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Spectroscopic study of a KrF excimer laser treated surface of the thin collagen films

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Abstract

The photochemical properties of collagen films before and after KrF excimer laser UV-irradiation with 248 nm were investigated using Raman, FTIR-ATR and fluorescence spectroscopy. It was shown that a single pulse of UV radiation ($\lambda = 248$ nm) can affect the conformation and photostability of collagen polypeptide chains. Raman and FTIR-ATR spectra analysis showed that UV laser light is capable of inducing conformational changes in the irradiated collagen films, mainly as a result of breaking of the hydrogen bonds network and losing of water molecules, accounting for the maintaining the structure organisation. Partial decomposition of the main collagen chain is also considered. Fluorescence measurements showed characteristic bands assigned to tyrosine aromatic compound and also to the products of its photochemical degradation given by laser irradiation.

The obtained results indicate that the interaction between collagen film and UV laser radiation can be considered as the result of the photomechanical regime, with low thermal degradation, combined with some photochemical transformations.

Keywords: Collagen film; "Micro-foam" structure; Triple helix; Random coil; Raman spectroscopy; FTIR-ATR spectroscopy; KrF excimer laser; Laser radiation

1. Introduction

Collagen has outstanding molecular architecture and properties and it is a suitable material for laser–matter interaction research [1,2]. Collagen in the human body accounts for about 25% of all proteins and it is the main component of connective tissue, occurring in skin, tendons, cornea, bone and membranes [1–3]. It is a strongly hydrophilic protein that explains the ability of collagen materials to bind a large amount of water in its internal structure [3–6]. The newest investigations show that in the collagen family 20 types of collagen are known [7,8]. The triple helix structure is maintained by hydrogen bridges between –NH group of glycine and carbonyl group C=O of residues from another polypeptide chain or by hydrogen bridges with water molecules [9–11]. Moreover, the influence on stabilization of the collagen helix is also due to short-range interactions such as: Van der Waals, hydrophobic or electrostatic [3,12]. In fibrillar collagens, ~25% of the amino acids in the α-chain are proline or hydroxyproline that effectively block internal rotation of the collagen chain at these sites and stabilize further the triple-helical structure [4]. Because of its interesting biological properties like non-toxicity and its large availability, collagen is extensively used as a source biomaterial in medicine, pharmaceutical and cosmetic industries [13]. After extraction from natural tissue, collagen materials can be processed to obtain thin films, sponges, membranes and fibres.

In our recent papers we presented a new laser processing of the studied biopolymer film surfaces, collagen, collagen/PVP blend and chitosan. A single KrF laser pulse of sufficient energy density for ablation excites the skin depth of the film (~15 μm) yielding a new micro-foam layer [14–19] having some promising biomimetic properties that could be used in cell culturing. This new and interesting result was unexpected since shorter but similar wavelength ArF laser at 193 nm gives only the traditional

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clean ablation which is now extensively being used in refractive cornea surgery. Studies of laser treatment at 248 nm are scarce because of the low absorptivity of cornea, a collagen rich tissue, which does not permit the so called clean ablation. However, we demonstrate with dry collagen films new interesting phenomena are observed at this wavelength.

In this framework we show the way in which single pulses of UV radiation ($\lambda = 248$ nm) can affect the conformation and photostability of collagen polypeptide chains. We will try to answer the question whether a partial loss of the unique three-dimensional protein structure, the triple helix, could be explained by means of known ablation mechanisms and other one photon photochemistry. The laser induced micro-foam structure was therefore investigated by several methods, including vibrational and electronic spectroscopies with the sake of tracking of the possible molecular damage and alteration to the molecular structure of collagen.

2. Experimental approaches

2.1. Materials

Collagen (Type I) was obtained in our laboratory from the tail tendons of young albinos rats. After washing in distilled water, tendons were dissolved in 0.4 M acetic acid solution. Non-dissolved parts of tissue were separated by centrifugation at 7500 rpm. Thin biopolymer films, of ~35 μm thickness, were obtained by casting the prepared aqueous solution onto glass plates and drying in air at room temperature overnight. The films under the study contain only molecules in both triple-helical and random coil conformations. They do not contain fibrils, as we could not find any fibres using X-ray diffraction methods [20].

