Finnish Institute of Occupational Health

Surface chemistry and size affect the toxicity of cellulose nanofibrils

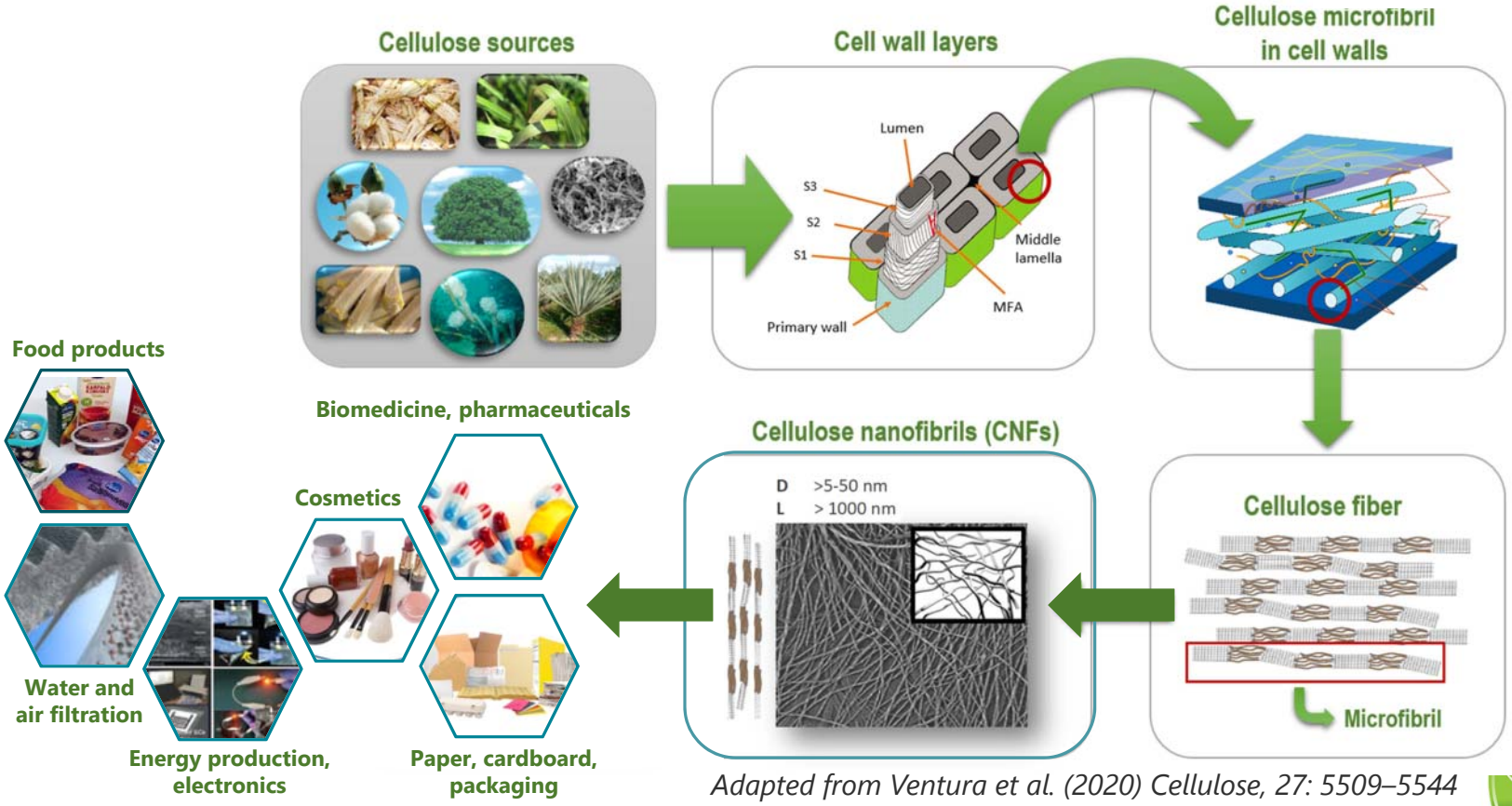
Aimonen K[1], Imani M[2], Hartikainen M[1], Suhonen S[1], Vanhala E[1], Moreno C[3], Rojas OJ[2], Norppa H[1] and Catalán J[1,3]

[1]FIOH, [2]Aalto University, [3]UNIZAR

TAPPI Nano 2021 Virtual Conference
15-16 June 2021

Finnish Institute of Occupational Health

Cellulose nanofibrils

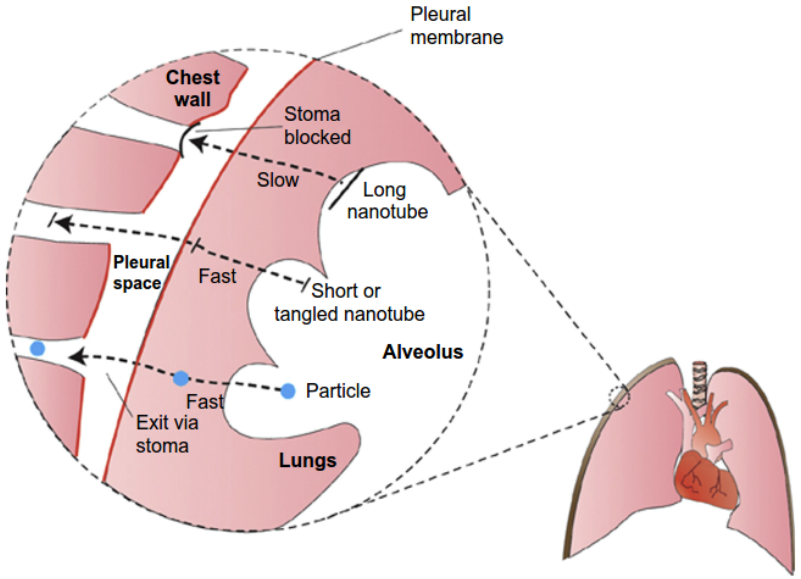


Adapted from Ventura et al. (2020) Cellulose, 27: 5509–5544

Finnish Institute of Occupational Health

Fibre pathogenicity paradigm

*"Any **high aspect ratio** material (HARM) that is **long**, **thin** and **biopersistent** has the potential to be carcinogenic at the pleura and in the lung leading to **mesothelioma** and **lung cancer** if the exposure was great enough for the dose to accumulate sufficiently to initiate these processes."*

