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ORIGINAL CONTRIBUTION

Microstructure and Thermal Behavior of a Biocompatible Naturally Occurring Novel Cellulose Film

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Abstract:

Naturally occurring biopolymer based thin films have enormous importance in environmental and medical engineering. This investigation deals with a new cellulose sheet which is naturally occurring biopolymer and mainly composed of cellulose. The film is supported by several fibers of uniform diameter (3 μm) and distance between each fiber is 2 - 6 μm. Each fiber is formed by the aggregation of several nanofibers (200 nm diameter). DSC and TGA analyses have been employed to investigate the decomposition processes and decomposed products of the film. The thermograms show that the film stables in ambient pressure and above room temperature. The decomposed temperature of the film is 318 ºC. This film does not exhibit any cytotoxic effect on mammalian cells which has been evaluated by hemolytic assay and cell cycle analysis using colon cancer cell, HCT 15. Thus it may have potent application in food packaging industry and medical sciences.

Keywords: cellulose film, cytotoxicity, food packaging, hemolytic assay, thermal behavior

Abbreviations: DSC (differential scanning calorimeter); HCT15 (human colon cancer cell line); RPMI medium (Roswell Park Memorial Institute medium 1640); TGA (thermo-gravimetric analysis); Tm (melting temperature).

1. INTRODUCTION

Cotton is an enormously important commodity throughout the world. Cotton is used to make a number of textile products including medical and hygienic purpose[1]. Cotton fibers are mostly composed of the long chain carbohydrate polymer with 95% cellulose. Cellulose is a linear homopolymer, consisting of ß-1, 4-glycosidic linked D-glucopyranose units which occurs in small crystalline microfibrills and arranged in multilayer structure[2]. Several film producing industries commonly use petrochemical-based product as their raw materials. These petrochemical based films have a good mechanical strength and elasticity but they are totally non-biodegradable leading to serious environmental problems. Since the last two decades, scientists and technologists are trying to develop biodegradable packaging materials from renewable biomass like various biopolymers from plant-based agricultural waste materials[3]. Study of thermal decomposition of film is useful to understand the fire-resistant properties of the materials[4]. Differential scanning calorimeter (DSC) and thermo-gravimetric analysis (TGA) are most valuable techniques to evaluate the thermal properties of textile materials[5]. However, DSC spectra only provide changes of temperatures of the material in pyrolysis process. TGA shows weight loss of fibers under different temperature, but not the concrete pyrolysis processes and products. Similar studies in this area have been made by several researchers[6-7]. Several studies have been reported on the biodegradable packaging films made of cellulose, chitosan, and gluten biopolymer[8-10]. The present investigation deals

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with a new naturally occurring biopolymer as cellulose films that have been attached with their seeds like wings. The aim of this study was to characterize its physical properties, thermal behavior and biocompatibility as an alternative food packaging films and applications in medical sciences.

