolecular bonds are made, without requiring the intervention of chemical agents.

Atomization drying is used for denaturated collagen extracts, especially for hydrolysates.

Procedures for obtaining gel or colloidal solutions require technological filtration and condition in sterile environments so that the bioproducts are not contaminated with microorganisms.

Regarding the process of obtaining biomaterials from non-denaturated collagen (pastes and gels or collagen solutions) key-steps are represented by: restructuration, chemical alteration, compatibilization with various bioactive compounds and drying or conditioning as a finished product.

In general, through the procedures used, the main aim is to maintain the specific biological properties of collagen, especially in what regards structural conformation and hydrophilia. These characteristics are important so as to ensure a high degree of biocompatibility of bioproducts used as biomaterial.

Collagen pastes and gels can be chemically or physically altered so as to ensure a better thermal or mechanical stability.

Biomaterials used in the tissue industry can be obtained from natural and synthetic polymers and require a certain internal micro-architecture of microporous structures. The processes through which such structures can be obtained are based on conventional [2-7] or unconventional [8] techniques for the computerized manufacturing of the desired solid shape through layer-by-layer formation.

Conventional techniques refer to:

- Matrix formation through washing the solvent that was used for the dissolution of biodegradable synthetic polymers;
- Foams obtained from polymer solutions containing high pressure CO₂, with the size of particles between 100-500µm;
- Tied fibrous masses, obtained through textile technology which allow the manufacturing of non-tissues from polyglycolic and polyacrylic fibres;
- Phase separation in a polymer solution, through solvent sublimation, which leads to the formation of scaffold biomaterials, which incorporate bioactive molecules into their structure;
- Pouring of polymer melts, which have been brought to glassy transition temperature, into matrices; in this manner, scaffold biomaterials with well defined forms, which might also contain fibres that can be used for the manufacturing of composites with specific biologic properties;
- Lyophilisation of polymer solutions or emulsions, a process which leads to the formation, both from natural polymers (collagen, chitin) and synthetic polymers or composites deriving from them.
 - **Unconventional techniques** refer to direct manufacturing techniques from computer-generated models, which allow for the improvement of scaffold biomaterial design through the control of several parameters: pore dimension and distribution, incorporation into an artificial vascular system which increases oxygen and nutrient transport inside the scaffold, thus maintaining cell development in the entirety of its mass in conditions of maximum hydration. These techniques allow for the manufacturing of scaffold biomaterial with the desired external measurements and predefined and reproducible interior morphology which, does not only control the dimension and distribution of pores, but also enables the obtaining of structures that lead to an increase in mass, oxygen, and nutrient transfer inside the biomaterial.

Results and Discussions

The Physical and Chemical Characteristics of Collagen Biomaterials

Collagen Fibres

Fibres are obtained from collagen solutions/gels through restructuring and drying techniques through lyophilisation. In fact, a fibrous wave is obtained (individual and cluster fibres), which can also be called fibrous collagen.

Due to the specific tridimensional structure of the skin, long individual fibres cannot be obtained. Rather, short fibres or fibres organized in bunches of varying width can be obtained (Fig. 1). Through formaldehyde tanning, fibres are better distinguished (Fig. 2).

Using the electronic microscope, subunits of the fibre, more specifically collagen fibrils can be obtained. Collagen fibres precipitated from a 0.2% collagen solution were studied under the TEM electronic microscope with double contrasting with phosphowolframic acid and uranyl acetate. Collagen fibrils are long, dense, and relatively even in regards to width and they form knot-like clusters. Also, it can be observed on certain portions of the photo, that there is a tendency for fibres to wrap

in a helicoidal manner. Fibres are elastic, which allows them to curve under different shapes, the most frequent one being represented by the semicircle shape.



Fig. 1 Non-tanned collagen fibre
(size 40x) [1]

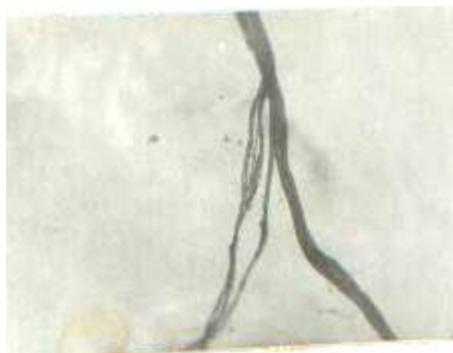


Fig. 2 Collagen fibres tanned with formaldehyde
(size 40x) [1]

Collagen fibres are pure products containing 90% collagen protein (table 1).