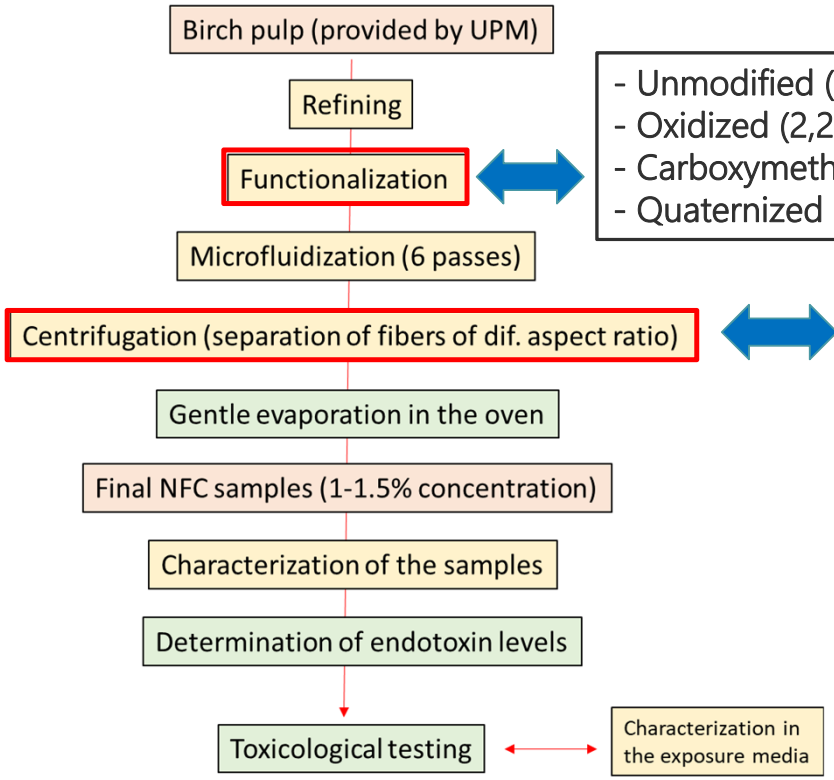


Donaldson & Poland (2009). Nat Nanotechnol, 4: 708-10

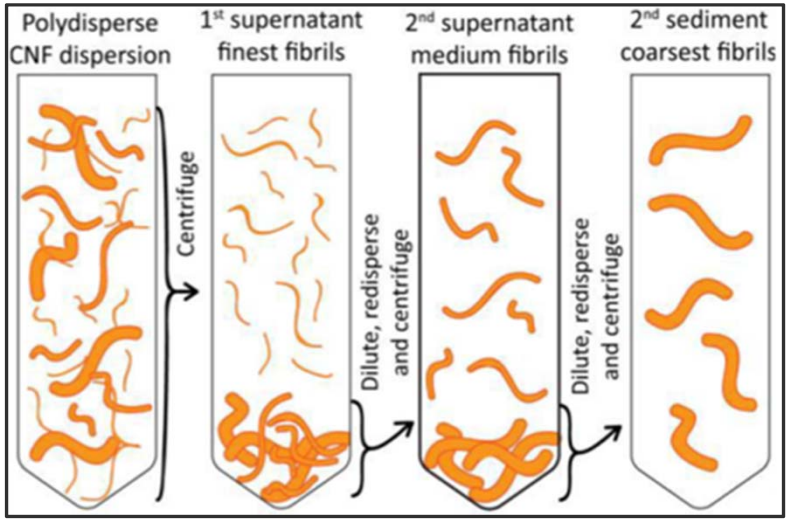
- **Do cellulose nanofibrils (CNFs) induce toxic effects?**
- **How do size and surface chemistry modulate the interaction of CNFs with cells?**

