In Vitro Cytotoxicity and Genotoxicity Assessment of Novel Cellulose Nanomaterials using Intestinal Cells

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INTRODUCTION

Cellulose nanomaterials (CNMs) are emerging materials with multiple potential applications in food and food packaging Industry

› ↑ human exposure via oral route › ? adverse health outcomes on the gastrointestinal tract (GIT). ›

AIM

Evaluation of cytotoxicity and genotoxicity of a microfibrillated cellulose (CMF – ENZ 140) and a nanofibrillated cellulose (CNF – T-1300), obtained from *Eucalyptus globulus* kraft pulp, using the HT29-MTX-E12 human intestinal cell model.

MATERIALS AND METHODS

Cellulose Source
Bleached *Eucalyptus globulus* kraft pulp

CMF – ENZ 140
Enzymatic hydrolysis

CNF – T-1300
Catalytic oxidation with TEMPO radical (2,2,6,6 tetramethylpiperidine-1-oxyl)

High-pressure homogenization

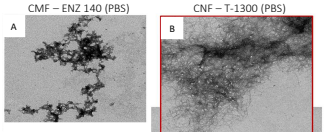
Characterization of primary and secondary CNMs properties (DLS and TEM)

Citotoxicity

1. MTT Assay (24 hours of exposure)
2. Clonogenic assay (9 days of exposure)

Genotoxicity

3. Comet Assay/FPG-modified Comet Assay (3h and 24h hours of exposure)
4. Cytokinesis-Block Micronucleus Assay (CBMN) (52 hours of exposure)



Pinto et al., 2022 https://doi.org/10.3390/nano12091432

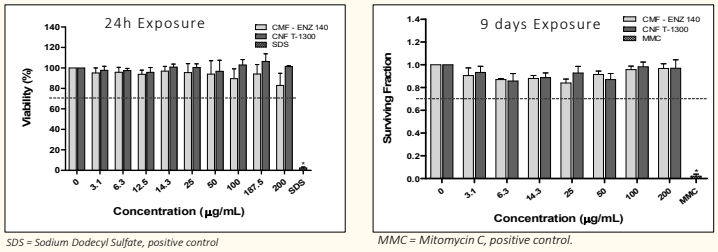
Micro/nanocellulose sample	Fibril diameter (nm) PBS	Zeta potential (mV) Stock	Zeta potential (mV) PBS (25ºC)	Zeta potential (mV) DMEM + 10% FBS (37ºC)
CNF – T1300	10.7±1.9	-44.9±2.31	-24.6±1.22	-11.6±2.71
CMF- ENZ 140	29.7±7.3	-33.9±1.4	-11.6±1.24	-6.03±0.41

RESULTS

1 MTT Assay

2 Clonogenic Assay

CMF – ENZ 140 and CNF – T-1300



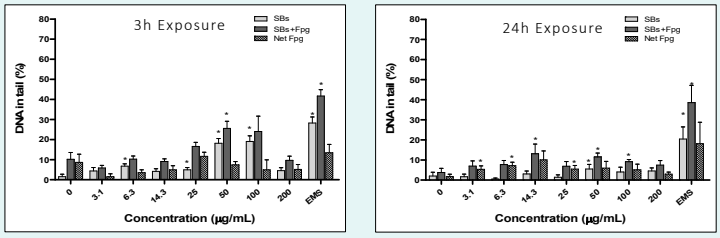
SDS = Sodium Dodecyl Sulfate, positive control

MMC = Mitomycin C, positive control.

No cytotoxic effects after 24h or long-term exposure to CNMs

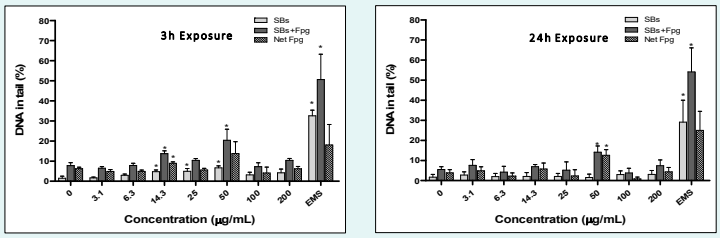
3 Comet Assay/FPG-modified Comet Assay

CMF – ENZ 140



- **Increased DNA damage** after 3h (6, 25, 50 and 100 µg/mL) and 24h (50 µg/mL) exposure (p< 0,000001 for 3h; p= 0,000038 for 24h (ANOVA));
- **No oxidative DNA damage** with the FPG-modified comet assay, after 3h.

CNF – T-1300

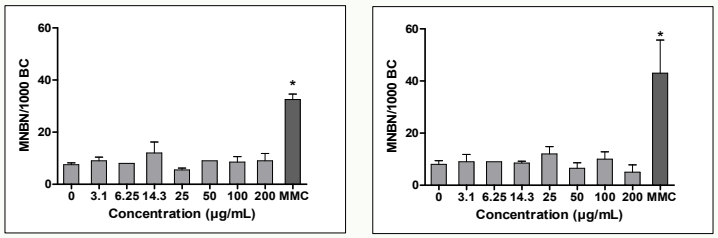


- **Increased DNA damage** after 3h exposure (14.3, 25, 50 µg/mL)(p< 0,000001 for 3h; p= 0,667 for 24h (ANOVA));
- **Induction of oxidative DNA damage** after exposure to 50 µg/mL, after 3h and 24h treatment.

4 Cytokinesis Block Micronucleus Assay (CBMN)

CMF – ENZ 140

CNF – T-1300



MNBN = Micronucleated binucleated cells; MMC = Mitomycin C, positive control.

No increase in chromosomal damage after 52h of exposure to CNMs

CONCLUSIONS

- CNMs tested are not cytotoxic.
- No increase in chromosomal damage was observed (clastogenic or aneugenic events).
- A genotoxic effect was revealed as single- and double-strand DNA breaks (comet assay), after short exposure to CMF – ENZ 140 and to CNF T-1300. Oxidative DNA damage does not seem to mediate the observed effect.
- Thus, the *in vitro* comet assay is shown as a useful and sensitive tool for detecting DNA lesions induced by CNMs in intestinal cells.
- Further studies, e.g. using the *in vitro* mammalian cell gene mutation tests, may clarify potential induction of mutations following exposure to CNMs.
- Ongoing studies with the use of *an in vitro* simulated digestion process before the toxicity testing, may disclose if the ingestion of these CNMs impacts its genotoxicity, allowing a more comprehensive assessment of CNMs safety.

In a SSbD perspective, both CMF-ENZ 140 and CNF-T-1300 raise some concern and highlight the importance of using genotoxic endpoints, other than cytotoxicity testing, at an early stage of CNMs development for food-related products.

Safer sustainable materials, Safer sustainable products
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Synthesis/production

NM Design

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