SUSTAINABILITY Masterclass

6 | 7 | 8 | 9

NOVEMBER

2023

Co-Organizers:

HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837

RUTGERS
School of Public Health

Massachusetts Institute of Technology

Program at a Glance (1/2)

Outline of Program
November 6, 2023

DAY 1

FRAMING THE SUSTAINABILITY CHALLENGE

10:00-11:30 — **Session 1: What is Sustainability: Economic Development, Employment, & the Environment**
Nicholas Askounes Ashford, PhD, JD
Professor of Technology, Policy Director Technology & Law Program, Massachusetts Institute of Technology

11:30-13:00 — **Session 2: Intersections of Planetary & Human Health**

Sustainability, Planetary and Human Health: Challenges and Opportunities
Philip Demokritou, PhD
Henry Rutgers Chair and Professor of Nanoscience and Environmental Bioengineering, Rutgers University & Adjunct Professor, Harvard University

Helmut Zarbl, D.C.S., Ph.D., ATS Fellow
Chair and Professor of Toxicology, Department of Environmental and Occupational Health. School of Public Health, Research Dean Rutgers University

The Regulation of Environment & Global Climate Change
Nicholas Askounes Ashford, PhD, JD
Professor of Technology, Policy Director Technology & Law Program, Massachusetts Institute of Technology

13:00-14:30 — **Lunch & Networking**

14:30-15:45 — **Session 3: Economic Development, Globalization (Trade) & Sustainability**
Nicholas Askounes Ashford, PhD, JD
Professor of Technology, Policy Director Technology & Law Program, Massachusetts Institute of Technology

15:45-16:00 — **Coffee Break**

16:00-17:15 — **Session 4: Global Megatrends, Sustainability, & the SDGs [By Zoom]**
Wendy M. Purcell, PhD FRSA
Professor, Rutgers University & Academic Research Scholar, Harvard University

17:15-17:30 — **Closing Remarks and Discussion - Day 1**
Philip Demokritou, PhD, N. Ashford, PhD W. Purcell, PhD

Outline of Program

Outline of Program
November 7, 2023

DAY 2

INDUSTRIAL POLICY, ENERGY CONSIDERATIONS AND PARTNERSHIPS FOR SUSTAINABILITY

09:00-9:30 — **Refreshments & Networking**

9:30-9:45 — **Introduction to Day 2**

Philip Demokritou, PhD
Henry Rutgers Chair and Professor of Nanoscience and Environmental Bioengineering, Rutgers University & Adjunct Professor, Harvard University

9:45-11:15 — **Session 5: Industrial Policy: Technology, Innovation, & Employment**
Nicholas Askounes Ashford, PhD, JD
Professor of Technology, Policy Director Technology & Law Program, Massachusetts Institute of Technology

11:15-11:30 — **Coffee break**

11:30-13:00 — **Session 6: Energy Consideration and Pathways to Sustainability**
Nicholas A. Ashford, PhD, JD
Professor of Technology, Policy Director Technology & Law Program, Massachusetts Institute of Technology

13:00-15:00 — **Lunch & Networking**

15:00-17:15 — **Session 7: Partnerships for Sustainability – Universities, Business, & Community**

Introductory lecture: Universities Driving Sustainability in Partnership
Wendy M. Purcell, PhD FRSA (45 mins)
Professor, Rutgers University & Academic Research Scholar, Harvard University

Integrating Public and Ecosystem Health Systems: Challenges and Opportunities to Move from Knowledge to Action
Kathleen Rest, PhD, MPA (20 mins)
Senior Fellow, Institute for Global Sustainability, Boston College

Greening the University Campuses: The University of West Attica Vision
John Kaldellis, PhD (20 mins)
Professor, University of West Attica

National Strategy on Research, Technological Development and Innovation 2021-2027. Research and Innovation Priorities for the support of Circular Economy and Sustainability
Dr Antonios Gypakis (20 mins)
Head of the Policy Planning Department / Planning and Programming for Research and Innovation Directorate, General Secretariat for Research and Innovation

Implementation of Food Waste Management in Greek Municipalities under a Circular Economy Perspective
Lyberatos Gerasimos, PhD (20 min)
Professor, National and Technical University of Athens

17:15-17:30 — **Closing Remarks and Discussion - Day 2**

Wendy M. Purcell, PhD FRSA
Professor, Rutgers University & Academic Research Scholar,

November 8, 2023

DAY 3

SUSTAINABILITY AGRICULTURE AND FOOD SYSTEMS

09:00-9:30 — **Refreshments & Networking**

9:30-9:45 — **Introduction to day 3**

Philip Demokritou, PhD
Henry Rutgers Chair and Professor of Nanoscience and Environmental Bioengineering, Rutgers University

9:45-11:30 — **Session 8: Sustainable Agriculture and Food systems**

Sustainable Agriculture
Jason White, PhD
Director of the Connecticut Agricultural Experiment Station & Clinical Professor of Epidemiology (Environmental Health, Yale School of Public Health)

11:30-11:45 — **Coffee Break**

11:45-12:30 — **Sustainable Nanotechnology: Nature-derived sustainable materials for agriculture, food systems, and beyond.**
Philip Demokritou, PhD
Henry Rutgers Chair and Professor of Nanoscience and Environmental Bioengineering, Rutgers University

12:30-14:00 — **Lunch & Networking**

14:00-15:00 — **Healthy Diets from Sustainable Food Systems: The Mediterranean Diet**
Stefanos Kales, MD
Professor, Harvard Medical School and TH Chan School of Public Health

15:00-16:00 — **Technological Advances in Food Safety [By Zoom]**
Benedetto Marelli, PhD
Associate Professor of Civil and Environmental Engineering, Massachusetts Institute of Technology

16:00-16:30 — **Smart farming Decision Support Systems. A key factor for sustainability and growth in agriculture**
Dimitris Kapnias, *Senior manager - Large Scale Projects, NEUROPUBLIC – GAIA EPICHEIREIN*

16:30-17:00 — **Global food systems under risk: Are we facing a permanent crisis?**
Yannis E. Doukas, PhD
Assistant Professor of Agricultural Economics and Policy, National and Kapodistrian University of Athens, Greece

17:00-17:30 — **Closing Remarks and Discussion - Day 3**

Philip Demokritou, PhD, Jason White, PhD, Stefanos Kales, M

DAY 4

SUSTAINABILITY IN VARIOUS SECTORS IN GREECE

09:30-10:00 — **Refreshments & Networking**

10:00-10:15 — **Opening Remarks**
Margarita-Niki Assimakopoulos, PhD
Associate Professor, Physics Department, National and Kapodistrian University of Athens

Maria K. Koukou, PhD
Asst. Professor, Department of Agriculture Development, Agri-Food and Natural Resources Management, National and Kapodistrian University of Athens

10:15-11:15 — **Sustainable Buildings**
Margarita-Niki Assimakopoulos, PhD
Associate Professor, Physics Department, National and Kapodistrian University of Athens

11:15-11:45 — **Standarisation activities and guidelines to decarbonise buildings construction and operation**
Alkis Triantafyllopoulos, *Mech. Eng. ASHRAE Region XIV SA Chair*

12:00-12:30 — **Sustainability in industrial company WILO**
Panagiotis Stapas, *Managing Director Wilo Hellas and Vice President ASHRAE Hellenic Chapter*

12:30-12:45 — **Sustainability & EUROPA. Looking into the future.**
Dikaiou Eleni, Energy Efficiency Consultant M.Sc., PMP, Europa Profil Aluminium S.A.