Production of cellulose nanofibrils

Aalto-yliopisto
Aalto-universitetet
Aalto University

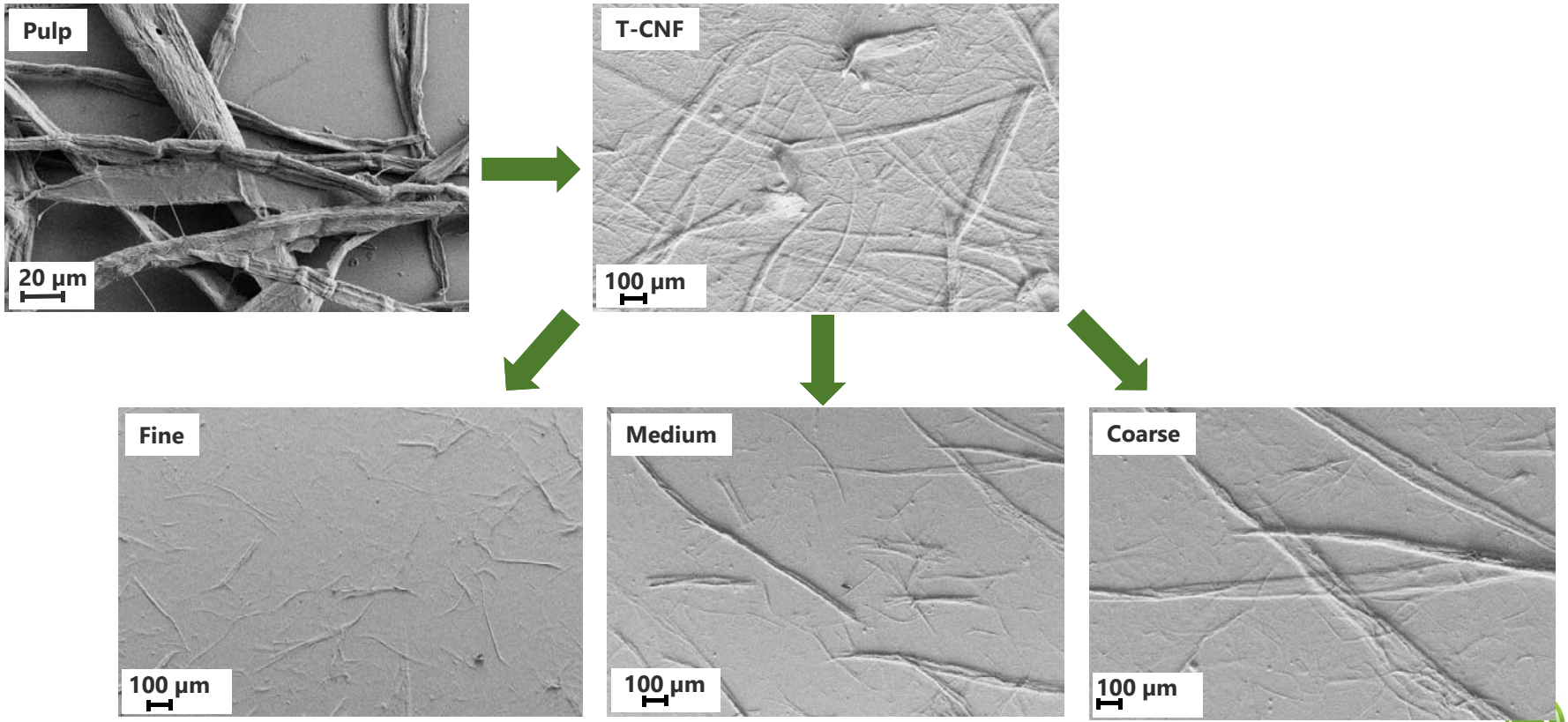


- Unmodified (non-functionalized) → U-CNF
- Oxidized (2,2,6,6-tetramethyl-piperidin-1-oxyl, TEMPO) → T-CNF
- Carboxymethylated → C-CNF
- Quaternized (epoxypropyltrimethylammonium chloride, EPTMAC) → E-CNF



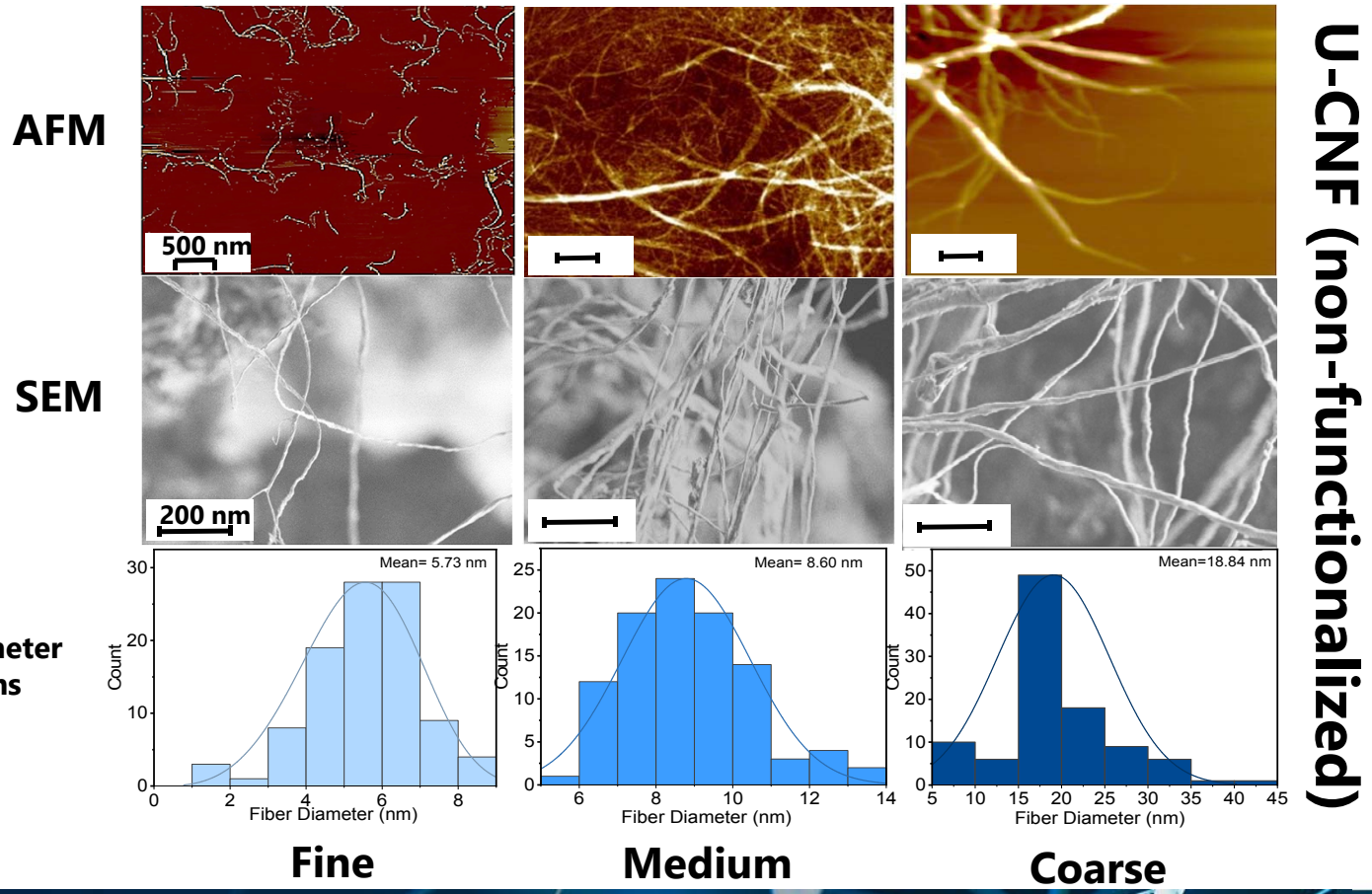
Toivonen et al. (2018). Adv Mater, 30(16): 1704050.

