4TH INTERNATIONAL CONGRESS ON OCCUPATIONAL AND ENVIRONMENTAL TOXICOLOGY

ARE CELLULOSE NANOFIBERS SAFE FOR BIOMEDICAL APPLICATIONS?

TESTING THE SAFETY OF TWO TYPES OF NANOCELLULOSE FIBERS FOR APPLICATION IN BONE REGENERATION

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"There is plenty of room at the bottom"

Feynman, 1959

NANOSCALE

NANOMATERIAL

1 nm to 100 nm

"A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm."

European Commission (2011)

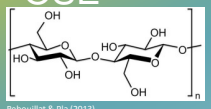
NANOMATERIAL

MACROMATERIAL

Same chemical composition but distinct physicochemical characteristics -> improved mechanical, optical, electric and magnetic properties

Louro, Borges & Silva (2013)

CELLULOSE



Rebouillat & Pla (2013)

- Affordable
- Abundant
- Renewable

Halib *et al.* (2017)

NANOCELLULOSE
(ORIGINS)

- Plants (cellulose in association with lignin and hemicellulose)
- Bacteria (*Acetobacter, Agrobacterium, Alcaligenes, Pseudomonas, Rhizobium* e *Sarcina)*
- Fungi
- Algae

Halib *et al.* (2017)

NANOCELLULOSE
(PRESENTATIONS)

- Nanocellulose nanocrystals (CNC)
- Cellulose nanofibers (CNF)
  - Diameter of 20 to 40 nm
  - Length of micrometers
  - High aspect ratio -similar to carbon nanotubes and asbestos

Ilves *et al.* (2018), Halib *et al.* (2017) & Nordli, Chinga-Carrasco, Rokstad & Pukstad (2016)

NANOCELLULOSE
(BIOMEDICAL APPLICATIONS)

Wound dressings

- CNC and CNF
- Burn wounds with excessive fluid loss
- Capability of maintaining a wet environment
- Delivery of bioactive substances such as antibiotics and anti-inflammatory drugs

Halib *et al.* (2017)

NANOCELLULOSE
(BIOMEDICAL APPLICATIONS)

Cartilage regeneration and replacement

- Cartilage is avascular, it has limited potential for healing
- Porous, mimics extracellular matrix and allows diffusion of nutrients
- CNF used as bioink for 3D printers

Halib *et al.* (2017)

NANOCELLULOSE
(BIOMEDICAL APPLICATIONS)

Bone and periodontal regeneration

- Bone loss associated with age
- High aesthetic demands
- CNC and CNF
- Great mechanical properties for scaffolds and membranes
- Porous, mimics extracellular matrix and allows diffusion of nutrients and cell proliferation

Andrei, Dinischiotu, Didilescu, Ionita & Demetrescu (2017) & Halib *et al.* (2017)

NANOTOXICOLOGY

- Greater presence means higher exposure risk in all phases of a nanomaterial's life cycle
- Inhalation is the most important route of exposure
- CNF have a high aspect ratio
- Oral exposure is also a concern
- Because of their size these particles are able to reach organs such as the liver and the brain through the blood and lymphatic systems

Schmalz, Hickel, Landuyt & Reichl (2017), Carriere, Sauvaigo, Douki & Ravanat (2016) & Louro, Borges & Silva (2013)

NANOTOXICOLOGY

- Nanomaterials are able to accumulate in practically every cell in the organism
- Once they penetrate the cells, some are able to enter the nucleus, having direct of indirect effects on the genetic material
- Two paradigms about nanotoxicology:
  - Inflammatory potential
  - Oxidative stress/genotoxicity
- Different physicochemical characteristics will alter pharmacokinetics and pharmacodynamics

Fornaguera & García-Celma (2017), Carriere, Sauvaigo, Douki & Ravanat (2016) & Louro, Borges & Silva (2013)

OBJECTIVE

- To analyze the safety of two CNF, obtained from *Eucalyptus globulus*, in bone producing cells – osteoblasts -, in order to be used in bone and periodontal regeneration procedures
  - Evaluation of the cytotoxic effects through the MTT assay (Mosmann, 1983)
  - Evaluation of the genotoxic effects through the cytokinesis-block micronucleus assay (Fenech, 2007)

MATERIALS AND METHODS

NANOCELLULOSE PRODUCTION

- Two samples obtained from industrial bleached eucalyptus kraft pulp
  1. CNF TEMPO – mechanical treatment followed by TEMPO-mediated oxidation (Saito & Isogai, 2007)
  2. CNF enzymatic – mechanical treatment followed by an enzymatic hydrolysis with endoglucanases (Tarrés *et al.*, 2016)

