

COLLAGEN RECONSTITUTION

BACKGROUND OF THE INVENTION

Fibrillogenesis or reconstitution of soluble tropocollagen into native collagen fibers and aggregate is well known in the art. For example, U.S. Pat. No. 3,075,961 describes one method for reconstituting collagen from acid precursor gelatin. Volume 5 of the *Molecular Biology* series entitled "The Macromolecular Chemistry of Gelatin" by Arthur Veis (1964) also describes in detail the production and processing of collagen.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an improved process for the reconstitution of soluble collagen molecules into homogeneous collagen gels or aggregate.

It is a further object to provide an improved method for the production of uniform collagen biomaterials for repair and replacement of human tissue and organs.

In accordance with the objectives, the invention is an improved method of producing a reconstituted collagen gel from a solution of solubilized tropocollagen molecules wherein a homogeneous collagen gel matrix is precipitated from the solution under gravitational force of less than one gravity. Preferably, the gravitational force is about zero gravity.

The invention further comprises performing the improved process within or on a die or mold whereby a homogeneous collagen biomaterial device is obtained. For example, an eye lense prosthesis (an artificial cornea, contact lens or interocular lens for the eye) may be formed directly if the improved method is carried out in an eye lense mold or die. Moreover, biomaterial parts can be fabricated from gel sheets made by the process and may be further shaped, for example by cutting, abrading, heating and chemical treatment.

Additives or impurities to selectively alter material properties may also be incorporated into the homogeneous reconstituted collagen matrix by introducing the additive into the solution prior to fibrillogenesis.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 and FIG. 2 are graphs of absorbance as a function of position of a collagen gel reconstituted at one gravity in a tube.

FIG. 3 is a similar graph for a gel reconstituted at three gravities.

DETAILED DESCRIPTION OF THE INVENTION

Collagen is the principal proteinaceous component of the white fibrous connective tissue. Collagen comprises about 30% of the organic matter in mammals and nearly 60% of the protein content. Because of this varied utilization of collagen, the fibers in various tissue are organized in different ways, are produced from different types of cells and are associated with various other substances. Thus purity and structure are important properties of the collagen fibers in respect of their ultimate use and suitability. The present invention proposes to improve the structure of reconstituted collagen fibrils and aggregate by processing under reduced gravity environment. Because of its compatibility with body tissue, the reconstituted collagen is useful in making replacement tissue and body organs. The homogeneous collagen gel produced by the present improved fibrillo-

genesis process is expected to result in vastly improved biomaterials for such replacement.

Reconstitution of insoluble collagen fibrils as known in the art comprises the steps of, (1) dissolving the soluble collagen molecules from any collagen source, for example, the tissue of young animals, (2) separating and purifying the soluble collagen molecules, and (3) precipitating and recovering the reconstituted collagen gel matrix by inducing a "polymerization" of the soluble molecules into insoluble collagen fibrils and subsequent three dimensional organization by unit assembly of the collagen fibrils into the collagen gel matrix.

The individual soluble collagen molecules extracted from animal tissue are primarily macromolecular monomer units known as tropocollagen (collagen-former). These tropocollagen units have a three chain helix structure and are on the order of 2800 Å in length and 15 Å in diameter. The insoluble collagen fibrils grow from these tropocollagen molecules which are caused to join in staggered layers by an exogenous change in the environment, for example, by a change in pH or temperature of the solution. The collagen fibrils then aggregate into a gel matrix by unit assembly and possible crosslinking.

Since lyophilized tropocollagen is commercially available, it would be possible to reconstitute collagen fibrils by merely redissolving the lyophilized tropocollagen and precipitating the collagen fibrils. However, the purity of available tropocollagen is somewhat less than that which can be produced in the laboratory, and we prefer to extract and purify the tropocollagen. Moreover, the extraction and purification can be customized in the laboratory such that certain desired "impurities" from the collagen source may be selectively retained with the tropocollagen to produce a reconstituted collagen with particular properties. Alternatively, additives such as complex carbohydrates (e.g. mucopolysaccharides), salts (e.g., CaPO₄), proteoglycans, or other proteins (e.g., elastin), can be added to the soluble tropocollagen to be incorporated into the precipitated collagen gel resulting in the optimization of certain properties such as strength or flexibility.

EXTRACTION

The preferred process of extracting and purifying tropocollagen is as follows. A source of collagen such as rat tail tendon or young animal skin is cleaned, cut up into small pieces and placed in an acid extracting solution. Acetic, citric, glycolic and propionic are examples of the acids which may be used as the extractant.

Tropocollagen, being soluble in acid solution, is dissolved along with other soluble impurities. Agitation over two or three days may be required to solubilize the tropocollagen.

Separation is accomplished by filtering and centrifugation, after which the supernatant contains the tropocollagen. The supernatant is recovered and tropocollagen is then precipitated therefrom by raising the pH with a NaCl solution. The tropocollagen is finally recovered by centrifuging the solution.

Purification of the recovered tropocollagen begins by redissolving in acetic acid and then salting out. This may be repeated several times at different pH. Subsequently, the salt is dialyzed out of the tropocollagen in an acid bath. At this point, the tropocollagen may be further purified by ultracentrifugation and the supernatant of pure collagen may be directly used in fibrillo-