12:45-13:15 — **Decarbonization of European Islands. The Scientific Experiment of Tilos**
John Kaldellis, PhD
Professor, University of West Attica

13:15-13:45 — **Sustainable geothermal applications – the case of Polichnitos**
Maria K. Koukou, PhD
Asst. Professor, Department of Agriculture Development, Agri-Food and Natural Resources Management, National and Kapodistrian University of Athens

Michail Gr. Vrachopoulos, PhD
Professor, Department of Agriculture Development, Agri-Food and Natural Resources Management, National and Kapodistrian University of Athens

13:45-14:15 — **Closing Remarks and Discussion - Day 4**

Philip Demokritou, PhD, Margarita-Niki Assimakopoulos, PhD

Session 8: Sustainable Agriculture and Food Systems

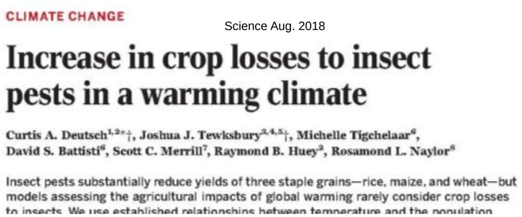
Drrs J. White & P. Demokritou

Agri-food systems: Major single determinant of Planetary and Human Health

- At present, the world is off track, headed toward extreme **climate change** and devastating **pandemics of infectious diseases, obesity, and diabetes coupled with pollution** that **reversed the gains** in public health of the last century and **undermined economic progress**
- **AgFood systems**: Tremendous impact on greenhouse gas emissions, freshwater and energy use, nitrogen cycling, and carbon sequestration. – CLIMATE CHANGE, BIODIVERSITY, POLLUTION
- **Food production** will need to increase by 70-100% by 2050 to sustain the population growth (USDA, 2019)
  - **Negative pressure from a changing climate and a loss of arable soil**: Current agricultural practices have experienced a consistent decline in yield over the last 4 decades
- **Food safety and waste**: 30-40% of food is wasted across the "farm to fork" continuum, 48 million annual cases of food-borne illness in the US, leading to 128,000 hospitalizations and 3,000 deaths
- **AgFood and Planetary Health**: Low efficiency of current agrichemical delivery and utilization (fertilizers, pesticides, etc), which is currently at 2-20% results in environmental issues.- Lack of PRECISION (3Rs- Right place, right time, right dose)
- **Great Food Transformation is needed: Healthy diets from SUSTAINABLE food systems**
- Nanotechnology can and will play a significant role in this effort in transforming AgFood systems ; **particularly dealing with the inefficiencies in agri-chemical delivery, functional food and food safety**


Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems

Walter Willett, Johan Rockström, Brent Loken, Marco Springmann, Tim Lang, Sonja Vermeulen, Tara Garnett, David Tilman, Fabrice DeClerck,

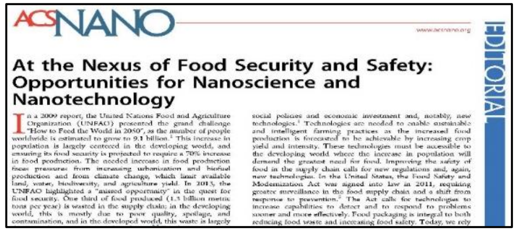

CLIMATE CHANGE

Science Aug. 2018

Increase in crop losses to insect pests in a warming climate

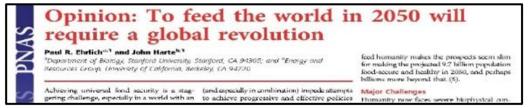
Curtis A. Deutsch, Joshua J. Tewksbury, Michelle Tigchelaar, David S. Battisti, Scott C. Merrill, Raymond B. Huey, Rosamond L. Naylor

Insect pests substantially reduce yields of three staple grains—rice, maize, and wheat—but models assessing the agricultural impacts of global warming rarely consider crop losses


ACS NANO

EDITORIAL

At the Nexus of Food Security and Safety: Opportunities for Nanoscience and Nanotechnology


PNAS

Opinion: To feed the world in 2050 will require a global revolution

Paul R. Ehrlich and John Harte


Environmental Science Nano

TUTORIAL REVIEW

Nanotechnology for sustainable food production: promising opportunities and scientific challenges

Sónia M. Rodrigues, Philip Demokritou, Nick Dokoozlian, Christine Ogilvie Hendren, Barbara Karn, Meagan S. Mauter, Omowunmi A. Sadik, Maximilian Safarpour, Jason M. Unrine, Josh Viers, Paul Welle, Jason C. White, Mark R. Wiesner and Gregory V. Lowry

Declining Global Food Safety & Security!!!

Jason White

Sustainable Nanotechnology: Bio-inspired, nature derived and non toxic nanomaterials for agri-food systems

Philip Demokritou, PhD (and group)

Henry Rutgers Chair and Professor in Nanoscience and Bioengineering, RBHS

Chair, Division of Environmental and Population Health Biosciences, EOHSI

Professor MAE, Rutgers School of Engineering

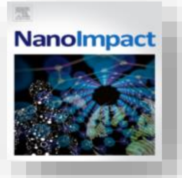
Adjunct Professor, Chan School of Public Health, Harvard University

Director, Nanoscience and Advanced Materials Center, RBHS

Founding Director, Center for Nanotechnology and Nanotoxicology, Harvard University

Founding Co-Director, Nanyang/Singapore-Harvard SusNano Initiative

Co-Editor in Chief, NanoIMPACT (ELSEVIER, IF 6.1)



Nanoscience & Advanced Materials Center (NAMC)

SusNano

"….. development of nanomaterials in safe and responsible way, with considerations of environmental health impact, in order to sustain economical, social and environmental health benefits and address emerging societal challenges…."

AgFood Research at our Nanocenter (NAMC)

FUNDAMENTAL & TRANSLATIONAL RESEARCH

- Development of Nature-derived biopolymers for agri-food systems using synthetic biology or extracted as part of **circular economy** from food waste and crop residues
- Development of scalable techniques to engineer natural polymers into functional materials for agri-food system applications.
- Development of nanoplatforms for **climate smart** agri-chemical delivery using biodegradable, nature derived biopolymers (**agriCHEM project**)
- Development of sustainable, nano–enabled, biodegradable, non toxic Smart Food Packaging (SFP) materials to enhance food safety and quality (**susPACK project**)
- Development of ingestible nature derived non toxic, nanoplatforms to modulate bioavailability of nutrients in the gut (**susFOOD project**)
- **Development of nature inspired antimicrobial platforms to enhance food safety and quality (EWNS)**

Sustainable by design, public, and regulatory acceptable materials and technologies:

- **Assessing and minimizing the environmental and toxicological footprint of materials across their life cycle as part of the development phase**

SusNano

Nanoscience & Advanced Materials Center (NAMC)

Funding Sources

USDA NIFA

NIH National Institutes of Health
Turning Discovery Into Health

NIEHS
National Institute of
Environmental Health Sciences

BARD

STERIS

Panasonic

nature's touch

Nanyang Technological University – Harvard T. H. Chan School of Public Health
Initiative for Sustainable Nanotechnology

Disclaimer

DIETRICS

TASTY · HEALTHIER · NATURAL

Safer by design, public, and regulatory acceptable Nanomaterials and technologies

- **Sustainable Biopolymer based nanoplatforms for nutrient delivery and modulation of "unwanted" substances (SUSFOOD)**