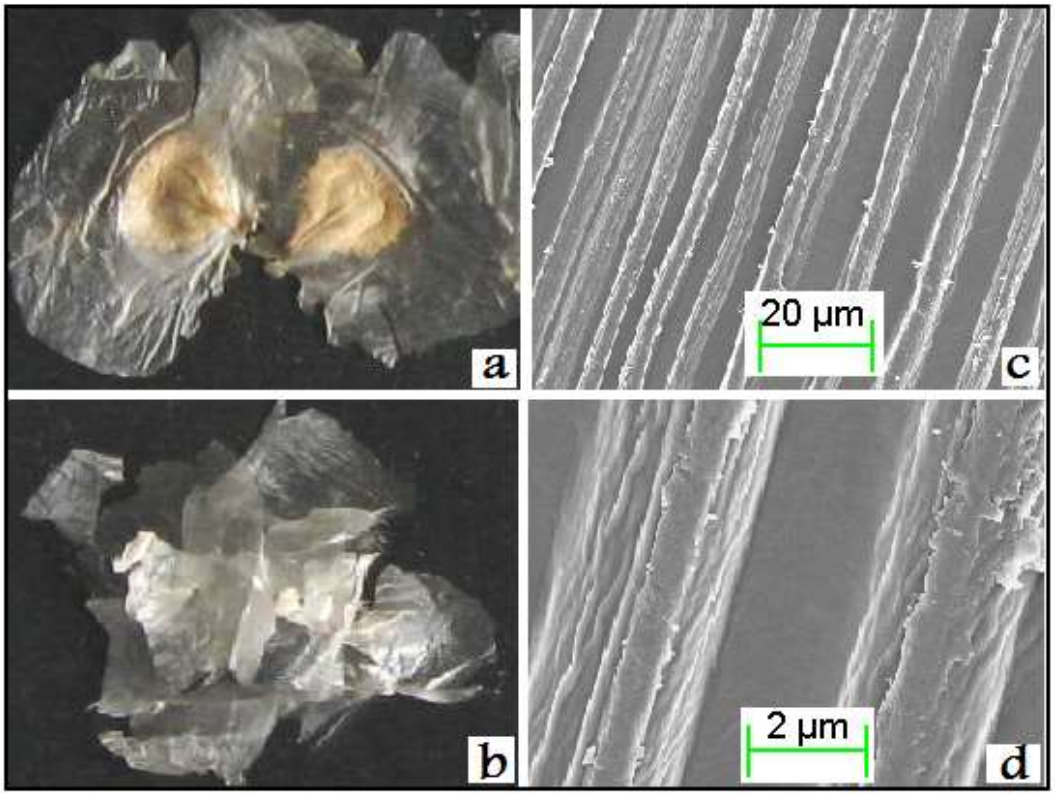


Figure 1: Photographs of ground dry cellulose films taken with digital camera (a) with seed and (b) without seed. (c) FESEM images of the film with (d) a magnified image of nanofibers

2. MATERIAL AND METHODS

2.1. Thickness and solubility

The sample was collected from the silk cotton plant (*Ceiba* sp.), located at IIT-Kharagpur campus, India. Thickness and solubility of ground dried cellulose film was determined. Film thickness was measured by using a digital micrometer (accuracy ± 0.001 mm). Measurements were performed 10 times, and the average value was calculated. Portion of the film weighting 0.051 g was put into 6 ml of distilled water and allowed to be swollen for 24h. Subsequently it was taken out and dried in an environment at a temperature of (30 ± 2) °C and a relative humidity of (60 ± 4) % for 48 h before taking it final weight. The solubility (%) values were calculated as: solubility (%) = [(initial wt.-final wt.)/ initial wt.] ×100.

2.2. Thermal behavior

The thermal transitions that occurring in cellulose fibers were characterized by using a DSC of M/S Perkin Elmer, (Diamond DSC). The sample weight was about 2-4 mg and the measurement was carried out under N_2 atmosphere with a heating rate of 5 °C/min. TGA analysis was carried out from room temperature to 500 °C at a heating rate of 5

