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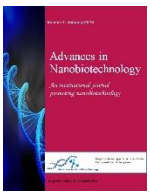

Comparative study of cytotoxicity effect between cellulose nanocrystal and cellulose nanofiber

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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 most important challenge in this study is to compare these nanomaterials based on the effects of their structures and compositions on the cytotoxicity properties. The characteristics of these nanomaterials such as morphology, structure, and composition and also the cytotoxicity effects of these nanomaterials on living cells were investigated.

1. Introduction

Kenaf is a natural tropical plant that has been grown commercially to generate a secondary source of income for developing countries including Malaysia. Its high cellulose content, ranging between 44 and 63.5% in kenaf, [1-3] 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 [1].

These microfibrils have disordered (amorphous) regions and highly ordered (crystalline) regions as illustrated in figure 1. Based on the Figure 1, to produce different forms of nanocellulose including CNC and CNF, the chemical and mechanical methods are used, respectively. When the chemical method is used, the amorphous part is omitted while at mechanical technique this part is remain in the structure of nanocellulose. 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 [4].

Cellulose is the most abundant polymer in nature and has long been a major renewable source of materials. Cellulose is a linear natural polymer of anhydroglucose units linked at the one and four carbon atoms by b-glycosidic bonds [5].

Apart from their use as a reinforcing filler for polymers, cellulose nanocrystal (CNC) and cellulose nanofiber (CNF) have been used to fabricate a wide range of other functional materials, including transparent barrier films [6], photonic crystals [7], shape-memory polymers [8], drug carriers, composite materials [9], optical and electronic devices [10], and super capacitor electrodes [11].

2. Experimental and Results

2.1. Production of natural nanofibers

Here, CNC and CNF were produced by use of acid hydrolyzing and mechanical methods, respectively. For Preparation of CNC, firstly, acid hydrolysis will be conducted under mechanical stirring by use of H_2SO_4 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 subsequently dialyzes against distilled water until a constant pH attain. Ultrasonic treatment then carries out to disperse the nanocrystals [12]. For fabricating CNF, water retted Kenaf bast fibers coded as RF will be cut to short pieces and then cook in a JSR-212 rotatory digester with NaOH and anthraquinone (AQ) solution at 160°C for 2 h [13]. AQ will 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 will be washed and be screened thoroughly.

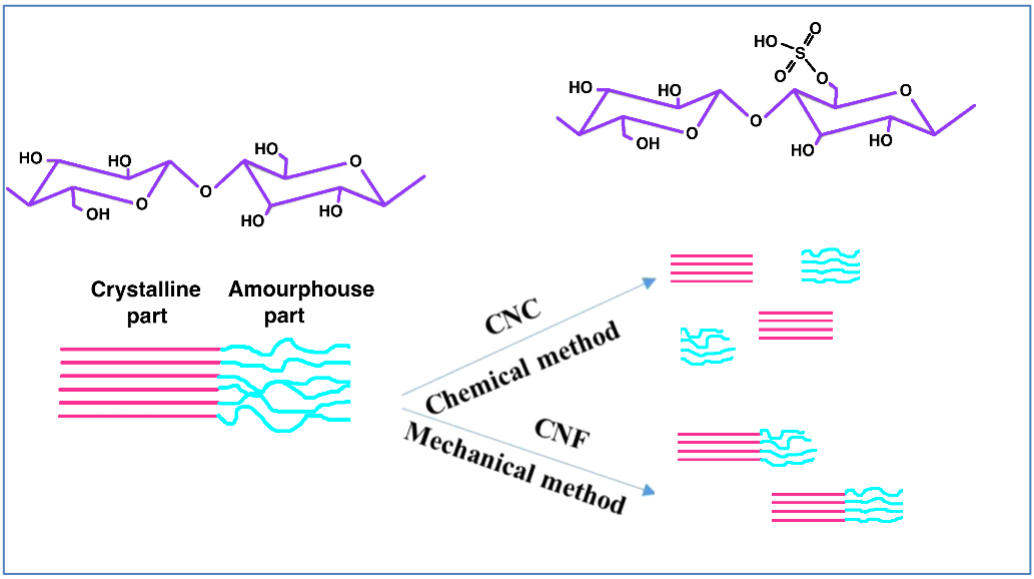

Fig. 1. Chemical and mechanical methods for producing CNC and CNF from cellulose

Extraction of nanofibers will be done by further mechanical destruction using super-masscolloider (several times). Aqueous suspensions with the concentration of 3wt% will be prepared and prepared and blend until formation of a homogeneous mixture. Consequently, the suspensions will be fed into the grinder and the process repeated until a gel was formed.

The FESEM and TEM images of the produced nanomaterials were shown in Fig 2. CNCs present a simple needle-like structure with an average length of 200nm, a diameter of 20nm. CNFs exhibit 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.

