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Polarization Imaging and Characterization of Chitosan for applications in tissue engineering

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ABSTRACT

In this study we described the optical properties of Chitosan as a biomaterial using light polarization Mueller analytical model which are not well understood by conventional characterization techniques. Despite the physical, mechanical and chemical properties measured by Scanning Electron Microscope (SEM), X-ray Powder Diffraction (XRD) and FTIR (Fourier Transform Infrared) Spectroscopy, optical properties are very useful to investigate the biodegradable Chitosan based scaffolds in tissue engineering. It has been observed from the SEM, XRD, FTIR and polarimetric results (diattenuation, retardance, depolarization, and degree of polarization) that Chitosan has porosity, amorphous, compactable and anisotropic material.

1. Introduction

Study of biomaterials has become an active area of research due to its useful applications in our daily life. Now a day's biomaterials are used in tissue engineering, bone cartilage, wound healings, dressings and many other fields [1–3]. The biomaterial being study is Chitosan, a natural biocompatible polymer derived from the naturally occurring polysaccharide based biopolymer, Chitin, by deacetylation with an alkali leaving behind a free amino group. Chitosan naturally exists only in a few species of fungi but it is mainly extracted from the cuticular and exoskeletons of invertebrates like crustaceans, mollusks, crabs and shrimps. It has very good properties as a biomaterial, being a biodegradable, biocompatible, non-toxic and anti-thrombogenic. These properties made Chitosan widely applicable in the pharmaceutical and biomedical fields for controlled release of drugs, wound management and space filling implants etc. [4–7].

It is worthwhile to investigate the optical properties using Mueller matrix polarimetric method to observe optical behavior of Chitosan. The polarimetric method is the technique of measuring the polarization state of a light beam and the diattenuating, retarding and depolarizing properties of materials. We used a Mueller matrix formulism for characterizing polarization elements because it contains within its elements all the polarization properties: diattenuation, retardance, depolarization, and degree of polarization [8–13].

Beside optical property, the physical properties of Chitosan were also determined with Scanning Electron Microscope (SEM). The Scanning Electron Microscope is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity etc. [14].

The mechanical property of Chitosan were determined by X-ray Powder Diffraction (XRD), which is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample [15].

To investigate the chemical property of Chitosan, Fourier Transform Infrared Spectroscopy (FTIR) is used. It is an analytical technique used to identify organic and in some cases inorganic materials. This technique measures the absorption of infrared radiation by the sample material versus wavelength. The infrared absorption bands identify molecular components and structures [16].

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2. Theory

Polarimetry is a sensitive, nondestructive technique for measuring the optical activity exhibited by inorganic and organic compounds. A compound is considered to be optically active if linearly polarized light is rotated when passing through it. The polarimetric method is a simple and accurate means for determination and investigation of structure in macro, semi-micro and micro analysis of expensive and non-duplicable samples. Since polarization ellipse describes any state of completely polarized light and time average of the polarization ellipse after doing some manipulations we can get [17–20].

$$(E_{ox}+E_{oy})^2-(E_{ox}-E_{oy})^2-(2E_{ox}E_{oy}\cos\delta)^2=(2E_{ox}E_{oy}\sin\delta)^2 \quad (1)$$

The terms in parentheses are written as:

$$S_0=E_{ox}+E_{oy} \quad (2)$$

$$S_1=E_{ox}-E_{oy} \quad (3)$$

$$S_2=2E_{ox}E_{oy}\cos\delta \quad (4)$$

$$S_3=2E_{ox}E_{oy}\sin\delta \quad (5)$$

Above four equations are known as Stokes polarization parameters for a plane wave.

For partially polarized light [21],

$$DOP=\frac{\sqrt{S_1^2+S_2^2+S_3^2}}{S_o} \quad (6)$$

where *DOP* is defined as the degree of polarization. The stokes parameters can be arranged in a column matrix for any elliptically polarized light as:

$$S=\begin{pmatrix}S_o\\S_1\\S_2\\S_3\end{pmatrix}=\begin{pmatrix}E_{ox}^2+E_{oy}^2\\E_{ox}^2-E_{oy}^2\\2E_{ox}E_{oy}\cos\delta\\2E_{ox}E_{oy}\sin\delta\end{pmatrix} \quad (7)$$

It is not a mathematically vector, but for custom and common use it is called vector. Mueller calculus is a matrix method for manipulating Stokes vectors, which represent the polarization of incoherent light. It was developed in 1943 by Hans Mueller, then a professor of physics at the Massachusetts Institute of Technology. Light which is unpolarized or partially polarized must be treated using Mueller calculus, while fully polarized light can be treated with either Mueller calculus or the simpler Jones calculus.

The incident beam propagates through the polarizing element and emerges with a new intensity and polarized state. Both the incident and the emerging beam are characterized by four stokes parameters S_i and S_i', respectively, where $i=0, 1, 2, 3$.

$$\begin{pmatrix}S_0'\\S_1'\\S_2'\\S_3'\end{pmatrix}=\begin{pmatrix}m_{00}&m_{10}&m_{20}&m_{30}\\m_{10}&m_{11}&m_{21}&m_{31}\\m_{21}&m_{21}&m_{22}&m_{32}\\m_{30}&m_{31}&m_{32}&m_{33}\end{pmatrix}=\begin{pmatrix}S_0\\S_1\\S_2\\S_3\end{pmatrix} \quad (8)$$

or

$$S'=MS$$

where S' and S are the Stokes vectors of output and input beams respectively, and M is the 4×4 Mueller matrix. All the elements of the matrix have real values. A Mueller matrix provides significant information about the sample that is not observed by conventional technology [21].

The Scanning Electron Microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. Accelerated electrons in a SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. Thus characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is considered to be "non-destructive"; that is, X-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly [22].

X-ray Powder Diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d\sin\theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. A key component of all diffraction is the angle between the incident and diffracted rays [23].

Fourier Transform Infrared Radiation (FTIR) is a type of spectroscopy IR analysis that uses infrared radiation to record molecule movements via computer-based programs. To identify a component of certain compounds, they are exposed to high energy such as Infrared Radiation (IR). The reaction results to emission of energy showing the reactions of the molecules, which are automatically plotted to a graph by one of the programs embedded in spectroscopic instruments. Using the generated graph, organic chemists analyze the plot and detect distinctive peaks that can be attributed to the components of the compound [24].

It is in the field of health that the many properties of Chitosan (bacteriostatic, immunologic, antitumoral, cicatrizant, haemostatic and anticoagulant) are of interest. For example, because of its biocompatibility with human tissue, Chitosan's cicatrizant properties have proven its effectiveness as a component, notably, in all types of dressings (artificial skin, corneal dressings, etc.), surgical sutures, dental implants, and in rebuilding bones and gums. Applications currently being developed include artificial skin, surgical sutures that are absorbed naturally after an operation, and corneal contact lenses. Finally, Chitosan delivers and time-releases drugs used to treat animals and humans. There are many potential Chitosan applications in the health field but their development calls for the use of components that comply with strict pharmaceutical-grade requirements.

Not only due to its multitude of applications but due to increasing environmental awareness of the population, biodegradable, and non-toxic products from 'natural' sources such as Chitin and Chitosan are going to be more and more appealing for the replacement of synthetic compounds. Moreover, in cosmetic and in biopharmaceutical industries, Chitosan has exclusive properties which are not found in other synthetic products.

3. Material and methods

We have used AxoScan™ Mueller matrix polarimeter system developed by Russell Chipman and Matthew H. Smith to determine the characteristics of the Chitosan [25,26].