Natural Nanomaterials: Synthesis and Cytotoxicity Analysis

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Abstract

In this research, natural nanomaterials including cellulose nanocrystal (CNC) and cellulose nanofiber (CNF) with different structures, sizes and surface areas were produced and analyzed. The aim of this study was to compare these nanomaterials based on the effects of their structures and compositions on the cytotoxicity properties.

Introduction

Kenaf is a natural tropical plant that has been grown commercially to generate secondary source of income for developing countries including Malaysia. Its high cellulose content, ranging between 44 and 63.5% (Jonoobi et al.,2009; Sanadi et al., 2001; Zampaloni et al., 2007) has generated interest in exploiting the material as nanofillers in composites. The nanocrystals and nanofibers can be obtained via acid hydrolysis, mechanical treatment, or enzymatic reaction. The chemical composition of kenaf bast is around 63.5 % cellulose, 17.6 % hemicellulose and 12.7 % lignin (Janoobi et al. 2009).

Cellulose is the most abundant polymer in nature and has long been a major renewable source of materials [1]. Cellulose is a linear natural polymer of anhydroglucose units linked at the one and four carbon atoms by b-glycosidic bonds (Mandal and Chakrabarty , 2011). The cellulosic microfibrils have disordered (amorphous) regions and highly ordered (crystalline) regions as illustrated in Figure 1. The nanofibrillar domains, generally referred to as nanocellulose are a promising raw material for new bio based composites due to their high mechanical strength, stiffness, large surface area, low thermal expansion, optical transparency, renewability, biodegradability, low cost and low toxicity (Brinchi et al.,2013).

Apart from their use as a reinforcing filler for polymers, cellulose nanocrystals (CNC) and cellulose nanofiber (CNF) have been used to fabricate a wide range of other functional materials, including transparent barrier films (Fukuzumi et al., 2008), photonic crystals (Kelly et al., carriers, composite materials (Walther et al.,2011) [10], optical and electronic devices (Mendez and Weder, 2010) and super capacitor electrodes (Nyström et al., 2009).

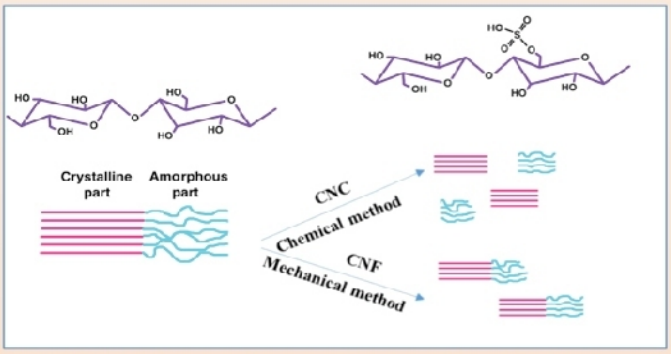

Fig. 1. Crystalline and amorphous region of cellulose and the mechanism of chemical and mechanical treatment for producing CNC and CNF from cellulose

Synthesis of Nanofibers

Here, CNC and CNF were produced by using acid hydrolysis and mechanical methods, respectively. For preparation of CNC, firstly, acid hydrolysis will be conducted under mechanical stirring by use of H2SO4 for 45 min. Then the suspension was diluted with cold distilled water and centrifuge for 10 min. This centrifugation step repeats three times. The aqueous suspension was subsequently dialyzed against distilled water until a constant pH was attained. Ultrasonic treatment was then being carried out to disperse the nanocrystals.

For fabricating CNF, water retted Kenaf bast fibers coded as RF was cut to short pieces and then cooked in a

JSR-212 rotatory digester with NaOH and anthraquinone (AQ) solution at 160°C for 2 h. AQ was be added to the cooking liquor to enhance the delignification rate and also protect the fibers from alkali degradation and so called end-wise degradation of cellulosic chains. The obtained pulp was washed and screened thoroughly. Extraction of nanofibers was then done by further mechanical destruction using a super-masscolloider. Aqueous suspensions with the concentration of 3wt% was prepared and blended until gel was formed.

The FESEM and TEM images of the produced nanocellulose were shown in Fig 2. CNCs present a simple needle-like structure with an average length of 200nm, and diameter of 20nm. CNFs exhibited a complex, highly entangled, web-like structure. Twisted/untwisted, curled/straight, and entangled/separate nano-fibrils and their bundles with diameters ranging from 50 to 200 nm in diameter can be identified from the micrograph.

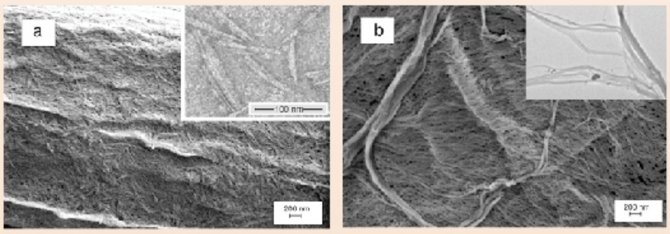

Fig. 2. (a) SEM and TEM images of cellulose nanocrystal (CNC) and (b) cellulose nanofiber (NFC)

Cytotoxicity Effect on Human Cells

Cell viability of produced nanocellulose was tested by MTT assay. The relative cell viability (%) related to control wells containing cell culture medium without nanoparticles was calculated by the following equation:

$[A]_{test} / [A]_{control} *100$

Based on the results shown in Figure 3, The CNF and CNC compound inhibited about 1.1% and 7% of cells at concentration of 100 µg/ml, respectively. At concentration of 12.5 µg/ml, CNF did not exhibit any toxicity towards the cell, as the cells were 100% viable. However this was in contrary to CNC where it was found that the CNC managed to inhibit 1.1% of cells at the same concentration.

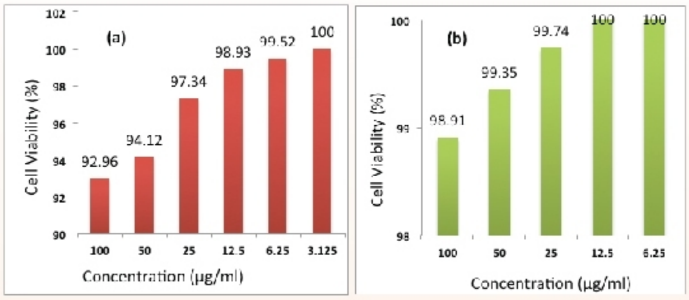

Fig 3. The cytotoxicity effects of (a) CNC, (b) NFC

Conclusions

In this research, we produced natural nanofibers (CNC and CNF) by using acid hydrolysis and mechanical techniques. SEM and TEM exhibited that CNC had smaller diameter size compared to CNF. The influences of morphology, size and structure of nanomaterials on their cytotoxicity properties were investigated. Overall, CNF had lower cytotoxicity effects rather than CNC which could be related to its preparation methods.

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