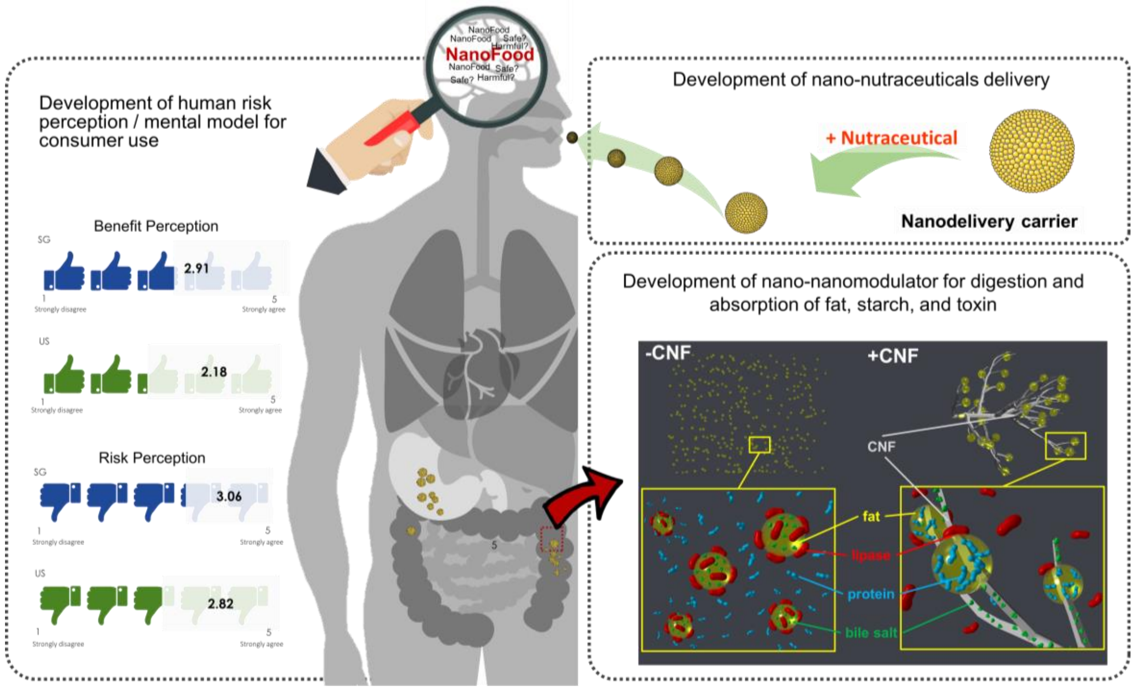
  Engineering interfacial processes in the gut to modulate the absorption of nutrients and target-specific delivery of nutrients using nature derived and non toxic nanoplatforms

- **Biopolymer based "smart" materials for agri-chemical delivery and food packaging (SusPACK & AGRICHEM Projects**
  - Abiotic and biotic triggered core-shell nanostructures for precise delivery of agrichemicals (**agriCHEM**)
  - Smart antimicrobial fibers for food packaging to enhance food safety and minimize spoilage(**susPACK**)

- **Engineered Water Nanostructures (EWNS):**
  - A water based, green, antimicrobial nanocarrier platform for food safety and beyond

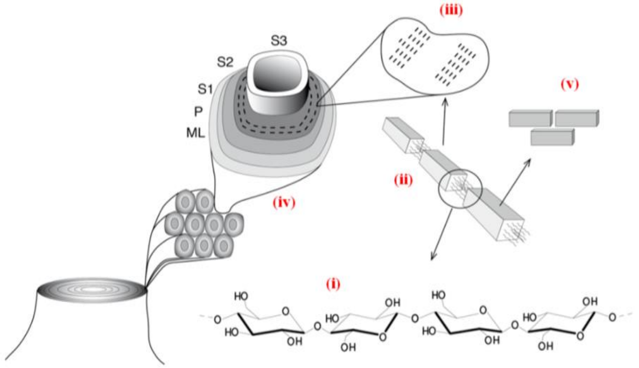
SusFOOD Objectives

- ❑ Extracting biopolymers from **food waste** as part of **circular economy** to be used in AgFood applications and beyond.
  - ❑ **Functional Food:** Designing ingestible biopolymer based **nano-platforms** to reduce GIT digestion and absorption of specific dietary substances and toxins (**nano-modulation**) and increase bioavailability of nutrients (nano-nutraceuticals)
    - ❑ **Unwanted substances**: Fat, carbohydrates (macronutrients)Toxins (e.g. pesticides, heavy metals)
- ❑ Assessment of their potential tox implications across biological systems
- ❑ Assessment of Public Perception of Nano-Enabled Food Products in US and Singapore



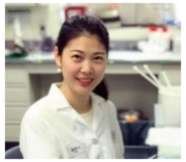
Sustainable, biodegradable, biopolymers

- **Biopolymers:** Nature derived polysaccharides and plant based proteins, non toxic and biodegradable, often are part of waste stream
    - **Celulose**- literally "grows on trees", fundamental building block of plants
    - **Chitosan-** a polysaccharide that is obtained from the hard outer skeleton of crustaceans (ie crab, lobster, and shrimp)
    - **Okara:** A polysaccharide byproduct of tofu manufacturing
    - **Kefiran** – Extracted from kefir, exopolysaccharides secreted by lactic acid bacteria
    - **Carbon dots:** Synthesized from any carbohydrate source
    - **Pullulan**: polysaccharide synthesized during enzymatic degradation of starch by microorganisms such as yeast.
- Development of **scalable** approaches to extract biopolymers from biomass waste as part of a circular economy
- Development of **scalable methods** to synthesize biopolymer based **nanoplatforms** for agri-food applications
    - **Synthesis methods:** Electrospray/electrospinning and rotary jet spinning



CASE STUDY:
Engineering interfacial processes in the GIT using Nanocellulose: Modulating fat digestion and absorption

Kunal Blattacharya
Research Associate

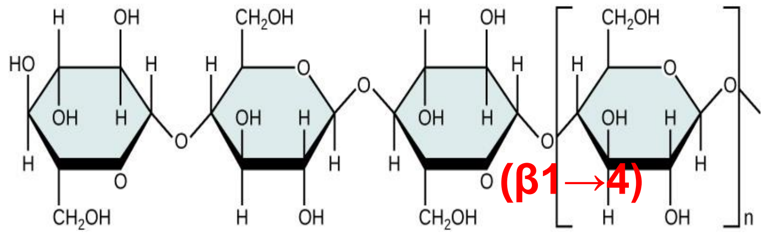


Xiaoqiong Cao,
Research Fellow

Glen Deloid
Research Associate

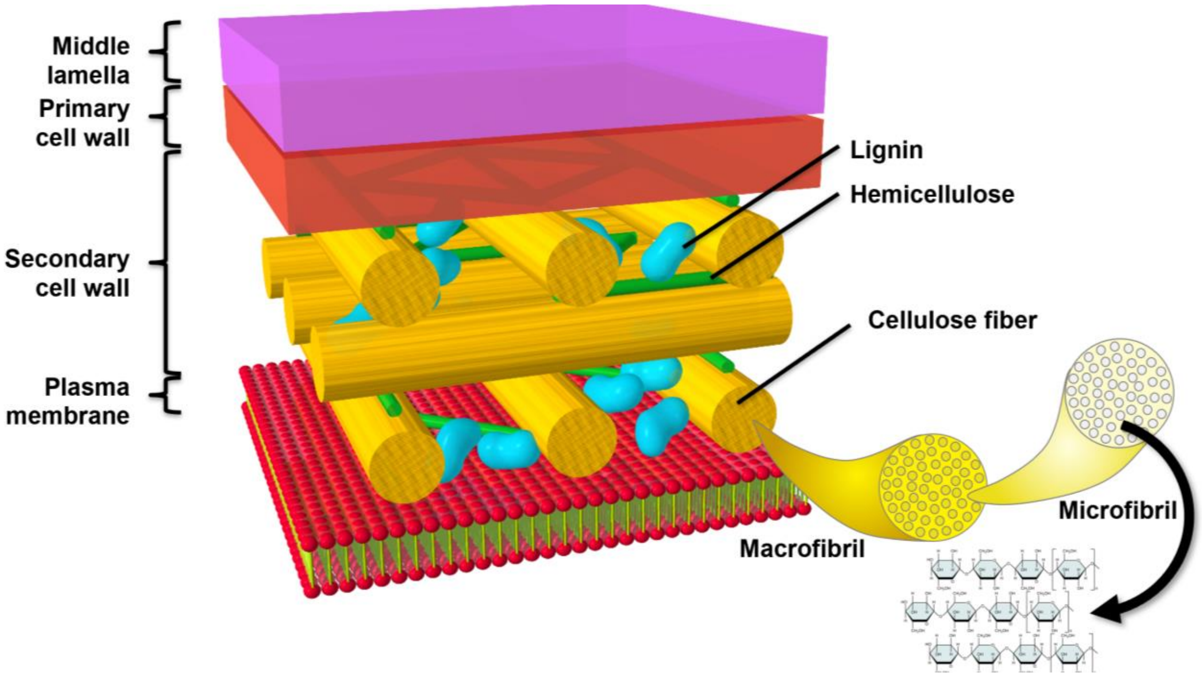
HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH
CENTER FOR NANOTECHNOLOGY AND NANOTOXICOLOGY
http://hsph.harvard.edu/nano

