Abstract

In tendons, collagen fibers are structured with a helical supra-molecular organization and interact with structured light depicting intrinsic and form birefringence (FB). FB is an optical anisotropy of the permittivity that is being used in the construction of man-made devices to filling up practical purposes. In the present case the used biomaterial displayed highest levels of FB.

Linear polarized monochromatic light, after traveling microscopic sections of collagen fibers, exhibit elliptically polarized front of light, with angular momentum, that after trespassing a second polarizer generated vortices images.

Advanced polarized microscopy, structured light, was used in this study to detect and characterize the production of vertices images by collagen fiber as structured biomaterial.

Microscopic sections used in the present study fulfilled all of the conditions for generating vortices.

Here, it is shown, for the first time that vortex structures are generated by chiral supramolecular collagen fibers in birefringent bundles, and can be observed with a polarizing microscope. Additionally, it is hypothesized that the vortices in biological structures are part of a mechanism of signal transference from extracellular space to cells.

Considering that the spatial distribution of collagen fibers and bundles in connective tissues may vary, it is assumed that the complexity of the corresponding vortex morphology may also vary.

Finally, it is recommended that the structured characteristics of collagen fibers chirality and their optical anisotropic properties could be used as inspiration for new man-made structured devices.

Keywords: Optical vortex; Form birefringence; Collagen fibers; Tendons

Introduction

Chirality, an optical activity of molecules, chemical composites and inhomogeneous birefringent elements, generates optical vortices [1-3]. In the field of data-transmitting channels for the Internet traffic, vortex properties are being envisioned to enable high-speed data transfer thus improving communication through fiber optic line [4].

The interaction of metamaterials with linearly or circularly polarized light, along with Gaussian beam propagation in complex materials has been assumed to occur with properties not found in nature [3]. However, this affirmation cannot be sustained in the face of the optically anisotropic properties exhibited by biological materials such as collagen fibers in tendons [5-9], which have been described as chiral bodies generated from chiral molecules [7,10,11].

Collagen fibers are constructed by a self-assemble mechanism of a three left-handed helices peptide chains that make a right-handed twist forming three coiled super helix molecule, so, it is a coil-coiled molecule 300x15 nm, rod like molecule, [12-16]. These molecules are organized in tandem forming a supramolecular structure (SMS) [12-16]. SMS depicts intrinsic and form birefringence (FB), and FT-IR dichroism [17-20]. Then, collagen fibers and collagen bundles are nanometric-structured supraorganization exhibiting high molecular order that generate optical anisotropic properties especially FB, as defined by Bêche and Gaviot [21] as “In optics, an anisotropy property on the permittivity tensor, called form birefringence, occurs in sundry multiple quantum wells consisting of two constituents.” Regarding collagen fibers in tendons, a more complex structure arises [11,18,19].

Form birefringence has been used in structural nano-fabrication of various devices with special optical properties [21-24]. Special attention must be paid to of Neale et al. [25], showing construction of a microgear movement of which depends on the photonic moment generated by form birefringence nano-structures.

Formed studies have revealed wave like birefringent structures (crimp) with determined molecular ordering originated by the nanometric collagen molecules [11,26,27] (Figure 1). This finding was related to the possibility of generation interference spiral like figures to be diagnostic as vortices.

In the present work we have examined optical anisotropy images in rat tendon sections to search for optical vortex generation in a non-linear birefringent chiral body such as tendon collagen bundles. Here, polarized light is considered to have been sufficiently coherent because it passed through a polarized and monochromatic filter and vibrated in phase, thus being capable to inducing light interference. Furthermore, collagen fibers, as birefringent elements, generate an elliptical polarized front of light with an angular moment. According to reports by Neale et al. [25], form birefringent elements produce photonic momentum.

If a laser light source were necessary, the introduction of a Glan-polarizer would be required [9] in order for effects analogous to those described here to be produced, including physiological effects such as those reported in [9].

Materials and Methods

Wistar adult rats were euthanized in a carboxic gas chamber

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according to an animal care protocol approved by the Unicamp Institutional Committee for Ethics in Animal Experimentation (Brazil) and in accordance with the Guidelines of the Canadian Council on Animal Care. The samples under analysis consisted of skinless tails, fixed in a 4% paraformaldehyde solution in 0.1 M phosphate buffer at pH 7. The materials were dehydrated and embedded in araldite M (Electron Microscopy Sciences, Fort Washington, USA). Sections cut perpendicular to the long axis of the rat tails were prepared using a low-speed saw (model 650, South Bay Technology Inc. USA). The sections were sanded to approximately 80 µm and polished (Vidal, 2003). The observation of the birefringent images of the collagen fibers in bundles was performed using an Olympus BMX-51 polarization microscope equipped with Image Pro Plus 6.3 software.