MATERIALS AND METHODS

MG-63 (ATCC® CRL-1427™): human osteoblasts obtained from an osteosarcoma

VIABILITY ASSAYS – MTT

- According to Mosmann (1983)
- Exposure to concentrations from 1.5 to 50 µg/cm^2
- 24h and 48h exposure

GENOTOXICITY ASSAYS – MICRONUCLEUS ASSAY

- According to Fenech (2007)
- Exposure to concentrations from 1.5 to 12.5 µg/cm^2

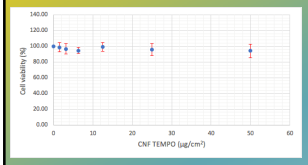
RESULTS

NANOCELLULOSE PRODUCTION

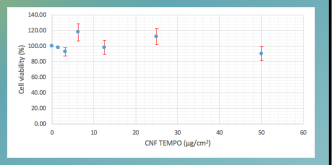
Sample	Batch	Dry content (%)	Yield (%)	DP
TEMPO	1	0.83	82.4	1177
Enzymatic	1	0.83	8.2	185
TEMPO	2	0.77	100.0	364.5
Enzymatic	2	0.85	*	1472.3
*not determined				

MTT ASSAY – CNF TEMPO

24h exposure

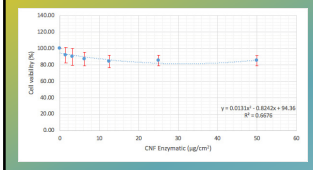


48h exposure

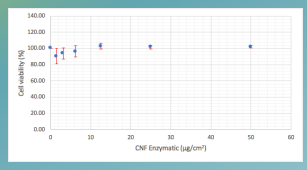


MTT ASSAY – CNF ENZYMATIC

24h exposure



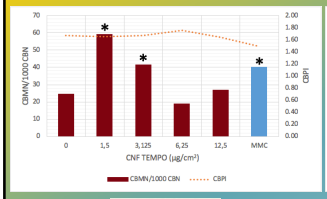
48h exposure

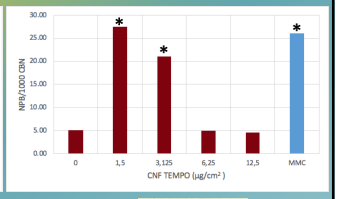


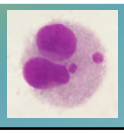
RESULTS

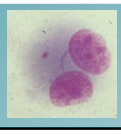
CYTOKINESIS-BLOCK MICRONUCLEUS ASSAY (CBMN)

CBMN – CNF TEMPO

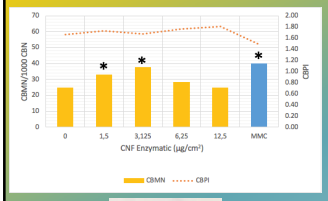


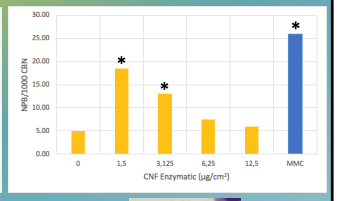


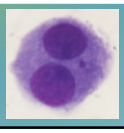


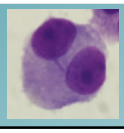


CBMN – CNF ENZYMATIC









DISCUSSION

- **Non cytotoxic for all concentrations and exposure times**, consistent with results obtained by Ventura, Lourenço, Sousa-Uva, Ferreira & Silva (2018), Souza *et al.* (2018), Lopes, Martinez, Strømme & Ferraz (2017), Rashad, Mustafa, Heggset & Syverud (2017), Nordli, Chinga-Carrasco, Rokstad & Pukstad (2016) & Pereira *et al.* (2013)
- Non-consistent with the results found by Ilves *et al.* (2018), who noticed a decrease in THP-1 macrophages cell viability post CNF enzymatic exposure

DISCUSSION

- **Higher frequency of micronucleated cells for the two lowest concentrations**, exactly as reported by Ventura, Lourenço, Sousa-Uva, Ferreira & Silva (2018) in their study with A549 cells exposed to *Eucalyptus globulus* CNF TEMPO.
- Lima *et al.* (2012) noticed DNA damage, as well, but using other assays.
- Catalán *et al.* (2016) didn't report higher frequency of micronuclei following exposure to CNF TEMPO obtained from fir tree.

DISCUSSION

- **Higher frequency of nucleoplasmatic bridges for the two lowest concentrations**, not reported by any authors.

CONCLUSION

- None of the studied CNF induced a loss of cell viability
- Both CNF demonstrated genotoxic potential, inducing the formation of chromosomal damage
- More endpoints need to be assessed in vitro, e.g., gene mutations, and in vivo, e.g. comet or micronucleus assay
- Although they show great potential to be used in the biomedical field, these two CNF still have to suffer changes in their production method in order to be considered a safe material

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