Cellulose



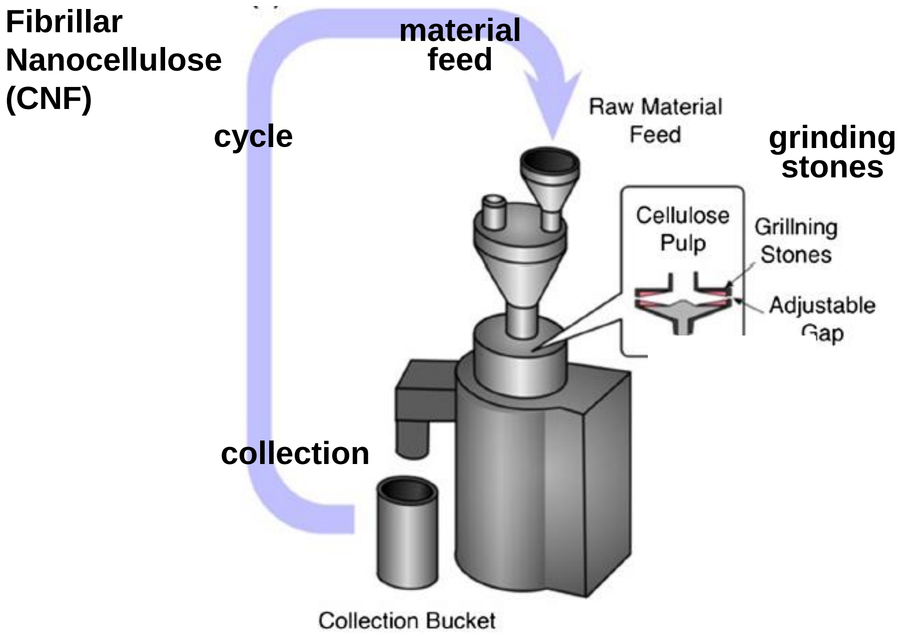
linear chains of glucose units linked by β-1,4-glycosidic bonds

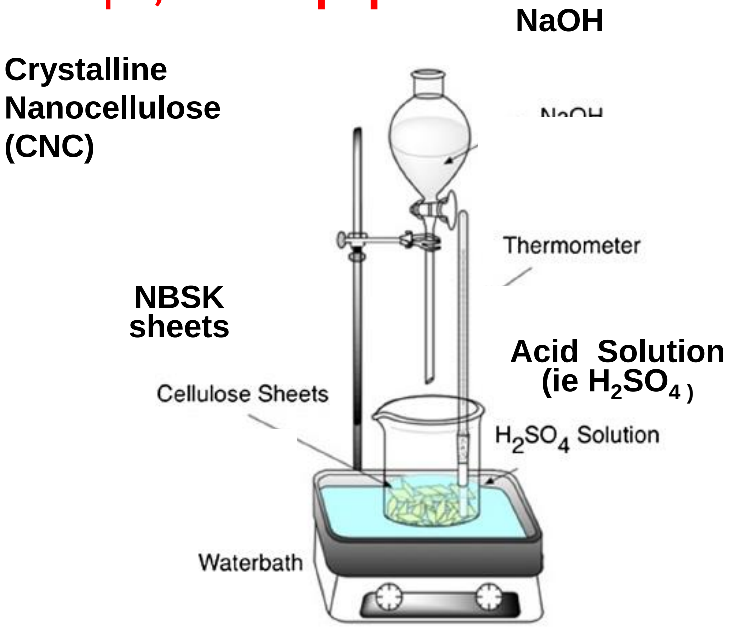
- **Cellulose**- literally “grows on trees” , fundamental building block of plants- part of secondary cell wall
  - Non **digestible** by humans- zero caloric value
  - Classified by FDA as **GRAS material** **(** generally regarded as safe for food use)
- **Nanocellulose (NC):**
  - **Two main nanoforms**: Nanofibrils (CNFs) and Nanocrystals (CNCs)
  - Currently used as a food additive and in food packaging films
- **Question:** Is nano-cellulose a GRAS material as its micron/macro size form?

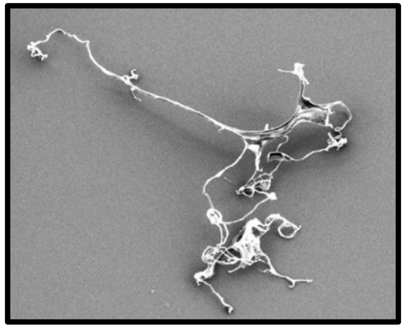


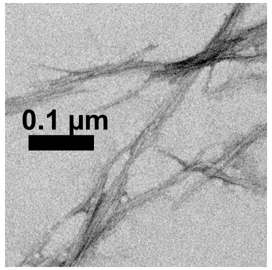
CNF and CNC synthesis: Mechanical and acid milling

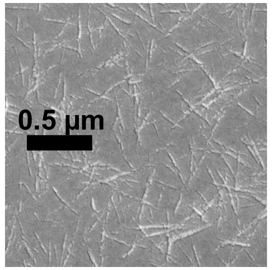
- **Starting material: Pulp** is a lignocellulosic fibrous material prepared by chemically or mechanically separating **cellulose** fibers from **wood**, fiber crops, waste **paper**