Nanofibrillitation yield



Characterization of cellulose nanofibrils

Aalto-yliopisto
Aalto-universitetet
Aalto University



Finnish Institute of Occupational Health

Characterization of cellulose nanofibrils

Surface modification	Degree of crystallinity (%)	Fiber Diameter and Length (nm)						Endotoxin level (UE/mL)[a]		
		Fine		Medium		Coarse		F	M	C
		D	L	D	L	D	L			
Non-functionalized	75	5.73± 0.09	989± 256	8.60± 0.13	1690± 640	18.84± 1.41	2768± 521	0.83	0.48	0.35
TEMPO Oxidation*	67	4.68± 0.90	892± 301	7.84± 0.28	1491± 125	15.39± 0.21	2168± 352	3.95	3.30	3.76
Carboxy-methylation	71	4.94± 0.07	1127± 231	8.95± 0.21	1920± 321	17.48± 0.10	2692± 232	0.14	0.15	0.15
EPTMAC quaternization	70	4.79± 0.14	1020± 505	7.56± 0.29	1702± 124	16.30± 1.04	1990± 129	0.08	0.07	0.07

[a]*Red/yellow: levels above the 0.5 EU/mL limit value established by the US Food Drug Agency (FDA) for inhalation studies*

*TEMPO fractions were autoclaved because their endotoxin level clearly exceeded the 0.5 EU/mL limit

ζ-potential (mV)

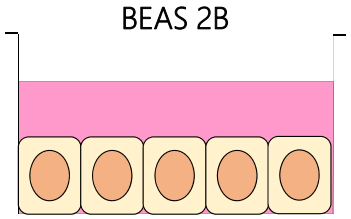
Surface modification	Fine		Medium		Coarse	
	Water	LHC-9	Water	LHC-9	Water	LHC-9
Non-functionalized	−16.5± 3.8	−7.2± 0.6	−22.2± 2.1	−11.3± 1.1	−20.3± 2.8	−9.8± 0.8
TEMPO Oxidation*	−36.5± 3.2	−1.8± 0.1	−41.5± 2.7	−3.9± 0.8	−20.0± 2.5	−2.5± 0.3
Carboxy-methylation	−36.7± 1.0	−14.2± 1.3	−37.7± 1.6	−16.1± 1.5	−19.9± 1.1	−11.4± 1.0
EPTMAC quaternization	10.6 ± 0.2	3.3± 0.5	31.6± 0.8	11.5 ± 0.9	35.5± 1.0	12.8± 1.4

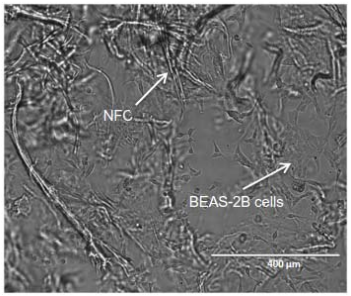
Aalto-yliopisto
Aalto-universitetet
Aalto University

Finnish Institute of Occupational Health

Toxicological analyses

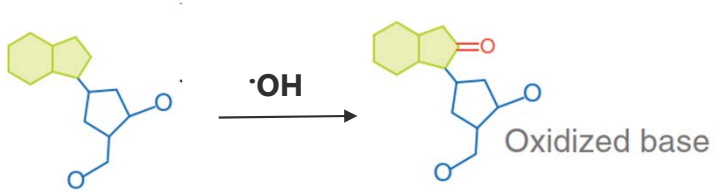
- *In vitro* lung model: Human bronchial epithelial BEAS 2B cells





- Cytotoxicity
  → CellTiter-GloVR luminescent cell viability assay (24-h and 48-h)
  → Replication index

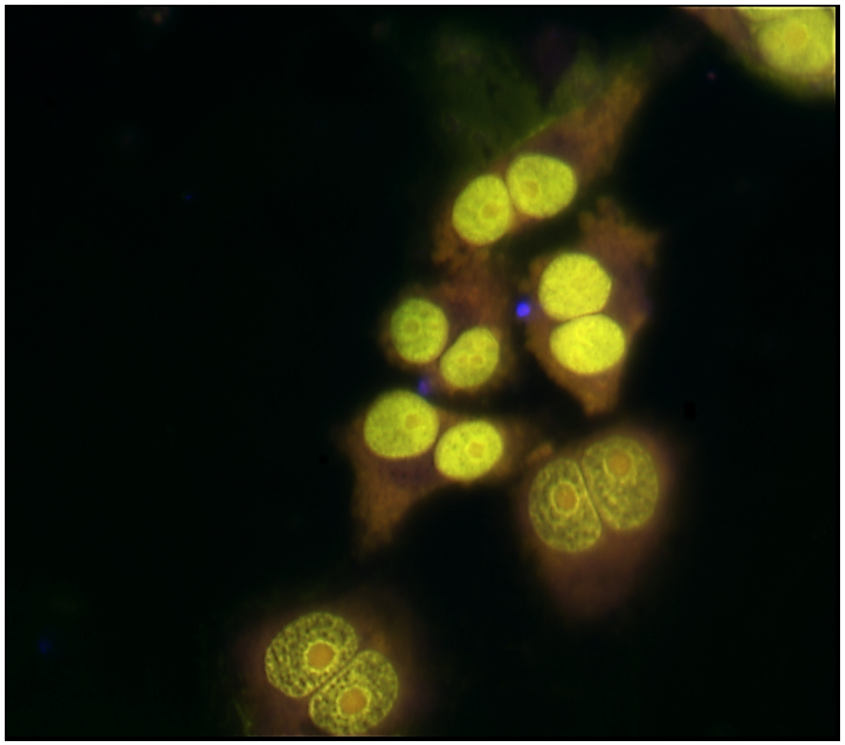
- Formation of intracellular reactive oxygen species (ROS)
  → CM-DCFDA assay (3-h, 6-h and 24-h)



