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Review

Cellulose nanocrystals as carriers in medicine and their toxicities: A review

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ABSTRACT

Cellulose nanocrystals (CNCs) are crystalline nanoparticles that present myriad applications. CNCs are produced from a variety of renewable sources, and they can be chemically modified. Although there are promising perspectives for introducing CNCs into pharmaceutical formulations, prior to achieving commercial products the influence of many parameters such as extraction and toxicity of the resulting products must be revealed. Since there is great physicochemical flexibility in the steps of obtaining and conjugating CNCs, there are uncountable and complex outcomes from the interactions of those parameters. We present a discussion that helps to unveil the whole panorama on the use of CNCs as drug delivery systems. The methods of producing CNCs are correlated to the resulting nanotoxicity from the cellular to organism level. This review points to relevant concerns that must be overcome to attain safe use of these nanostructures. We also discuss the patents and commercially available products based on CNCs.

1. Introduction

Nanocellulose is a general term for cellulose-based nanostructures, which have been increasingly studied in recent years mainly due to their flexibility in regard to chemical modifications, physical properties and the possible myriad applications. From a technological point of view, by searching in patent databases with the term “nanocellulose” the versatility and high potential of nanocellulose-based composites in different applications can be revealed (Charreau, Foresti, & Vázquez, 2013; Durán, Lemes, & Seabra, 2012; Trache, Hussin, Haafiz, & Thakur, 2017). These nanostructures were discussed in a previous article (Lin & Dufresne, 2014) that compared three different types of nanocellulose: (i) cellulose nanofibrils (CNFs), (ii) cellulose nanocrystals (CNCs) and (iii) bacterial cellulose (BC) in terms of production, chemical and physical properties and also for their potential applications in medicine. CNCs, or nanowhiskers, represent nanoparticles extracted from lignocellulose fibers by hydrolysis with strong acids, while CNFs comprise nanoparticles extracted by the use of strong mechanical (shear) forces (Charreau et al., 2013). In addition, BC represents microbially produced CNFs. The authors emphasized the importance of functional chemical and physical modification of nanocellulose, since this process will determine its toxicity and biomedical properties. On this latter aspect, since CNCs and CNFs are able to bind and release water-soluble molecules through ionic interactions, they can be thought of as drug delivery or drug depot systems. Recently, these perspectives were discussed (Plackett, Letchford, Jackson, & Burt, 2014). However, although there is consensus on the recent progress achieved on this topic, including investigation of the biocompatibility and fate of nanocellulose *in vivo*, Plackett et al. (Plackett et al., 2014) pointed out that most studies were carried out at an academic level, and hence did not focus on preparing specific drugs for practical disease treatment.

Another recent review discussed the chemical structure of CNCs and the available physical and chemical isolation procedures, and also described optical, mechanical and rheological features of CNCs (George & Sabapathi, 2015). In addition, the review highlighted the novel applications of CNCs in diverse fields such as electronics, material sciences, catalysis and biomedical engineering. Furthermore, (Rojas, Bedoya, & Ciro, 2015) presented and discussed recent progress in nanocellulose applications based on the production of face masks, bandages, skin replacements for implants, burns, cuffs for nerve surgery, artificial

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blood vessels, cell carriers and support matrices for the immobilization of enzymes. Jorfi and Foster (Jorfi & Foster, 2015) reported advances in the fabrication and design of advanced nanocellulose-based biomaterials with promising biomedical applications. The authors also discussed the material requirements for each therapeutic application, as well as the challenges that these nanostructures might face in the pharmacological field. More recently, cellulosic nanomaterials were reviewed regarding their production and the influence of the biomass source on the morphological characteristics of the generated nanostructure, as well as the chemical functionalization that can be performed from the hydroxyl radicals on their surface (Mondal, 2017). The nanocellulose physical and chemical properties were correlated with their applications in the contexts of biomedicine, energy, in nanocomposites and especially for environmental issues (Mondal, 2017).

A common concern among researchers is the toxicity of nanocellulose. Roman (Roman, 2015) reviewed the literature on the cytotoxicity, oral, pulmonary and dermal toxicity of CNCs. Apparently, no toxicity was demonstrated upon dermal and oral administration of CNCs. However, inconsistent results were reported for pulmonary administration of CNCs. Moreover, the author highlighted the necessity of further studies focused to evaluate the possible side effects upon ingestion or skin contact with CNCs. Also, some warnings were raised related to some factors that interfere after processing cellulose (e.g., the presence of endotoxins or toxic chemical impurities). These aspects could be responsible for some adverse health effects through various exposure routes (Roman, 2015). In face of this panorama, a better understanding of the potential human health risk of nanocellulose will come only from the compilation of all possible factors (Camarero-Espinosa et al., 2016). Firstly, it is important to quantify the toxic dose for human exposure, in particular by skin and inhalation routes. The same applies for environmental applications.

