

## NON-ANTIGENIC COLLAGEN AND ARTICLES OF MANUFACTURE

### CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional of application Ser. No. 744,536, filed Nov. 24, 1976, now U.S. Pat. No. 4,140,537, which is a continuation-in-part of application Ser. No. 624,678 filed Oct. 22, 1975, now abandoned.

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

Collagen is the principal structural protein present in vertebrates. It has many properties which make it particularly desirable for the fabrication of medically useful devices. Natural collagen is readily available from a variety of domestic animals. The major portion of its structure varies little between mammalian species; and the positions of the distinguishing and structurally significant amino acid residues (glycine, proline, and hydroxyproline), are uniquely consistent in the main helical portion of the atelopeptide collagen. This fundamental similarity is associated with characteristically low levels of immunogenic activity. Many immunogenic determinants are in the nonhelical protein appendages extending from the terminal portions of the native molecule. These non-helical extensions, telopeptides, represent less than five percent of the naturally occurring molecule and can be removed through limited proteolysis. The treatment produces a disaggregation of discrete undenatured collagen molecules from the fibrous matrix (i.e. solubilization) and a substantial reduction in the ability of such molecules to elicit an immunologic response in a host different from the collagen source.

While the telopeptides are important sites of immunogenicity and their presence in collagen is undesirable in medical applications, the telopeptides play an important structural role in naturally occurring collagen. The telopeptides are the primary sites of both intra- and intermolecular cross-links. It is this portion of the molecule which provides the structural integrity of native collagen fibers. Moreover, there is evidence that the telopeptides direct the process of fibrogenesis through promoting the orderly accretion of constituent molecules into large structurally significant fibers. Thus, the removal of the telopeptides also removes that portion of the molecule which in the natural state appears essential to the formation and subsequent stability of native collagen fibers.

#### 2. Description of the Prior Art

Two survey articles concerning collagen are by Bornstein: *The Biosynthesis of Collagen*, Annual Review of Biochemistry 43: 567 (1974), and by Stenzel, et al., *Collagen As a Biomaterial*, Annual Review of Biophysics and Bioengineering 3: 231 (1974). Patents concerned with forming atelopeptide collagen are U.S. Pat. Nos. 3,034,852; 3,121,049; 3,131,130; 3,314,861; 3,530,037; and 3,949,073.

Patents concerned with various articles of manufacture prepared from collagen include U.S. Pat. Nos. 2,920,000; 2,934,446-7; 3,014,024; 3,491,760; 3,562,820; and 3,563,228.

Other articles will be referred to in the text, when appropriate, in relation to specific aspects of the subject invention.

### SUMMARY OF THE INVENTION

The subject invention is concerned with the formation of fibers of collagen substantially free of the immunogenic nonhelical terminal portion, i.e. telopeptides. The fibrous products formed in the subject invention are of relatively large diameter which appear in scanning electron micrographs as twisted intertwined fibers with a rope-like structure. The collagen fibers of this invention have substantially the same structure as natural collagen and may be used as formed, or cross-linked, to provide a variety of medically useful products: sponges, prosthetic devices, films, membranes, sutures, etc.

Native collagen is liberated from noncollagen connective tissue constituents (lipids, sugars, proteins, etc.) and isolated after subjecting it to proteolytic enzymatic treatment by an enzyme other than collagenase. The enzymatic treatment is maintained for a time sufficient to achieve a substantial removal of the telopeptides and to provide a collagen material which is soluble in aqueous media of reduced pH.

The resulting collagen in solution is then treated in accordance with a regimen in which the acidic medium is modified to provide for a slow precipitation of the atelopeptide collagen while the fluid medium is simultaneously exposed to sustained shear. The collagen separates from solution as fibrous microaggregates ("native fibrous micropolymers") which may be freed of the salt solution or taken up in a different solution. The fibrous aggregates may be used directly for a variety of purposes or may be cross-linked to provide fibers having substantial structural integrity and macroscopic dimensions. Depending upon the intended use of the native fibrous micropolymers, the fibers may be treated in a variety of ways to prepare various articles of manufacture.

### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

A method is provided for preparing articles derived from nonhuman collagen which have low immunogenicity or are nonimmunogenic. Collagen may be obtained in commercially useful amounts from the connective tissues of a wide variety of domesticated animals such as cattle, swine, etc. The native collagen is most conveniently obtained from tendons or skin and is freed from extraneous matter such as lipids, saccharides and noncollagen protein, so as to leave the collagen protein free or substantially free of other connective tissue materials. native collagen fibers are composed of regularly arranged subunit structures are referred to as collagen molecules. Each collagen molecule is about 3000 Å long and 15 Å in diameter. This long, rigid rod-like structure consists of three polypeptide chains wound together in a triple helical configuration. Typically two of the constituent chains are identical in composition and the third is different. A characteristic distribution of amino acid residues along the length of any given polypeptide strand, wherein repeating triplets contain glycine at every third position, favors the formation of a helical configuration. The individual collagen units form fibrils which associate to form fibers.

The nonhelical terminal portions of the native collagen molecule, the telopeptides, extend as random coils from the amino and carboxy ends of the molecule.

The telopeptides appear to serve a number of functions in the formation of the native collagen fiber. The