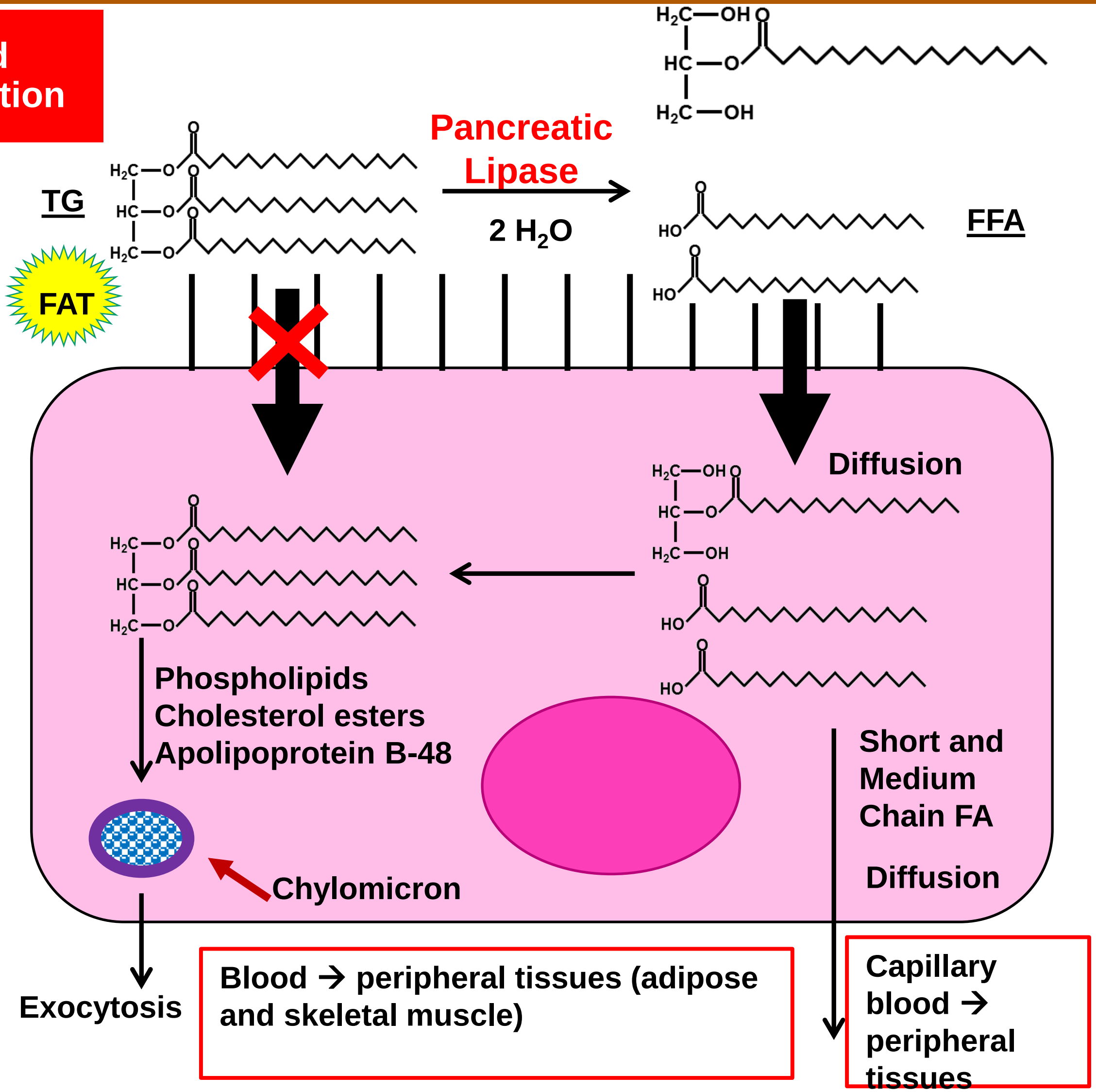


(Materials from Harvard -NIEHS ENM repository)

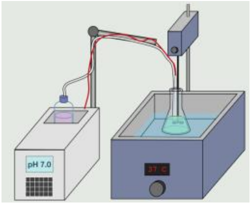
Georgios Pyrgiotakis,
Research Scientist

RESULTS: Using Nanocellulose to reduce Intestinal digestion and absorption of Fat

Fat digestion and Intestinal absorption



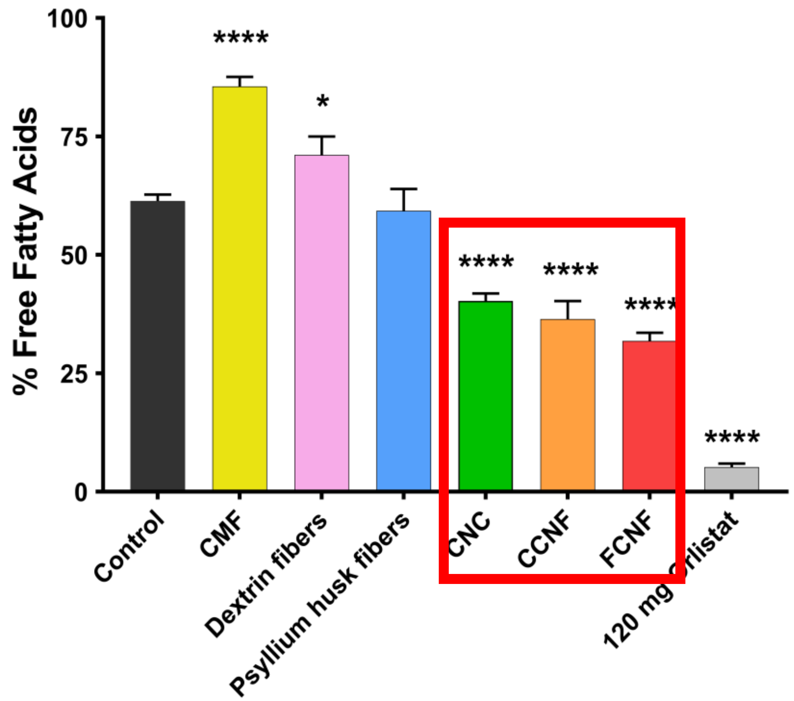
Small intestinal Phase of GIT simulator



- pH in small intestine: 7
- When TG are hydrolyzed, FFA are released and pH drops.
- pH Stat will inject Sodium hydroxide (NaOH) titrant as needed to bring pH to 7
- <u>Accidental discovery</u>: Less titrant is used for NC compared to other ENMs -> less FFA release due to less fat digestion
- NC Interference with interfacial process in the gut?

RESULTS: Effect of nanocellulose on triglyceride hydrolysis (bioaccessibility) using simulated digestions (¼)

Heavy Cream food model, Final 13.3% Fat, 0.75% fibers



% total FA hydrolyzed based on titrant volume used

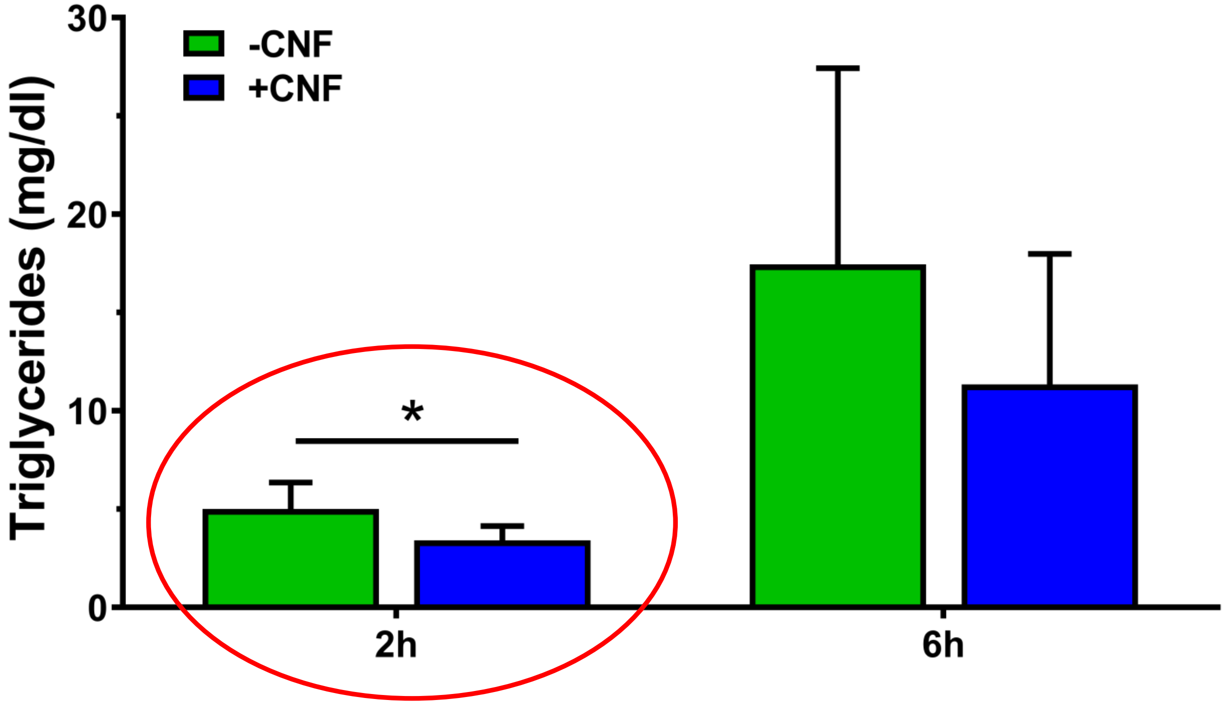
- ❑ **Materials:** Various forms of nanocellulose
  - ❑ FCNFs- 50 nm
  - ❑ CCNF- 80 nm
  - ❑ CNC – 250nm x 25 nm
- ❑ **Controls:** Heavy cream, bulk size cellulose (CMF), other dietary soluble fibers (Dextrin and psyllium husk).
- ❑ **Orlistat:** Chemical inhibitor of Lipase

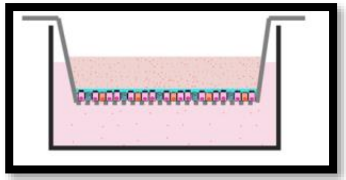
RESULTS:

- ❑ **Both CNF and CNC nanoforms reduce fat digestion (bio-accesibility for absorption)**
- ❑ **~50% reduction with FCNF (CNF-50 nm)**
- ❑ **Nano-specific effects!**

RESULTS: Effect on bioavailability of TG using a triculture small intestinal cellular epithelial model (Deloid et al. PFT 2017) (2/4)