Table 1 Physical and chemical characteristics of collagen fibres

Characteristics	Non-tanned collagen fibres	Collagen fibres tanned with formaldehyde
Aspect	fibrous	fibrous
Colour	White-yellow	White-yellow
Humidity, %	15,0	15,0
Total azoth content*, %	17,5	17,45
Protein concentration *, %	98,0	98,0
Minerals *, %	1,0	1,0
Absorption ability Water at 37°C Acetic acid 0,5M	Partially soluble Partially soluble	Partial swelling Partial swelling

*Values calculated at free humidity

Collagen fibres are used as standard semi-finished product for the manufacturing of collagen-based products used in medicine, pharmacy, and cosmetics. Formaldehyde tanned collagen fibres are used as support for biochemical analysis, cell cultures, and fibrous insertions in collagen matrices [1].

Collagen Hydrogels

Gels with water dispersion media or hydrogels are tridimensional networks of reticulated hydrophilic polymers, generally of a covalent or ionic nature, which interact with watery solutions, inflating to an equilibrium value. These can be relatively enduring, as in the case of cellulosic membranes used in dialysis or relatively less enduring, becoming less and less enduring as the water content increases, even though they vary according to the nature of the cross linking agent, the polymeric network, and crystallinity which can all influence mechanical behaviour [9].

Hydrogels are relatively subtle systems which balance themselves in the environment, thus enduring numerous alterations. The properties of such gels are highly dependent on reticular density and impurity content. In the case of synthetic polymers, they also depend on monomer traces, catalysts, and polymer stereospecificity.

Superficial and interfacial properties of hydrogels are relatively similar to that of biological gels and tissues. Also taking into account volume properties, these are really convenient in what regards medical and pharmacological application. Thus, there are numerous similarities between the gel/water interface and live cells/physiological solutions. Given the fact that the latter category is rather hard to study and interpret, the study of the former can lead to the uncovering of certain aspects regarding live nature.

Volume properties such as inflation can lead to the discovery of inflation and osmosis mechanisms in biological tissue, as well as to their use as implants that can inflate. On the other hand, their permeability to water soluble compounds with low molecular weight means that gels become a convenient method for the controlled release of drugs or for the transport of diluted substances, such as enzymes.

In what regards pharmaceutical applications, the mechanical properties of hydrogels are very important. For example, the integrity of drug release devices during the lifespan of the application is important for obtaining the FDA improvement in the USA. Moreover, the device is also designated as a biodegradable system. A drug release system destined to protect a sensitive therapeutic agent such as protein must maintain its integrity, in order to be able to protect the protein until it is released outside of the system.

Collagen Membranes

Gels and collagen solutions air dried at 25°C are transformed into films called membranes, due to resemblance to natural membranes (the placenta, the basal membrane, the dura mater, or the intestinal membrane). The duration of the drying process, the relative humidity of the air from the drying and the air circulation speed influence the morphologic structure of the collagen membrane. Membranes can be reticulated in gel phase or after their formation, through immersion in a fixation/reticulation solution containing aldehyde or another reticulation agent; less frequent, formaldehyde vapour reticulation is used.

Collagen membranes are semitransparent, even, elastic, and relatively enduring enough in order to be plied and rolled in tubes (fig. 3) [1]. They can either be one or multi layered and they can have textile insertions, which enhance their mechanical resistance.



Fig. 3. Collagen membrane

Composite collagen membranes can contain the same class of compounds used for obtaining of composite matrices. However, there is a restriction in what regards the percentage of added components. This depends on the nature of the compound and its solubility and reactivity towards the collagen gel. In composite membranes formed by powders of ceramic, hydroxyapatite, TiO₂, SiO₂ aerogels, modified natural silicates, cannot represent more than 5-10% of these compounds. The formation of composite collagen membranes from natural polymers or water soluble vegetable composites is more significantly influenced by intermolecular interactions which can lead to the co-precipitation of components [1]. Amongst collagen membrane-based bioproducts, the most important are related to:

- Gradual release capacity (drug delivery) through transdermal patches [11-13];

- Diffusion, electrical, and piezoelectrical properties, which manifest themselves with implantable bioproducts (tubes used for the regeneration of peripheral nerves);
- abdominal tissue and membrane substitutes used for guided tissue regeneration [14-16];
- Till present date, on a national level, the following types of collagen membranes have been obtained:
- simple, uni and tri-layered membrane, used as myelin substitute in neurosurgery;
- tissue insertion membrane used as a substitute for the abdominal wall.