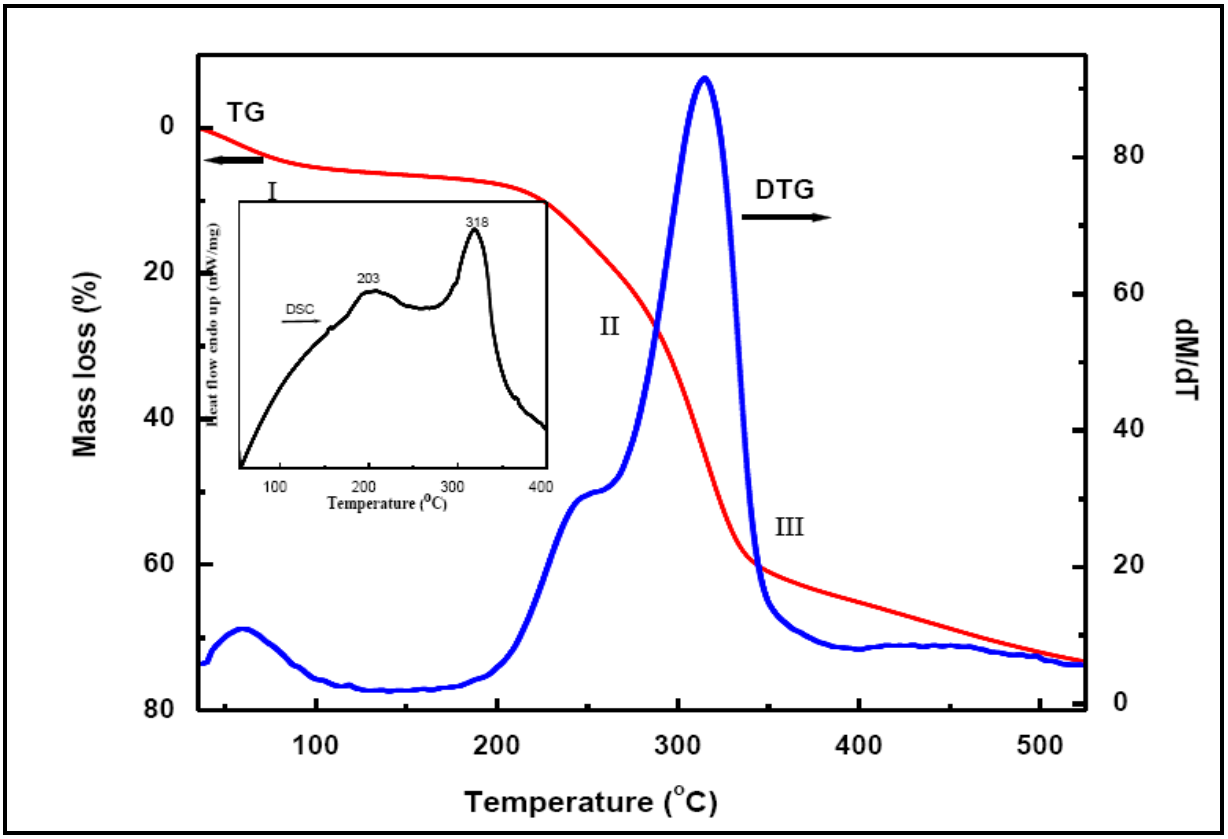


Figure 2: TG-DTG thermograms of film. DSC thermogram (inset) was obtained by measuring at 5 °C/ min heating rate in N_2 atmosphere

°C/min using M/S Perkin Elmer (Pyris Diamond TG/DTA) in air. Microstructural surface morphology of the films were studied under a field emission-scanning electron microscope (FE-SEM) of Carl Zeiss, model Supra 40, with an accelerated voltage 5-20 kV. The samples were gold sputtered to give electronic conductivity under a vacuum prior to such observations.

2.3. Scanning electron microscopy and Fourier transform infrared spectroscopy

The Fourier transform infrared spectrum (FTIR) was studied of dried raw film. A Nexus^{TM} 870 FT-IR (Thermo Nicolet, USA) spectrophotometer equipped with a deuterated triglycine sulfate thermo electric cool (DTGS-TEC) detector was used to collect the data.

2.4. Cell Cycle Analysis and Hemolytic Assay

Cell cycle analysis was determined of HCT 15 cell line after grown in RPMI-1640 medium and the distribution of cells in the different cell cycle phases was analyzed from the DNA histogram using Becton-Dickinson FACS Caliber flow cytometer and Cell Quest software[11-12]. Hemocompatibility study was performed using standard protocol with some modification. In brief, blood was obtained from 6-week-old BALB/c male mice and Red blood cells (RBC) were collected by centrifugation (1500 g, 5 min, and 4° C) of the blood. The