Overall, the aspects that might affect the toxicity of CNCs are (i) size and morphology, (ii) degree of crystallinity, (iii) surface chemistry and (iv) colloidal stability. Another important aspect is that new protocols are necessary for establishing facile and reliable identification of the size and surface chemistry of the nanoparticles, in order to provide a realistic comparison between different studies. This necessity has been previously expressed for other carbon-based nanomaterials, such as oxidized carbon nanotubes (CNTs) and graphene oxide (GO) (Faria et al., 2012; Padovani et al., 2015; Seabra et al., 2014; Seabra, Paula, & Durán, 2013). Rather than having a defined chemical composition, they can have a variety of chemical groups functionalizing the surface, distributed in a random fashion. As a consequence, for these nanostructures there is random distribution of surface charges as well as complex stereochemical behavior. Similar behavior occurs for functionalized nanocellulose-based nanostructures. Furthermore, CNFs and CNCs intrinsically present a polydisperse size distribution, which also makes it difficult to compare different toxicological studies.

Recently, some authors raised important concerns that justify the study of biointeractions and possible impacts of nanocellulose exposure to humans. This would provide consistent and useful knowledge that can guide the outgrowth of regulatory (Camarero-Espinosa et al., 2016; Endes and Camarero-Espinosa, 2016). The acute and/or chronic toxicity of nanocellulose during occupational exposure at normal conditions or exacerbation of pre-existing disease conditions must be studied.

From the currently available discussions on nanocellulose-based materials in the literature reviews, one can only grasp a general panorama involving the wide spectrum of applications as well as the many physical and chemical processing methods to attain products. However, since nanocellulose includes a class of nanostructures with different morphological, physicochemical and biological properties, more detailed discussions must be presented for members of this class. From this perspective, CNCs are promising in the biomedical context, especially for drug delivery/depot systems. There are many modification/functionalization methods for CNCs, which impact their application as drug delivery/depot systems and their toxicological behavior. On this latter, many concerns were raised on several occasions when these nanocrystals were tested. Consequently, the use of CNCs as drug delivery/depot systems and the safety aspects involved should be discussed in detail. We have, therefore, compiled a large amount of data on this topic in order to present a suitable discussion on the most relevant scientific and technological results involving the use of CNCs in biomedical applications. Essentially, we discuss the recent progress in the design of CNCs as drug carrier systems, their nanotoxicity, patents and commercially available CNC products. They are obtained from renewable sources and CNCs can be extensively modified with the well-established protocols from organic chemistry. This leads to materials with specific mechanical, chemical and biological properties. CNCs can play an important role in future pharmaceutical formulations and medical procedures. However, prior to achieving commercial products based on CNCs, the production–processing–safety relationship regarding CNCs must be well understood. This review largely contributes towards it.

2. CNCs as drug carriers in aqueous suspensions

This section introduces and summarizes important CNC-based materials that have been explored to date. CNCs have largely been studied when chemically conjugated to other molecules or particles, by covalent or non-covalent bonds, in an attempt to provide several functionalities for the system. CNCs were bound to significant amounts of tetracycline and doxorubicin (DOX), water-soluble and ionizable drugs, which were released during a period of 1 day (Jackson et al., 2011). In order to increase the hydrophobicity of CNCs, cetyl trimethylammonium bromide (CTAB) was attached to the CNC surface, which increased zeta potential values in a concentration-dependent manner (e.g., from −55 to 0 mV). These functionalized CNC crystallites bound significant quantities of the hydrophobic anticancer drugs docetaxel, paclitaxel and etoposide, which were liberated in a sustained manner in a period of 2 days. In addition, the authors observed uptake of CNC–CTAB complexes by KU-7 bladder cancer cells (Jackson et al., 2011). The surface of torispherical CNCs was also modified by CTAB in order to improve the loading capability of the water-insoluble anticancer drugs luteolin and luteoloside (Qing et al., 2016). Although surfactants, such as CTAB, have been used in conjugation with nanomaterials, including CNCs, in order to increase the nanoparticle loading with hydrophobic drugs (such as anticancer agents), caution must be taken with this approach (Alkilany & Murphy, 2010). Due to its chemical property, CTAB might interact with the phospholipid bilayers of the cells, leading to a destabilization of the cell membrane. This destabilization might result in cell death. Depending on its concentration, CTAB might create holes in the phospholipid bilayer of the cells (Ulitzur, 1970).

An interesting conjugation was performed from surface-modified CNCs with cationic porphyrin (Por) (Feese, Sadeghifar, Gracz, Argyropoulos, & Ghiladi, 2011). The resulting commonly insoluble crystalline material CNC-Por, although only suspended in an aqueous system, showed high efficacy towards the photodynamic inactivation of *Staphylococcus aureus* and *Mycobacterium smegmatis*, with only discrete activity against *Escherichia coli*. This work describes the synthesis of novel, bioactive and photobactericidal materials that are toxic against several bacteria, with possible utilization in the food and health care industries (Feese et al., 2011). A similar CNC-Por structure showed high efficacy towards the photodynamic inactivation of several microorganisms (e.g., *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Acinetobacter baumannii* (MDRAB) (Carpenter, Feese, Sadeghifar, Argyropoulos, & Ghiladi, 2012).

Curcumin (CUR)-conjugated cellulose nanoparticles (cellulose-CUR; 5.2 nm) demonstrated a high level of cellular uptake, causing maximum ultrastructural changes on apoptosis (presence of vacuoles), such as in prostate cancer cells, in comparison with free CUR (Yallapu,