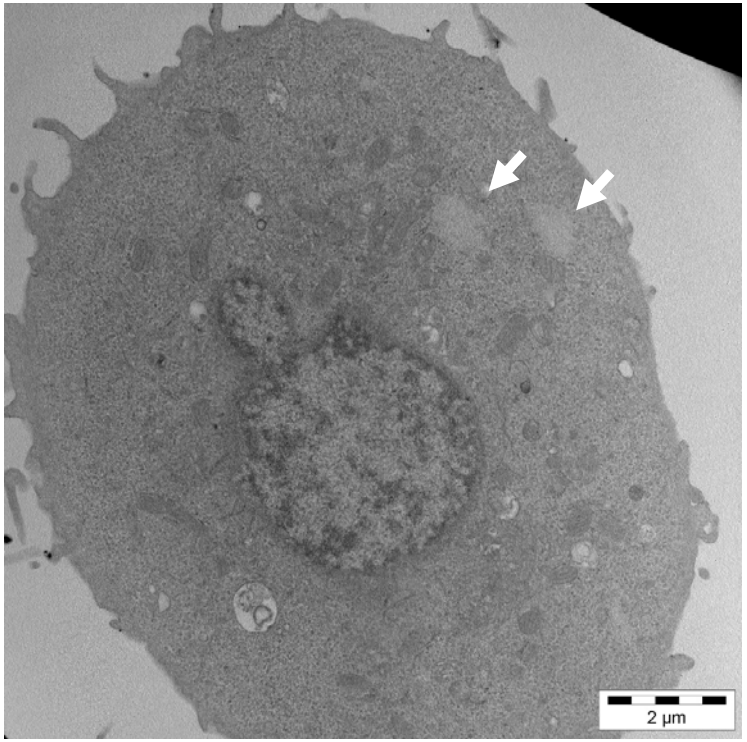
- **Genotoxicity assessment**:
  - ✓ DNA damage → Alkaline comet assay (24-h)
  - ✓ Chromosome damage → Micronucleus assay (OECD TG 487; 48-h)

Cellular uptake

Calcofluor staining



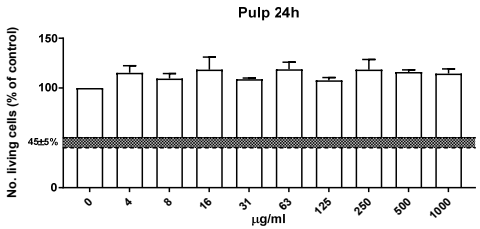
TEM analyses

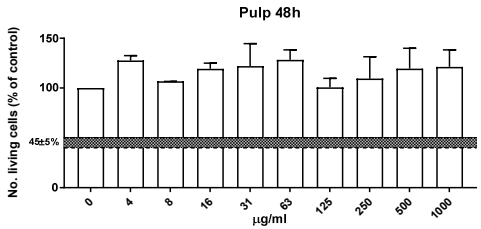


BEAS 2B cells treated with 111 µg/ml of the fine fraction of E-CNF for 48 h (Calcolfluor staining) and 6 h (TEM)

Finnish Institute of Occupational Health

Cytotoxicity

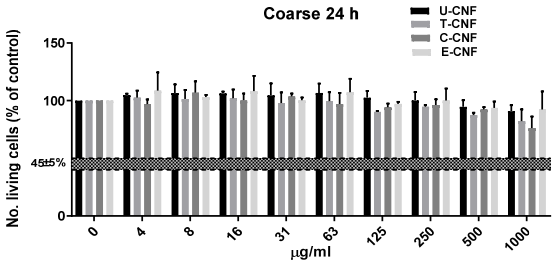


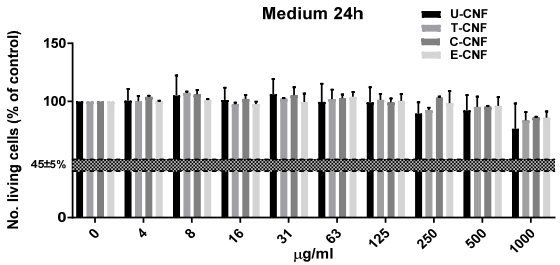


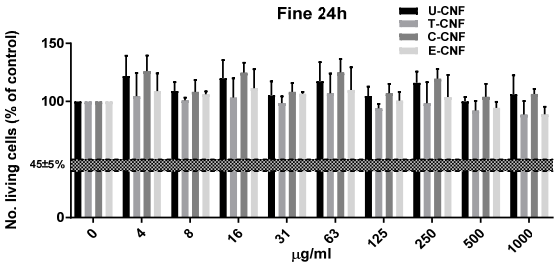
C-CNF
48 h/ 1000 µg/ml
- Finest: 43.6%
- Medium: 3.2%
- Coarse: 4.9%

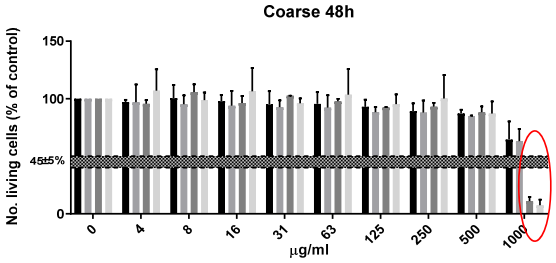
E-CNF
48 h/ 1000 µg/ml
- Finest: 4.7%
- Medium: 50.2%
- Coarse: 7.4%