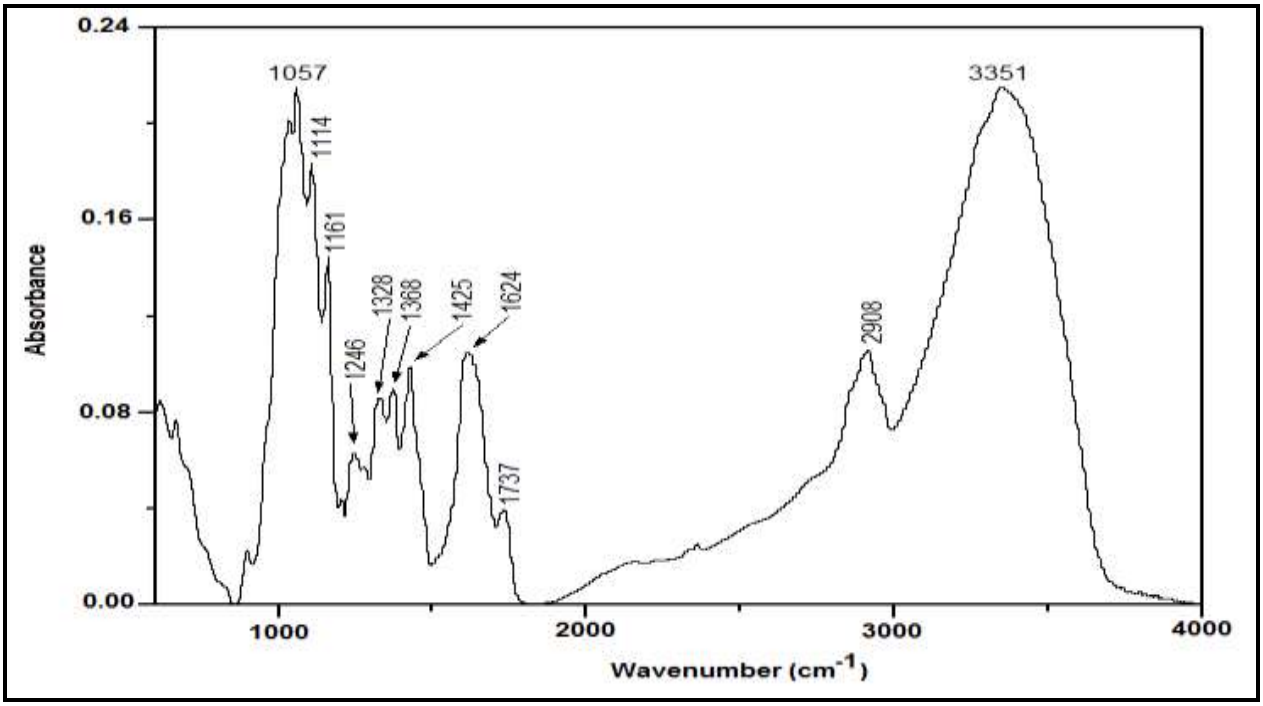


Figure 3**:** FTIR spectrum of dried isolated film

collected RBC pellet was diluted in 20 mM HEPES buffered saline (pH 7.4) to make a 5% (v/v) solution. The RBC suspension was added to HEPES-buffered saline (-ve control), 1.0% Triton X-100 (+ve control) and the film, and incubated for 30 min and 60 min at 37°C. After centrifugation (Heraeus table top centrifuge 5805R) at 12,000 rpm at 4°C, the supernatants were transferred to a 96-well plate. Hemolytic activity was determined by measuring the absorption at 570 nm (Biorad Microplate reader 5804R). Control samples of 0% lysis (in HEPES buffer) and 100% lysis (in 1% Triton X-100) were employed in the experiment (2). All assays were performed in triplicate. Hemolytic effect of each treatment was expressed as percent cell lysis relative to the +ve control cells (% control) defined as: [(Abs570 samples)/ (Abs570 +ve control cells)] × 100, where absorbance is abbreviated to Abs.