2.2. Methods

2.2.1. Laser system

The source of UV radiation was krypton-fluoride (KrF) excimer laser (Lambda Physik, LPX 220i) emitting the wavelength of 248 nm and pulse duration of 25 ns, with a method of beam shaping as described in a recent paper [14]. The irradiation was carried out in air and at room temperature, by using single pulses of UV light isolated out manually from the output of the laser working at a repetition rate of 1 Hz.

2.2.2. Raman spectroscopy

Raman spectra of collagen films before and after laser irradiation (fluence 1.0 J/cm^2, 1 pulse) were recorded in air at room temperature using a Confocal Raman Microspectrometer (Horiba Group), equipped with an argon laser delivering 10 mW of power at the wavelength of 514.5 nm. Data collection and plots were achieved with the LabSpec program supplied by manufacturer.

2.2.3. FTIR-ATR spectroscopy

Infrared spectra of the examined collagen films were recorded in the 500–4000 cm^{-1} window using Nicolet Magna IR 560 spectrometer with a probe cell equipped with a diamond crystal prism working in the attenuated total reflection (ATR) mode. These measurements could be done up to 5 pulses and were not measurable for more because of the too large surface roughness which is increasing with delivered pulses number. All spectra were acquired in air at room temperature and each measurement was an average of 300 scans.

2.2.4. Fluorescence measurements

The intrinsic fluorescence studies were performed on a Spex Fluorolog 212 (Horiba Group) spectrophotometer. Measurements were done using mainly 270 and 350 nm excitation wavelengths, before and after laser irradiation of the collagen film surface at a fluence of 1.0 J/cm^2. Absorption and emission bands were assigned to aromatic amino acids, mainly tyrosine and to their degradation products.

3. Results and discussion

3.1. Raman spectra

The peptide bonds give rise to several classical and well documented vibrational modes in the Raman spectra which are reported as amide I–VII, A and B bands. Amide I and III bands have relatively high Raman intensity and are more sensitive to conformation change than others. These bands were analysed to determine the changes in the internal structure of the collagen molecules presented in this work. The amide I band consists of C=O, C–N stretching (approx. 80% and 10% contribution, respectively) vibrations and N–H bending vibrations, whereas the amide III band represents mainly C–N stretching, N–H bending and CH_3–C stretching vibrations (approx. 40%, 30%, 20% contribution, respectively) [21–23]. In order to determine the protein or polypeptide chain conformation using amide I and III bands, the frequencies of these bands should be related to Ramachandran angles (ψ and φ-angles of rotation) which result from the possibilities of polypeptide backbone to rotate around C_α–C and C_α–N bonds [21–24]. The conformation of a protein molecule such as collagen is mainly determined by interaction of amide backbone with amino acid side chains, which are of steric nature. The conformation of the polypeptide chain is maintained by means of intermolecular hydrogen bonding between oxygen from the carbonyl group of each peptide bond and hydrogen atom coming from the amine group of every fourth amino acid. The internal hydrogen bonds form periodically within the same main chain at each turn of the helix [22,24]. Establishing the conformational changes in the protein molecule (for example: conversion from helical to random coil structure) based on Raman spectra obtained, should include an interpretation of the two amide bands (I and III), simultaneously. Analysis of amide III band only, owing to its complexity, can lead to a false conclusion concerning structural changes in the material studied.

In Fig. 1 the Raman spectra obtained for the non-irradiated collagen film and the sample irradiated with a single laser pulse at a fluence of 1.0 J/cm^2 are shown. The main bands seen in the spectra are located at the following wavenumbers: 1669–1638, 1454, 1265–1244, 1004, 937–921, 876–855 and 815 cm^{-1}. A