Validation #2: TG concentration in basolateral compartment measured by colorimetric assay



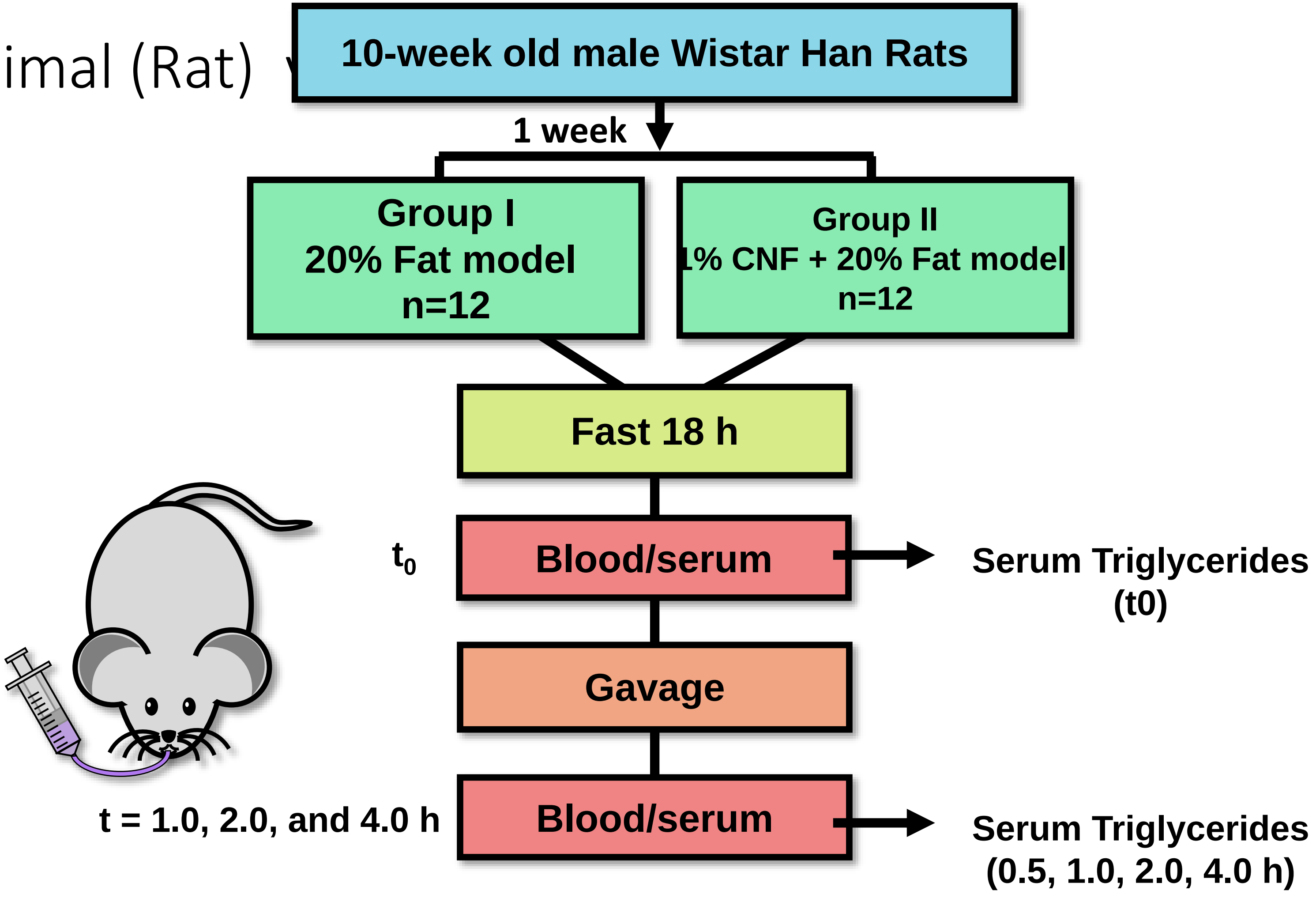


Advanced gut epithelium cellular model

- **CNF (.75%) reduces TG translocated in triculture cellular model , p < .05 at 2 h.**

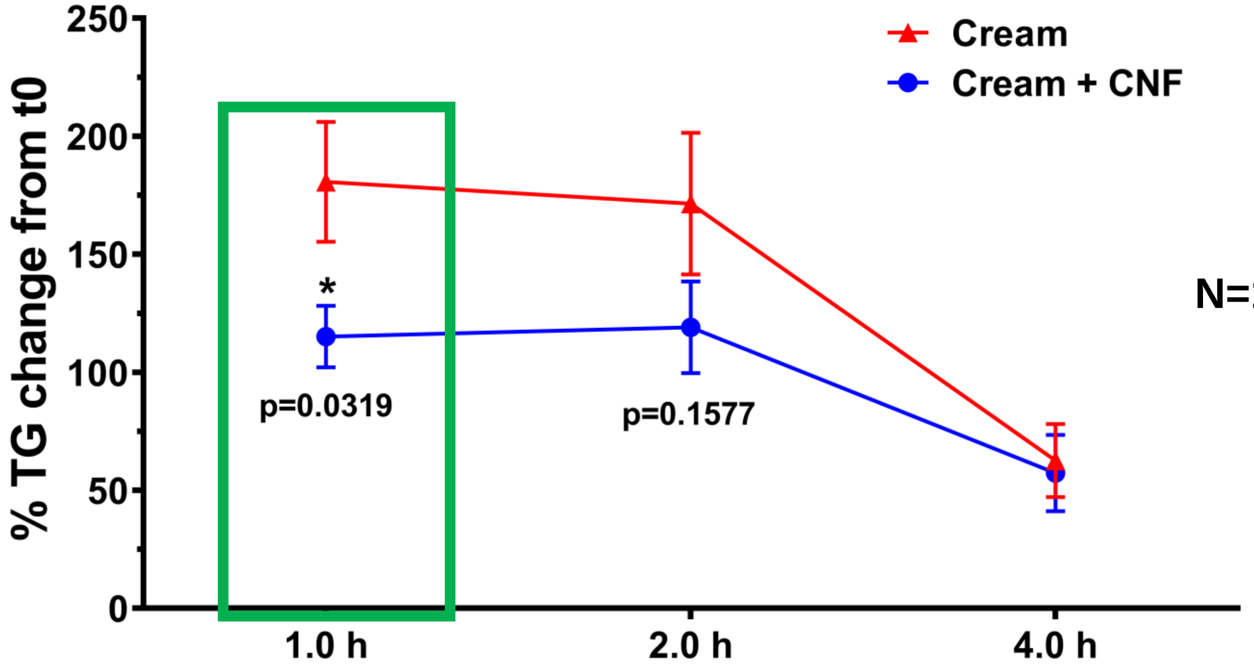
RESULTS: In vivo animal (Rat) validation (3/4)

ESULTS: In vivo animal (Rat) v



RESULTS: In vivo animal (Rat) validation (3/4)

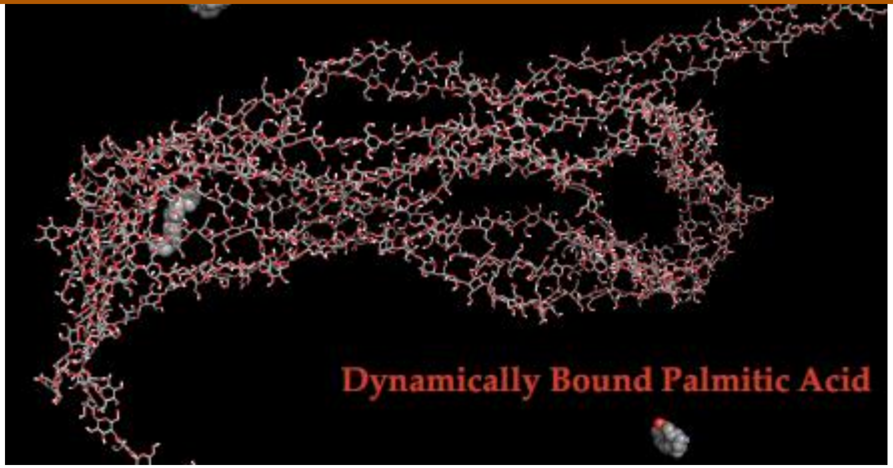
Food model = Heavy Cream, Final 23.3% Fat