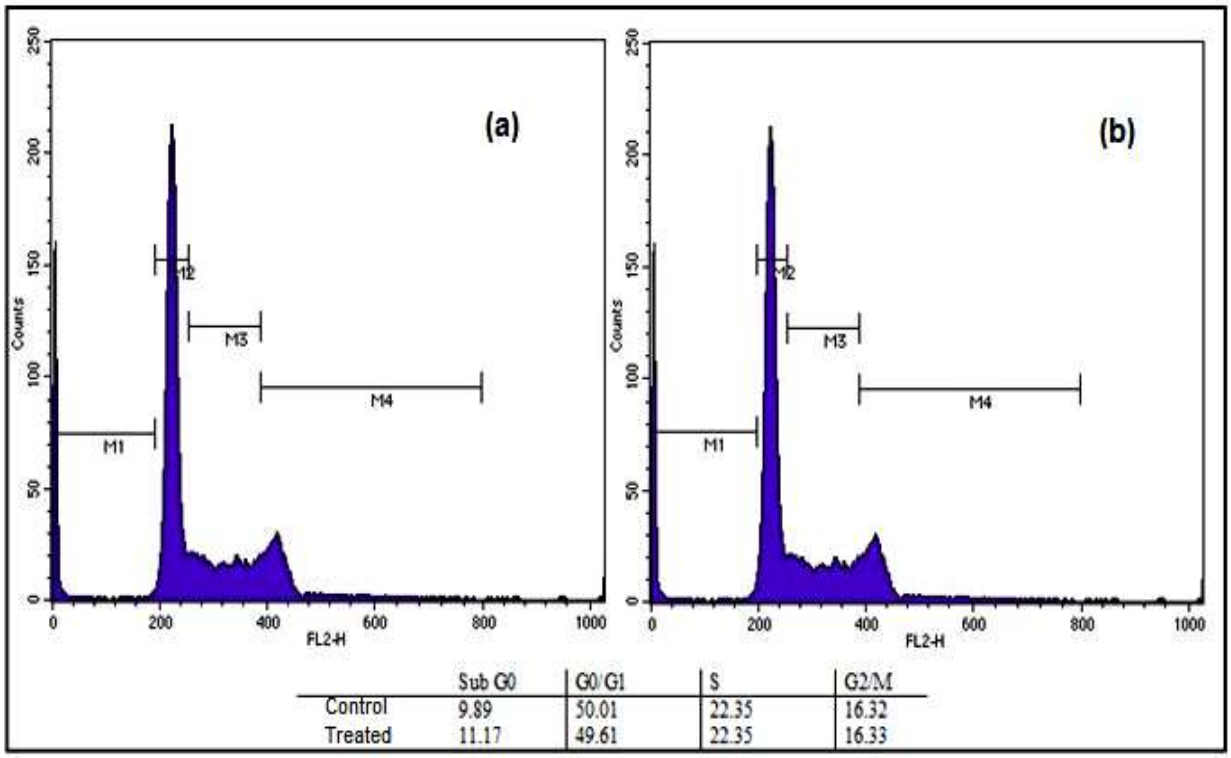
3. RESULTS AND DISCUSSION

3.1. Microstructure of film

A photograph (Fig 1a and 1b) of ground dry film was taken from with and without seed using a digital camera and it was found that the seeds were surrounded by a thin film like wings. FE-SEM images of the film surface showed the regular pattern of fibers (diameter ~ 3 μm) which was supported on a plain sheet (Fig 1c). The fibers are arranged in a parallel fashion and the average distance between each fiber is varied between 2 to 6 μm. A magnified FE-SEM image showed that each fiber was made by the aggregation of several nanofibers (diameter ~ 200 nm) which are arranged in a bunch manner (Figure 1d).

3.2. Thickness and solubility of film

The average film thickness was 0.008 ± 0.0001 mm. Most important properties of a packaging film depend on its solubility in aqueous solution. Potential applications of a food packaging film required low water solubility to enhance the product integrity and



	Sub G0	G0/G1	S	G2/M
Control	9.89	50.01	22.35	16.32
Treated	11.17	49.61	22.35	16.33

Figure 4: A; Cell cycle distribution pattern of HCT 15 cells grown in absence (a) and presence (b) of film for 24h

water resistance capacity. The solubility of the film is ~ 15 %, which is several folds lower than other synthetic biodegradable films[13].

3.3. Thermal analysis of film

DSC and TG/ DTG analyses of fibers revealed that the decomposition of fiber includes three stages: initial, main, and char decomposition (Fig 2). Subject to heating in air, a thermal decomposition occurs over room temperature to 500 °C. The decomposition may go from amorphous regions to crystalline regions in fibers. The TG curve yields a total mass loss ~74 % of the initial mass of the sample in three successive steps as marked over the curve by the symbols I, II, and III. The DTG spectrum showed a broad band over 45-150 °C, with mass loss ~8 % in the region I (Fig. 2). In this region, the most important changes of the fibers are due to some physical properties and the damage of the cellulose occurs mostly in amorphous region of the polymers. In the temperature range between 200-350 ºC (region II in Fig 2), the polymer fiber mass is decreasing sharply (mass loss ~58 %). DTG shows a solder over 150-290 °C, which follows a well-defined sharp peak at 315 °C. In this stage, the weight loss is very fast and significant. Most of decomposed products are produced in that stage. Glucose is one of the major products, together with all kinds of combustible gases like CO_y (y = 1-2). The decomposition takes place in crystalline region of cellulose fibers in this stage. In DSC thermogram, 318 °C is the melting temperature of the sample (inset in Fig 2). The char decomposition occurs at the temperature above 350 °C (region III in Fig 2). During this process, dewatering and charring reactions compete with the production of glucose. The mass decomposition continues to dewater and decarboxylization, releasing more water and CO_2 and producing carboxyl and