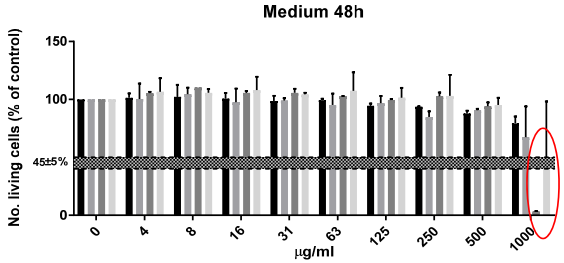
Replication Index > 70%

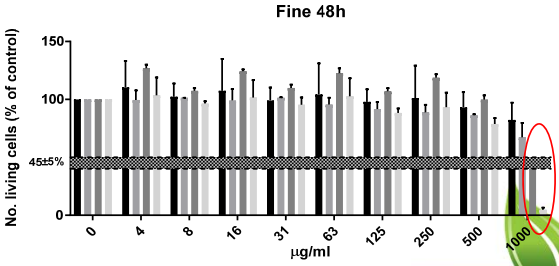




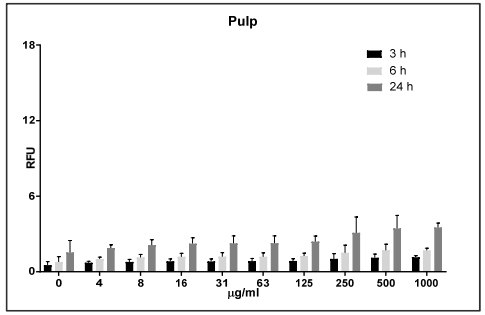


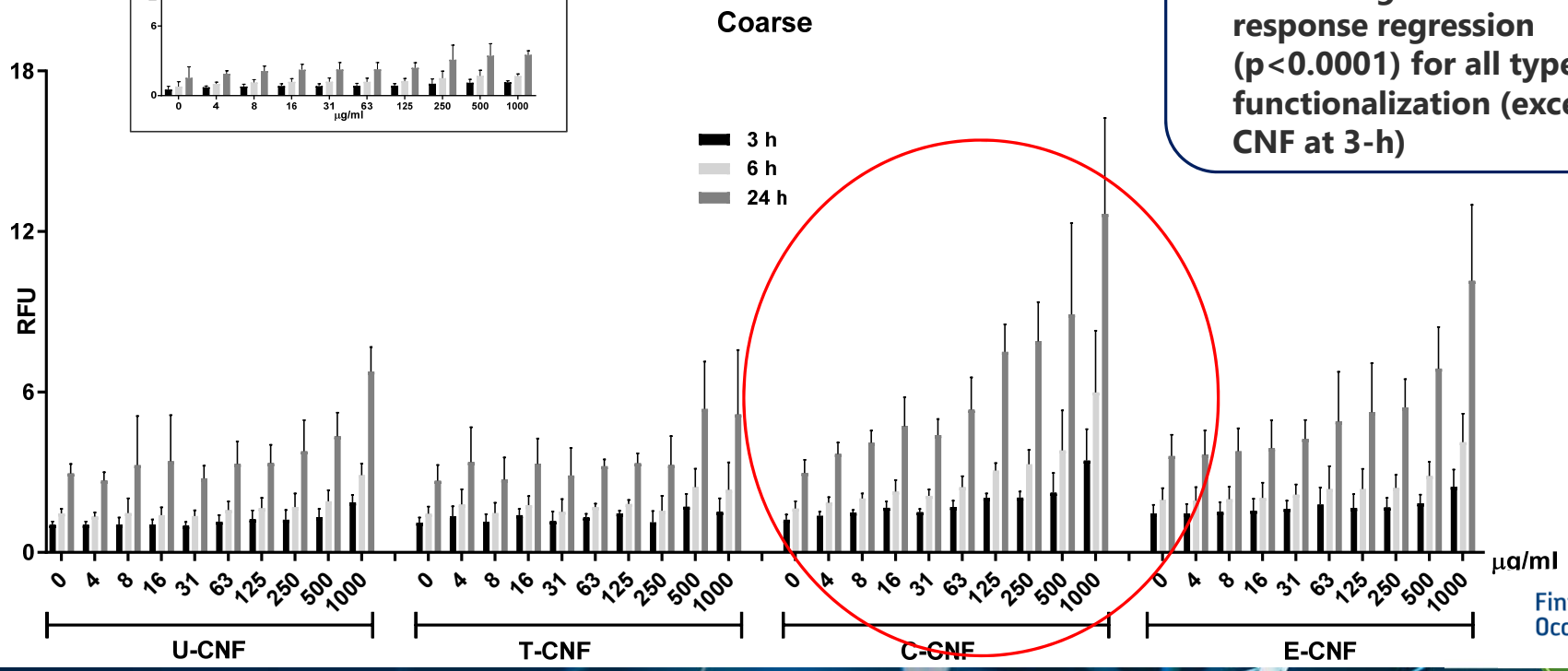






ROS formation

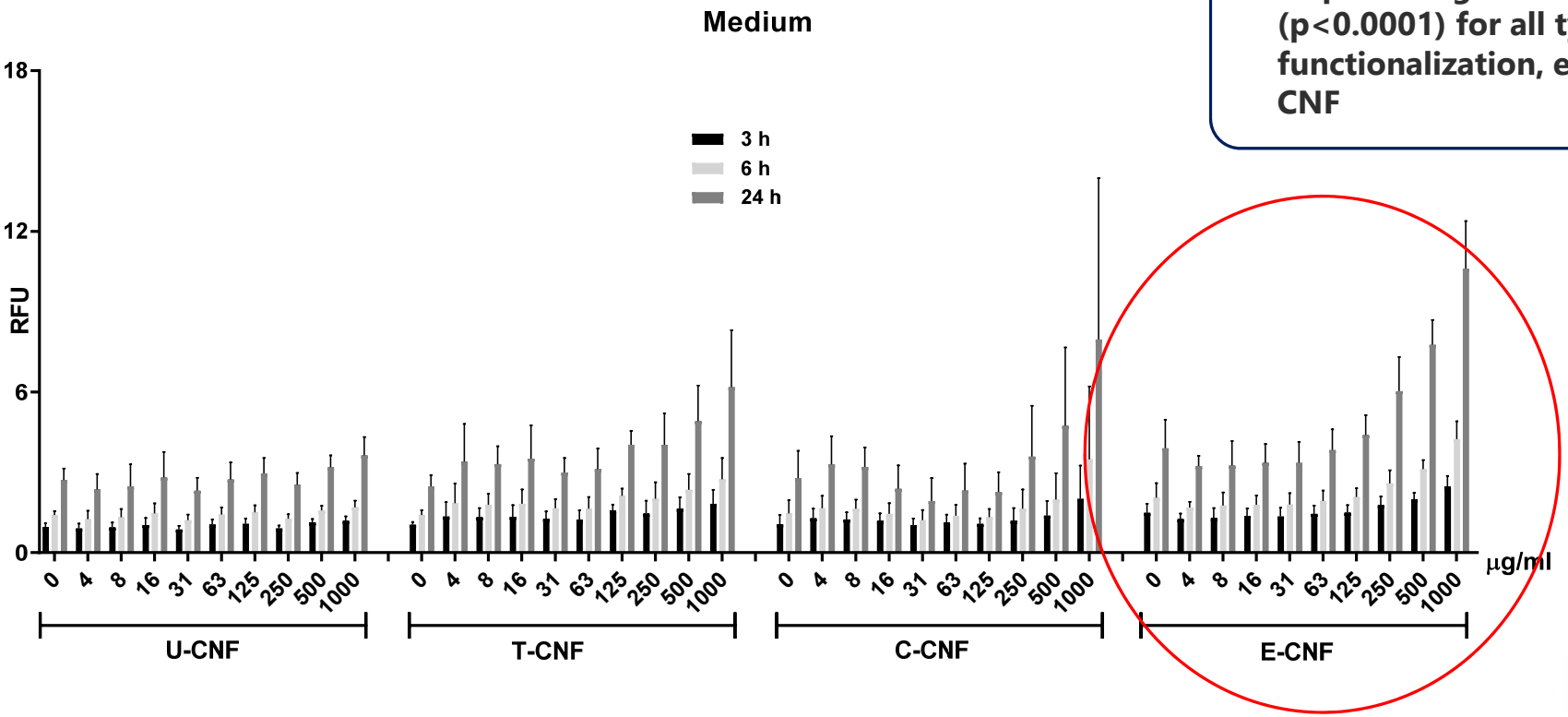




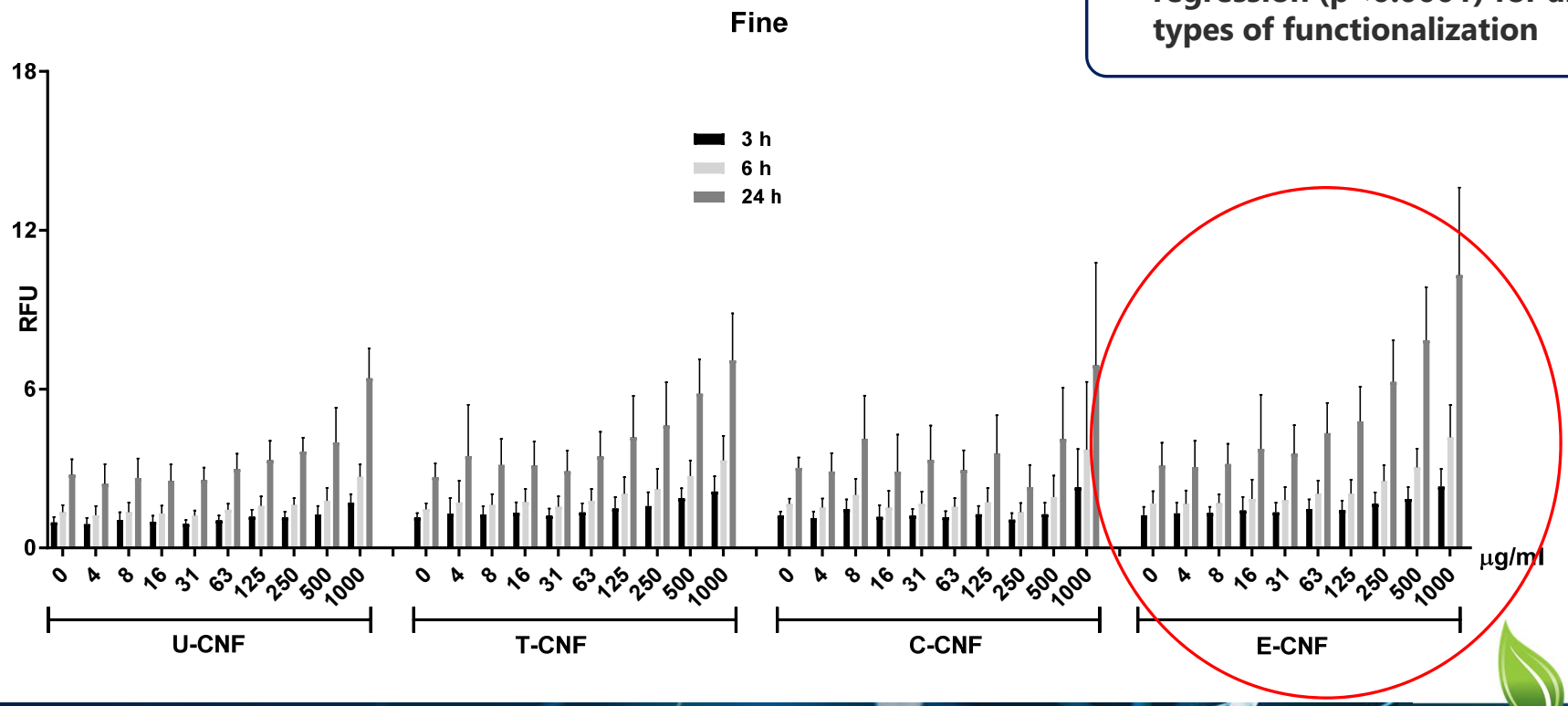
- CNFs behave differently depending on the size fraction