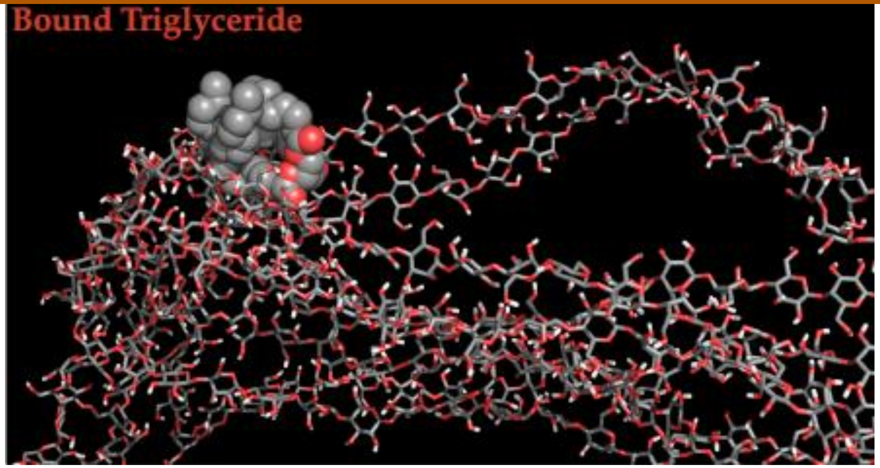


N=12 per group

- **1 h postprandial rise in serum TG levels after heavy cream gavage**
- **TG levels were reduced by approx. 50% ($p<0.05$) when 1% CNF-50 is added in the food.**
- **In agreement with in-vitro cellular data**

RESULTS: Molecular Dynamics Simulations: Sequestration of fat and bile salts by NC fibers?

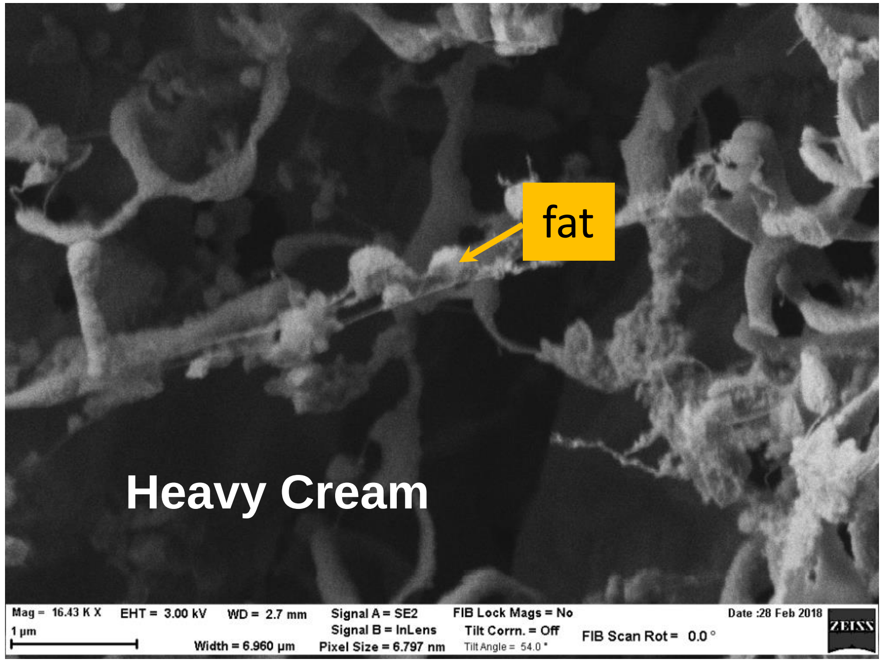




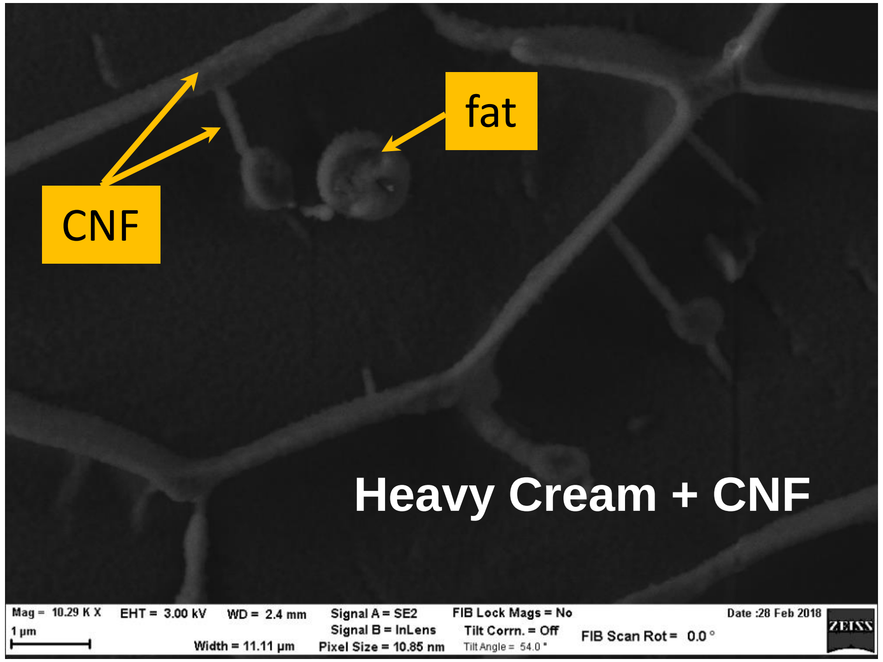
Nickolas Gardner ,
CEINT,DUKE

System	Cellulose Fiber Binding Energy (kcal/mol) ± SD
Cellulose Fiber - Palmitic Acid (FFA)	-2.2 ± 2.3 (weak, highly dynamic)
Cellulose Fiber - Palmitic Triglyceride	-4.4 ± 2.5 (Strong, dynamic
Cellulose Fiber – Glycodeoxycholate (bile salt)	-2.1 ± 0.7 (weak but stable)

RESULTS: Mechanisms: CNF induces coalescence of lipid droplets in the stomach:



Fat droplet diameter = 230±50 nm



Fat Droplet diameter = 700±170 nm

- ❑ CNF induces coalescence of lipid droplets in the stomach and increase lipid size by a factor of 3→ less available interfacial surface area for binding and action of lipase
- ❑ Honey comb cellulose structures are formed and induce trapping of fat globules within the fiber structure → Slow down mobility and accessibility of lipase and bile salts to fat globules

Ana Stevanovic, Research fellow

Mark Zhenuan, Research fellow

ACS NANO
Cite This: *ACS Nano* 2018, 12, 6469–6479
www.acsnano.org
ARTICLE

Reducing Intestinal Digestion and Absorption of Fat Using a Nature-Derived Biopolymer: Interference of Triglyceride Hydrolysis by Nanocellulose

Glen M. DeLoid,[*,†] Ikjot Singh Sohal,[‡] Laura R. Lorente,[†] Ramon M. Molina,[†] Georgios Pyrgiotakis,[†] Ana Stevanovic,[†] Ruojie Zhang,[§] David Julian McClements,[§] Nicholas K. Geitner,[‖] Douglas W. Bousfield,[⊥] Kee Woei Ng,[#] Say Chye Joachim Loo,[#] David C. Bell,[∇] Joseph Brain,[†] and Philip Demokritou[*,†]

The Harvard Gazette

NEWS +

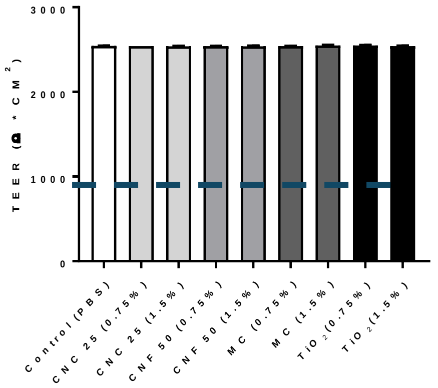
News from Harvard schools, offices, and affiliates

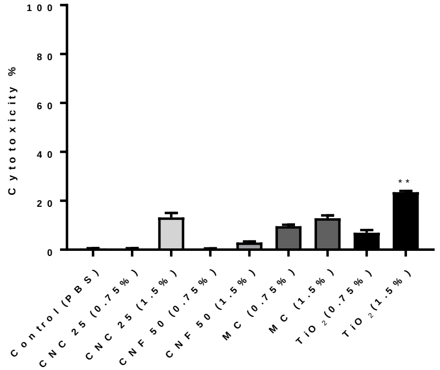
Could a nanofiber help fight the obesity epidemic?