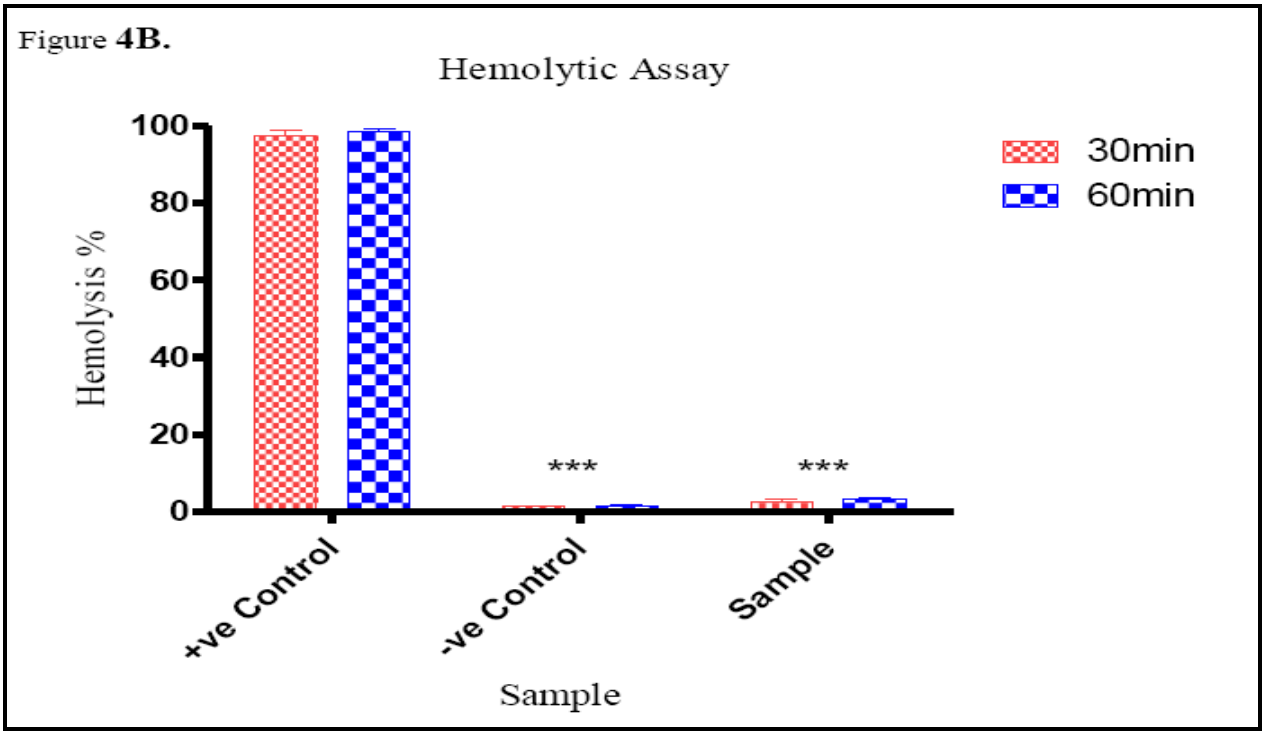


Figure 4.B: Hemolytic assay of the film. Red blood cells were collected by centrifugation of the blood and resuspended in HEPES-buffered saline. The cell suspension was added to HEPES-buffered saline, 1% Triton X-100 and sample then incubated for 30 and 60 min at 37°C. After centrifugation, hemolytic activity was determined by measuring the absorbance (570 nm) of the supernatant. Cellulose film does not exhibit any significant amount of hemolysis respect to the +ve control after 30 min and 60 min treatment. Control samples of 0 % lysis (in HEPES buffer) and 100 % lysis (in 1% Triton X-100) were employed in the experiment. The bars indicate the means ± SD (n = 3). Significant difference is shown as ***$p < 0.001$ versus +ve control.

carbonyl products. The carbon content in the decomposed products becomes higher and charred residues are formed. It is cleared that the melting temperature of this film is almost three fold higher than commercially available polyethylene (http://en.wikipedia.org/wiki/Polyethylene). The melting point of average and commercial low-density polyethylene is typically 105 to 115 °C (220 to 240 °F). Finally, the thermograms believed that fibers are stable in ambient pressure and until above room temperature.

