Collagen - Isolation and Perspectives of Application of Nature Nanomaterials

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***Abstract* – Through the extreme diversity of tissues and types of collagen it is difficult to develop a standard method of extraction for all types of collagen from different tissues. Two procedures based on acid- and enzymatic-soluble collagen isolation were combined and described some advantages and disadvantages of methods used in the present. Our results have demonstrated relatively low concentrations of collagen in the final solutions. There is 4,7 mg/ml from theoretically 10 mg/ml of acid-soluble fraction of collagen. Here are discussed the possibility to utilize the collagen, as fibrous structural protein with superior mechanical properties, that provides an intriguing example of a hierarchical biological nanomaterialas, for the construction of nanostructures with the required dimensions.**

***Index Terms* – collagen type I, methods of isolation and purification, nanomaterials, tissue engineering**

I. INTRODUCTION

Inspired by nature's ability to produce supramolecular nanostructures from the bottom-up, materials scientists have become increasingly interested in the use of biomolecules like DNA, peptides, or proteins as templates for the creation of novel nanostructures and nanomaterials [1].

Collagen is an exemplary type of robust biological nanostructure built from simple building blocks. It is the most abundant protein in modern vertebrates, comprising approximately 30% of the total protein content and 70% of the dry weight of human skin. Collagen provides the three-dimensional matrix for connective tissue types such as bone and cartilage [5].

Numerous studies have demonstrated that collagens can induce or regulate many cellular functions and processes such as cells differentiation, motion, communication and apoptosis [4, 5]. But its main function is the formation of insoluble fibrils with high strength characteristics.

Collagen is the major component of the extracellular matrix and more than 27 genetically isoforms have been identified. Collagen type I, II and III are the most abundant widely used as a plastic material in different medical domains, cosmetology, and in the pharmaceutical industry as a compound that provide drugs action [3, 4]. Type I collagen has been described as a natural scaffold and a potential candidate for tissue engineering and reconstructive medicine [6]. Such diverse functions are due to physical and chemical properties of collagen protein.

At physiological conditions, the individual collagen molecules of approximately 300 nm length and 1.5 nm diameter aggregate longitudinally and bilaterally to microfibrils and further to fibrils (fig. 1). Thus it is a nanostructured carcass with possibility to carry out the assembly of protein complexes [1]. Collagen consists of tropocollagen molecules that have lengths of L ~280 nm and diameters of ~1.5 nm, leading to an aspect ratio of ~190 [12–14]. Staggered arrays of tropocollagen molecules form fibrils, which arrange to form collagen fibers (Fig. 1).

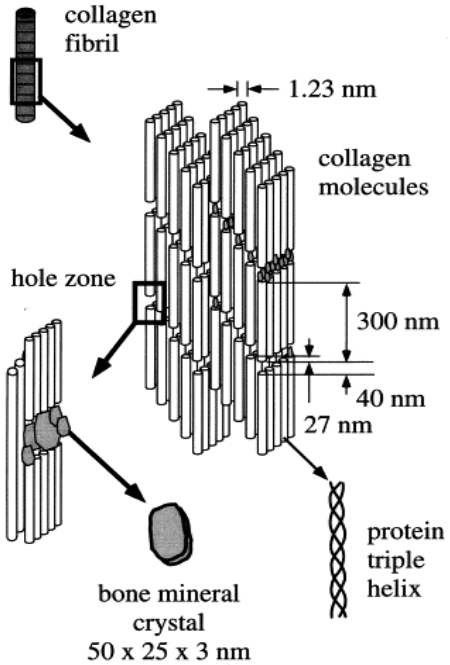

Fig. 1: Nanostructure of natural bone [Rho 1998]

Type I collagen is trimeric [$(\alpha 1)_2$ α 2] and exists as triple helix. The helices have the typical repeats for collagen Gly-X-Y (where X and Y are mainly Pro and Hyp). Thus, proline and hydroxyproline constitute about 23% of the total protein sequence and structure Gly-Pro-Hyp is often founded [4]. Its unique tertiary structure is a right-handed triple helix composed of three helical peptide strands (left-handed polyproline II-type).

Through the extreme diversity of tissues and types of collagen it is difficult to develop a standard method of extraction for all types of collagen. The number of the covalent intermolecular interactions in collagen structure increases in time and frequently determines almost full insolubility in solvents utilized to dissolve proteins [7].

The main task of our study was to analyze the known methods of collagen isolation and purification. Obtained solid-phase collagen could be a promising platform for generation of new and interesting nanostructured materials [11]

II. MATERIAL AND METHODS

*The collagen source***.** Type I collagen was isolated from steer (3 years old) flexor bovine tendon. Fixed mass of tendon was suspended in cold distilled water at 4^0 C and water was changed two times per day for three days. The tendon fibers were cut into small pieces (1 cm in length) and pulverized in a mill after that. Pieces were dried 24 h in the thermostat at 40^0 C.

Method of collagene isolation

The procedure is based on the extraction of collagen from the tendon pieces in organic acid (0,5M CH_3COOH) in the presence of 5mM EDTA and pepsin with concentration 0,05 g per 100 g of tissue, pH = 2,5 - 3,0 for 48-96 h at 4^0C.

III. RESULTS AND DISCUSSION

After testing several collagen isolation' procedures [2, 4, 5] we have chose the method based on acetic acid dissolution of collagen fibers with some modifications. One of them is introduction of the neutral salt or low ionic strength acidic solutions.

We have combined these two procedures based on acid- and neutral-salt extraction with enzymatic collagen isolation. Several types of soluble collagen are distinguished depending on the specific protein solvent: neutral salt-soluble collagen, acid-soluble collagen and enzymatic-soluble collagen. Thus a combined approach was developed which includes pepsin digestion in acidic solution.