- Significant dose-response regression ($p<0.0001$) for pulp and coarse fraction of U-CNF
- Coarse: significant dose-response regression ($p<0.0001$) for all types of functionalization (except T-CNF at 3-h)

Finnish Institute of Occupational Health

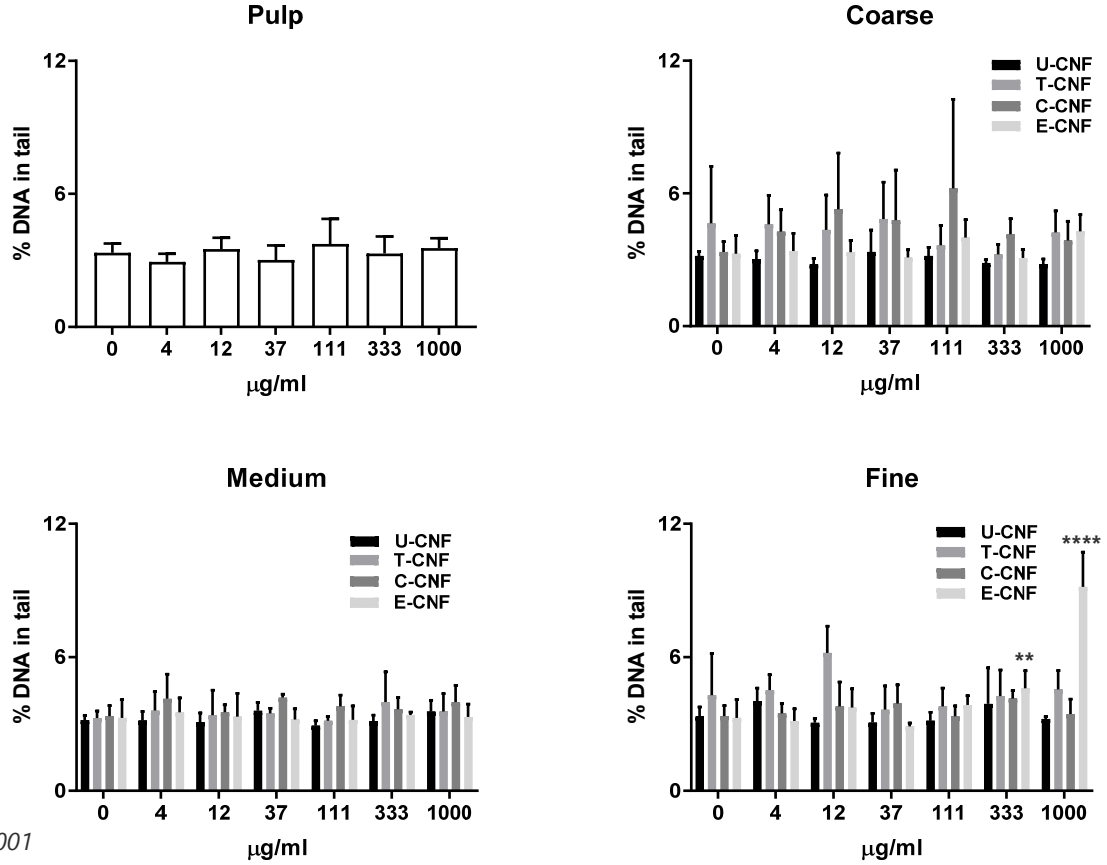
- **Medium: significant dose-response regression ($p<0.0001$) for all types of functionalization, except U-CNF**