3.4. FTIR analysis of film

FTIR spectroscopic approaches have been widely used to distinguish the broad categories of cellulosic, proteinaceous, regenerated natural fibers and many different kinds of synthetic fiber[14-16]. The FTIR spectrum of film (Fig 3) shows a broad band between 3600 and 3000 cm^{-1}, corresponding to vibrations of the hydroxylic groups. Methyl and methylene group vibrations around 2908 cm^{-1} were also present in the spectrum. Structural features arising from particular conformations around 1161 cm^{-1} of C-C ring breathing band from lignin; at ~1737 cm^{-1} of C=O ester band from pectin or carbonyl groups of oxycelluloses, each can be considered as representative of the proportion of that component within the film. The C-O-C glycosidic ether band at ~1057 or 1114 cm^{-1} arose from the polysaccharide components, mainly cellulose. The intensity of the C-H

stretching vibration at ~2908 cm^{-1} was taken as a measure of the general organic material content of the fiber[17]. The fibers were characterized by based on the intensities of the bands at 2900, 1057-1161, and 1737cm^{-1} were taken to represent the overall organic content, the lignin, pectin and the cellulose.

3.5. Cytotoxicity Study by Cell Cycle Analysis

Biocompatibility of a biomaterial refers to the extent to which the material does not have toxic or harmful effects on biological systems. In the pre-commercialization steps of a biomedical-grade biopolymer (natural and synthetic polymers) runs into a number of pre-toxicity tests, *in vitro* or *in vivo*. Cottons are generally used in medical science for wound dressing. It is necessary to determine the biocompatibility of any biomaterial in order to evaluate its strength, esthetics and feasibility for clinical manipulation. Thus, preliminary *in vitro* tests are continually being carried out to screen and characterize the potentially harmful effects of a dressing material before it is commercially used on humans[18]. Here, we have used the colon cancer cell, HCT 15 and checked the effect of film on morphology and cell cycle distribution on it. It was found that no morphological changes were observed after exposure to films for 0h, 24h, 48h and 72h. Moreover, it did not show any morphological changes like cell shrinkage, membrane blebbing, loss of their attachment to the substratum, rounding, and fragmentation compared to the control cells (Figure not shown) which indicates the compatibility of this film to the colon cancer cell used in the experiment. In addition, HCT 15 treated with the cellulose film for 0hr and 24hr did not produce significant change of cell number in different phase of cell cycle (Fig 4A).

3.6. Evaluation of Red Blood Cell Lysis

In this study, hemolytic assay was performed to examine the interactions of film with negatively charged red blood cell membrane. The membrane-damaging property of the sample was analyzed by the quantification of released haemoglobine. After 30 min and 60 min of incubation with film, it displayed a lower membrane-damaging effect causing a significantly lower haemoglobine release than the +ve control. Also no significant difference in hemolysis could be detected between sample and –ve control after 30 min of incubation (Fig 4B). Extending the incubation time of sample to 60 min did show the same result. These results are in agreement with the morphological and cell cycle analysis studies, which further confirm the biocompatibility of the film. It revealed that this naturally occurring cellulose film has no adverse effect on mammalian cells.

4. CONCLUSION

This naturally occurring novel cellulose film is reporting first time and demands an extensive studied for environmental or medical application. It may be considered to be the alternative for non-biodegradable food packaging films because of its characteristics similar to those of many other biopolymer-based films obtained from cellulose, xylan or lignin based. In medical applications, such thin film sheets can represent a scaffold for skin where mammalian colon cells grow without any cytotoxic effect and may be used in wound management as dressing materials like cotton.

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