Under the conditions of the observations, the collagen fibers oriented at 45° to the plane of the crossed polarizers exhibited the highest birefringence. After traveling through the collagen fibers the birefringent elements generated an elliptical polarized front of light with an angular momentum. The test to verify the elliptical condition of the emergent front of light was the use of the Senarmont's compensation, which transform elliptical polarized light in a plane polarized light with an angle to the analyzer to be compensate. The values of the angles could represent the angular momentum; these angles are not reported here; only a visual test was used.

**Results**

Examination of rat tail cross-sections by polarization microscopy demonstrated birefringence generation, which provides evidence of the supra-organization of the collagen fibers into bundles. To understand the birefringent images of the collagen fibers, an illustration was built that represents the relationship of the distribution of the collagen fiber axis with respect to the planes of polarized light (PPLs) formed by the crossed polarizer-analyzer (Figure 1). The birefringence observed in the cross-sections of the rat tail tendons shows domains of brilliance (birefringence intensity) and darkness (birefringence extinction) in the collagen bundles, forming a Maltese cross and revealing a spiral vortex image (Figures 1 and 2). The most intense brilliance was obtained for collagen fibers with their long axis oriented at 45° relative to the PPL (Figure 1). Surrounding the vortices, collagen bundles of the endotendon and peritendon display stronger birefringence intensity due to their greater aggregation (Figure 3).

The sections cut parallel to the long axis of the tendon revealed wave-like helical structures whose construction varied according to the helical pitch of the collagen bundles (data not shown); consequently, when these structures were examined in sections cut perpendicularly to the long axis of the tendon, a variable vortex morphology was captured (Figure 3). The periodic wave-like distribution of the collagen fibers, which depends on the microscopic wave-like collagen structure (crimp), was observed even macroscopically.

Linear polarized monochromatic light, after traveling through microscopic sections of collagen fibers, exhibited elliptically polarized front of light with angular momentum, which after passing through a second polarizer (the analyzer) generated vortices images.

Rotating the microscope stage while keeping the polarizer and analyzer crossed and motionless resulted in changes in the angle of the collagen fibers relative to the PPLs. This maneuver demonstrates the helical distribution of the collagen fibers structured as a vortex. The birefringence intensity at any specific point of the section changes with the rotation of the sample (Figure 3).

**Discussion**

Studies on the phenomena of the tendon’s intrinsic and form birefringence optical anisotropy have demonstrated that collagen bundles are helically structured [17,28]. An extruded collagen type I gel forms rods that exhibit optical birefringence typical of twisted-grain boundary liquid crystals [29]. The idea that chiral molecules generate a chiral body [10] applies to tendon collagen fibers [11].

In fact, polarized light traveling through birefringent collagen fibers in a tendon section emerge, exhibiting an elliptical polarized light with an angular momentum. Because this result occurs is the measurement of the optical path difference requires the use of Senarmont's compensation. This compensator converts the elliptical polarized light in the polarized plane with an angle that is measure [18,19]. For the detection of wave-like structures of collagen fibers by their birefringence and the corresponding molecular order, which is nano-scale, the use of this type of compensation is obligatory. Likewise, it is proved that form birefringent nano-motor can generate photonic momentum [25].

Because the birefringent images of the tendon collagen bundles contain morphological information that depicts a helical distribution for the collagen fibers [11,27-30], these images also provide evidence for the first time in biological matter that vortex structures are generated by chiral supramolecular collagen fibers in birefringent bundles and can be observed with a polarizing microscope. Morphology in this case contains information regarding the essential mechanisms of its generation, e.g., the molecular order, the supramolecular ordered aggregation state and the interaction of structured light with a structured biological sample.

There are strong indications that collagen bundles interact with cells by exchanging chemical signals [31,32]. It is hypothesized that the vortex structure acquired by collagen shown here may contribute to the mechanism of signal transference between cells and the extracellular matrix. Molecular order, if organized as an inducing vortex, could
Conclusions

The complexity of the corresponding vortex morphology may also vary in bundles in connective tissues may vary [29,32], it is assumed that the structures to achieve new optical properties. To date. Such structured biomaterials could also inspire man-made properties of collagen structured as in a vortex has not been proposed to improve signaling. Indeed, the exploration of anisotropic optical effects in the second harmonic signal of collagens I and IV. J Am Chem Soc 127: 10314-10322.

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