Collagen Matrices

As it has already been stated, applying the principle of lyophilisation drying of gels and collagen solutions leads to the formation of sponges with a macro, micro, and nano-porous structure. In this regard, collagen materials can be regarded as biofoams, xerogels, or aerogels. Specific to this structure is the very characteristic weight represented by approximately 0,02 g/cm³. The hydrophilic properties, represented by water absorption and water vapours, are specific to collagen matrices and account for a level of water absorption of up to 3000%. Matrices with various compositions and properties adapted to the field of use can be obtained: scaffold biomaterials, drug delivery implants and tissue replacements (skin, cartilage, bone) [17, 18]. The wide variety of matrices is obtained through the formation of composites at a micro and nanostructural level, when added components to the collagen gel do not surpass 30-40%;

Solubility of collagen matrices, either *in vitro* or *in vivo*, is regulated through solubility, particularly with aldehydes, through engraftment or the formation of intermolecular complexes. Collagen matrices can be used as a bioactive bandage to treat skin burns [19]. This collagen matrix is non-reticulated, soluble in the physiological environment. Because of this, it also represents an added protein supplement needed for the restoration of the skin's connective tissue. Collagen-based biomaterials have developed at an accelerated rate, demanded by the field of regenerative medicine, which requires a wide variety of implants, tissue replacements and prosthetics.

The Use of Collagen Membranes

Collagen membranes used as scaffold biomaterial for fibroblast cell cultures and skin replacements. When treating various skin lesions, complications arising for wound infestation can arise. In order to avoid this peril, collagen membranes are injected with antibiotics and other drug, which are slowly released in a time span of 48 hours. The purpose of local release is represented by the need to maintain a high concentration of the drug, without the innate toxic effects. In this case, collagen also has a protective role for the damaged tissues. Drug incorporation is realized before lyophilisation, in concentration around 0,1-5%, according to the composition and the mechanism of action of every drug or bioactive compound, such as cellular growth factors. Thus, collagen matrices containing 1-3% captopril, estradiol, diclofenac or gentamicin have been obtained [20]. The profile of release masses has been studied *in vitro*, in physiological serum for medicine contained in collagen matrices obtained at varying pH and varying drug concentration. The results were then correlated with the morphostructural properties of the matrices, represented by porosity and hydrophilic properties, so as to determine collagen-drug interaction models and to establish optimal concentrations for their immobilization in matrices [21, 22]. In order to ensure a constant release of drugs from collagen matrices, these must contain 0,2% diclofenac, 0,1 – 0,2% gentamicin [20].

The Use of Collagen Biomaterials in the Medical Field

Biomaterials are natural or artificial materials used to redirect, complete, or replace live tissue functions in the human body [23]. According to Williams [24], a biomaterial is a material used as an implant or medical device which will interact with the biological system. The use of biomaterials dates back to ancient times: Egyptian mummies were found to possess artificial eyes, ears, teeth, and noses [25]. The Chinese and Indian peoples used was, clay and tissues in order to reconstruct missing parts of the body. Throughout time, the development of synthetic material, surgical techniques and sterilization methods has allowed for biomaterials to be used for several purposes [26]. Currently, the medical practice uses a large number of implants and devices [27].

The use of collagen as biocompatible and bioresorbable material is well known, both internationally and nationally. Regardless of the novelties emerging in synthetic-based biomaterials, collagen as a natural polymer remains one of the most important biomaterial for connective tissue, where it represents the main protein. Collagen forms with these synthetic

biomaterials compounds with a large range of application in medicine [28]. Collagen as a biomaterial can be used in a variety of forms and applications: injectable solutions for the eye, or subcutaneous, for cosmetic purposes, powders with haemostatic properties, suture wires, membranes for surgical restoration, implant sponges and tubular shapes to be used as vascular prosthetics or organ reconstruction material (oesophagus, tracheae).

Alongside the collagen biopolymer, various compounds can be part of the process meant to create biomaterials for medical purposes: proteins (elastin, fibrin, actyne, and so on), vegetable polysaccharides (celluloses and derivatives), animal polysaccharides (hyaluronic acid, heparin), bacterial compounds (chitosan, xantan, manan, and so on), a wide variety of synthetic polymers (polyacryle, polyurethane, polyester), a wide variety of biologically active compounds (enzymes, lipids, sugars, drugs, growth factors, cells and others), as well as ceramics.

Conclusions

Collagen is one of the most widely used biomaterials [29], being perceived by the human body as a constituent and not a foreign object [30]. The main variants of collagen as biomaterial are: acid or neutral solutions for dermal implants, gels combined with liposome for the controlled release of drugs, control materials for transdermal release, implantable hydrogels, ophthalmologic membranes, spongy membranes used as bandage for burns and wounds, haemostatic agents, or cell culture support, microgranules and tablets for protein release, surgical suture wires, bony materials, transport agents for drugs and other physiologically active substances controlled release, and collagen-based penetrating networks.

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