2.2. Cytotoxicity effect on human cells

In order to analysis the cytotoxicity potentials of the different nanomaterials and cell viability, the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) dye reduction was used. The cytotoxic effect of the nanomaterials could be measured by using this assay based on the IC_{50} generated. A 100µL of 4T1 cells at 0.8 x 10^5 cells/well concentration was poured into a 96-well plate and kept in the RPMI medium for 24 hours.

The next day, nanomaterials were added to the wells and then incubated for 72 hours.

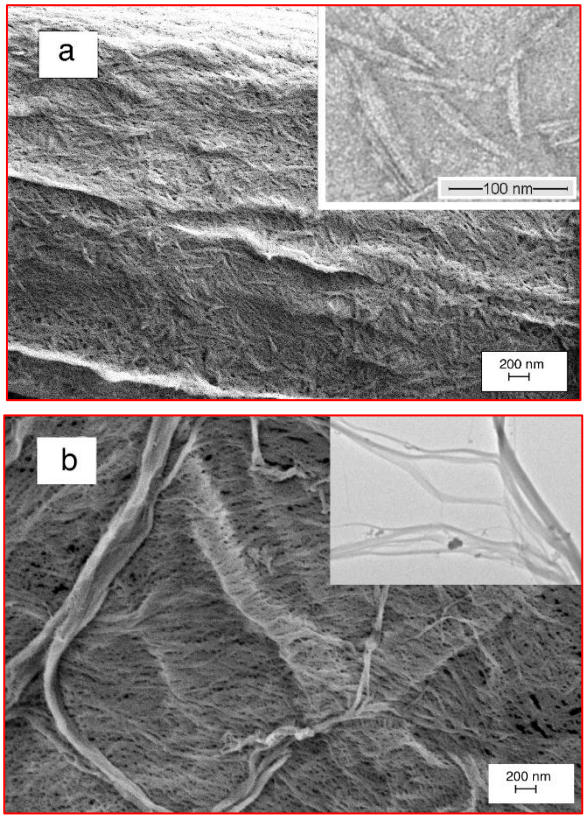

Fig. 2. (a) SEM and TEM images of cellulose nanocrystal (CNC) and (b) cellulose nanofiber (CNF)

MTT solution (5mg/ml) (Calbiochem) was added, separately, at a volume of 20µL into each well and incubated for 3 hours. Later, the solutions were removed from wells and 100µL of DMSO was added to solubilize the formazan crystals. Finally, the plate was read using an ELISA plate reader at a wavelength of 570 nm (Bio-Tek Instruments, USA) [14]. 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 and 4, The CNF and CNC compound inhibited/killed about 1.1% and 7% of cells at concentration of 100 ug/ml, respectively. At concentration of 12.5 ug/ml, CNF did not exhibit any toxicity towards the cell, as the cells were 100% viable. However, it was not the case for CNC compound as it still managed to kill 1.1% of cells.

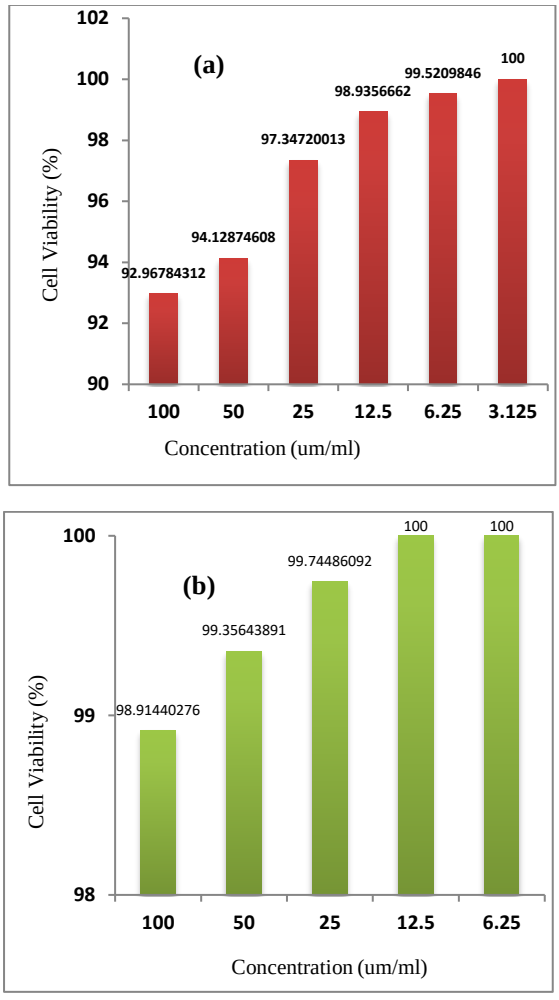

Fig 4. The cytotoxicity effects of (a) CNC and (b) CNF.

3. Conclusions

In this research, we produced natural nanomaterials (CNC and CNF) by use of acid hydrolyzing and mechanical techniques. SEM and TEM images not only confirmed the crystalline nature of CNC but also determined the smaller size against CNF. The influences of morphology, size and structure of nanomaterials on their cytotoxicity properties were investigated. Generally speaking, CNF has lower cytotoxicity effects rather than CNC may be related to their preparation methods.

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