- **Fine: significant dose-response regression ($p<0.0001$) for all types of functionalization**



DNA damage

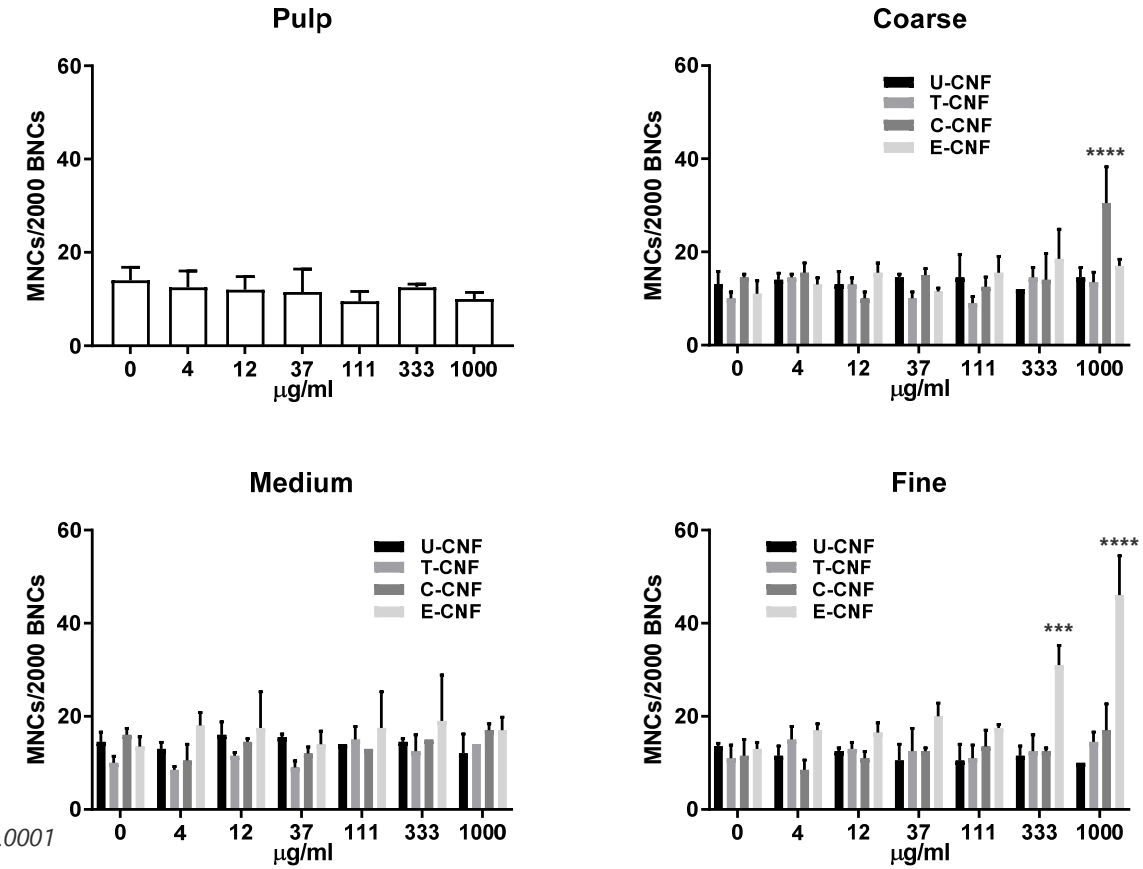


- No significant differences between pulp and coarse U-CNF
- CNFs behave differently depending on the size fraction
- Coarse: non-significant dose-responses
- Medium: non-significant dose-responses
- **Fine: significant dose-response regression only for E-CNF ($p<0.0001$)**

$^{**}p< 0.01$; $^{****}p< 0.0001$

Finnish Institute of Occupational Health

Chromosome damage



***p< 0.001; ****p< 0.0001

- None of the cellulosic materials exceeded 55 ± 5% cytotoxicity (RI)
- No significant differences between pulp and coarse U-CNF
- CNFs behave differently depending on the size fraction
- **Coarse: significant dose-response regression only for C-CNF (p<0.0001)**
- Medium: non-significant dose-responses
- **Fine: significant dose-response regression only for E-CNF (p<0.0001)**

Conclusions

- There is not a uniform pattern for the toxicological response displayed by cellulose nanofibrils as a function of their size or functionalization. Instead, the response depends on the interaction between both parameters
- All types of nanofibrils, as well as the pulp fibres, are able to induce the formation of ROS
- The increase of ROS did not result on an increased genotoxic damage, except for the fine fraction of E-CNF and the coarse fraction of C-CNF

Finnish Institute of Occupational Health

Thank you!

julia.catalan@ttl.fi

ttl.fi

@tyoterveys
@fioh

tyoterveyslaitos

tyoterveys

Tyoterveyslaitos