Our results have demonstrated relatively low concentrations of collagen in the final solutions – 4,7 mg/ml.

Although the method of extraction based on acetic acid and pepsin dissolution was standardized more than 40 years ago, it still has two major problems.

First, the collagen solubility is still ill-defined due to cross-link mediated aggregation, so that the reproducibility of the collagen preparations is poor. Secondly, the collagen peptides, especially the short non-helical regions of collagen, are susceptible to proteolysis/hydrolysis during the isolation [4, 9].

For this reason the utility of the acidic-extracted collagen is limited, since the isolated material must be stored in cold acetic acid solution or dried. The maximal obtainable concentration of collagen is also limited to 10 mg/ml [4] as estimated by wet weight and also by amino acid content. The methods of collagen isolation, purification and determination should be modified, using new strong detergents for deeper dilution of collagen fibers, on purpose to overcome the disadvantages of its partial degradation.

A major requirement of collagen purification the elimination of the antigenic components of the protein represented by the telopeptide regions of collagen type I that can be more efficiently when treated with pepsin. However, collagen extracted from animal sources presents a small degree of antigenity, that's why it is considered acceptable for tissue engineering in humans [4].

There is an important problem to control the construction of nanostructures from collagen with the required dimensions.

Despite significant research effort over the past couple of decades, the geometry and typical length scales found in collagen fibrils, the deformation mechanisms under mechanical load, and, in particular, the relationship between those mechanisms and collegen's molecular and intermolecular properties, are not well understood. Moreover, the limiting factors of the strength of collagen fibrils and the origins of toughness remain largely unknown [12].

Some experimental efforts focused on the deformation mechanics of collagen fibril at nanoscale, including the characterization of changes of D-spacing and fibril orientation [13-15], analyses that featured x-ray diffraction [13] and synchrotron radiation experiments [14]. Other experimental studies were focused on the averaged response of arrays of collagen fibrils, considering nanoscale deformation mechanisms [12, 24].

To develop a fundamental and quantitative understanding of collagen mechanics, it is critical to develop theoretical models encompassing the mesoscopic scales between the atomistic and macroscopic levels [12]. There exists no model that links the properties of individual molecules with the overall mechanical response of fibrils or fibers, considering the different types of chemical bonding and nanoscale mechanics and geometry. The role of the staggered structure and the reasons for the specific length scales and high aspect ratio of TC molecules remain unexplained.

An improved understanding of the nanomechanics of collagen may help in the development of biomimetic materials or for improved scaffolding materials for tissue engineering applications [17].

Buehler [12] has used a hierarchical multiscale modeling scheme based on atomistic and molecular simulation to describe the mechanical properties of collagen under large stretch, leading to permanent deformation or fracture. There was shown that the key to understanding the mechanics of collagen is to consider the interplay between the mechanics of individual tropocollagen molecules with characteristic length scales, the intermolecular chemical interactions, and the mesoscopic properties arising from hundreds of molecules arranged in fibrils. It was explored the mechanics of collagen by considering different nanostructural designs, and pay specific attention to the details of molecular and intermolecular properties and their impact on the mechanical properties.

Energetic effects rather than entropic contributions govern the elastic and fracture properties of collagen fibrils and fibers. The fracture strength of individual tropocollagen molecules is largely controlled by covalent polypeptide chemistry. The shear strength between two tropocollagen molecules is controlled by weak dispersive and hydrogen bond interactions and by some intermolecular covalent cross-links.

Some studies have suggested that the length of tropocollagen molecules and strength of intermolecular interactions plays a significant role in determining the deformation mechanics, explaining some of the structural features of collagen found in nature.

Key concepts that can be adopted from self-assembly found in nature include molecular recognition of the single building blocks and the formation of predictable three-dimensional nanostructures [1, 9, 10].

Pioneered by Braun, Belcher, and their coworkers, there have been numerous examples of DNA or viruses as scaffolds for complex nanostructured inorganic materials [8–11]. Since the nucleobase or amino acid sequence encodes how these scaffolds self-assemble, a variety of programmed nanostructures can be produced [1, 10, 11].

Although DNA can be readily synthesized, it is composed of a small number of similar monomers. As a result, some

approaches have combined DNA and proteins to create functional nanomaterials [1]. In contrast, peptides and proteins are built from 20 proteinogenic and a wide variety of non-natural amino acids. This leads to chemical diversity, evident by the display of aliphatic, acidic, basic, or aromatic side chains from a peptide backbone, and structural complexity, manifested by the multitude of possible molecular architectures like helices, β-sheets, and tubules [1, 8].

Working with live systems, we can use "natural nanotechnology", that mean an ability to link proteins individually with other proteins or other substances to form complexes with desired properties. These so named collagen-binding domains can be used:

- for creating artificial surface for the cultivation of eukaryotic cells;
- as drugs that accelerates wounds and burns healing;
- to prolong the drug effect;
- as drugs that promote early fracture consolidation;
- as composite materials, for implant coating [1, 5, 10, 11].

IV. CONCLUSIONS

Recent studies in cell biology, nanotechnology, and computation gave more new insights regarding the physical proprieties, that in complex with the chemical one, can regulate cell signaling and gene expression. Due to the importance of biocompatible matrixes for tissue engineering and their application in medical technology, the availability of native collagen should be studied by refining the collagens extraction procedure. It is very important to elaborate the method of collagen isolation that give us fully or partially soluble collagen that can be used in producing by tissue engineering of matrices, powder, sponges, fibers or filaments.

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