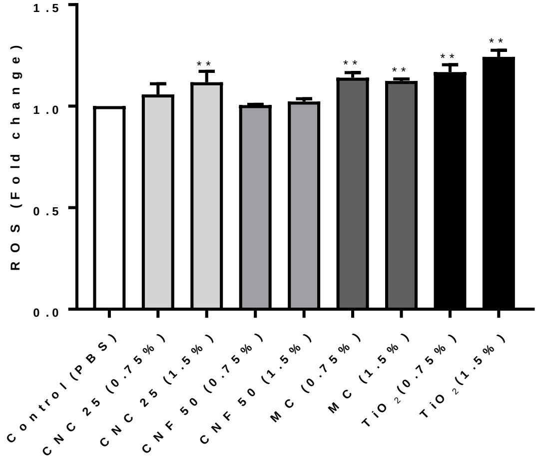
DATE
July 19, 2018

You won't smell it. You won't taste it. And you certainly won't see it. But a nanocellulose material derived from all-natural substances could potentially become a food additive that reduces fat digestion and absorption and aids in weight loss.

Question : Does Nanocellulose is SAFE similar to GRAS "bulk" size cellulose ?



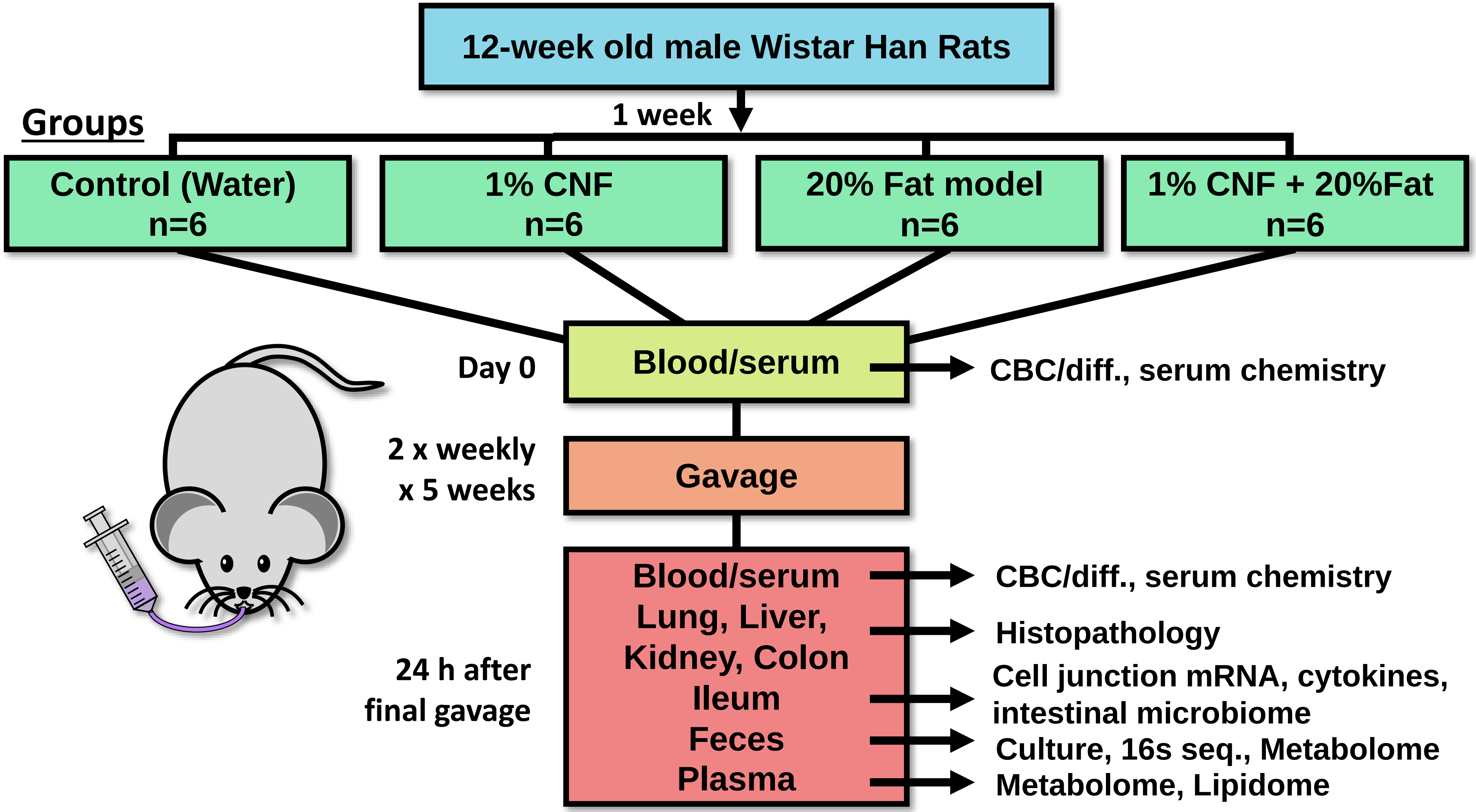




- **Materials:** Various forms of nanocellulose (CNCs, CNFs)
- **Concentrations:** Realistic food concentrations (0.75%, 1.5% w/w)
- **Controls:** Micron size cellulose (MC) and TiO_2 (E171)
- Use **Harvard iENM integrated methodology**[1] to prepare simulated digestas for cellular studies using the tri-culture gut model
- **Intestinal epithelium integrity:** Intact epithelium barrier (TEER values)
- **Cytotoxicity (LDH):** Minimum, at same or lessen levels compared to bulk cellulose **(MC)**
- **ROS generation:** Lower or same levels as bulk size cellulose

- **Appears to behave like GRAS "bulk"size cellulose in cellular tox. studies**

Results: in vivo tox assessment for CNF- No effects



Ingested nanocellulose: in vitro and in vivo Toxicology Papers

SUSTAINABILITY Masterclass
6 | 7 | 8 | 9 NOVEMBER 2023

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.elsevier.com/locate/nanoimpact

Frontier Article

Cytotoxicity and cellular proteome impact of cellulose nanocrystals using simulated digestion and an *in vitro* small intestinal epithelium cellular model

Xiaoqiong Cao [a,1], Tong Zhang [b,1], Glen M. DeLoid [a], Matthew J. Gaffrey [b], Karl K. Weitz [b], Brian D. Thrall [b], Wei-Jun Qian [b,*], Philip Demokritou [a,*]

Environmental Science Nano

PAPER

Cite this: DOI: 10.1039/c9en00184k

Toxicological effects of ingested nanocellulose in *in vitro* intestinal epithelium and *in vivo* rat models

Glen M. DeLoid, *[a] Xiaoqiong Cao,[a] Ramon M. Molina,[a] Daniel Imbassahy Silva,[a] Kunal Bhattacharya,[a] Kee Woei Ng,[bc] Say Chye Joachim Loo,[b] Joseph D. Brain[a] and Philip Demokritou*[a]

DIETRICS
TASTY · HEALTHIER · NATURAL

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.elsevier.com/locate/nanoimpact

Effects of ingested nanocellulose on intestinal microbiota and homeostasis in Wistar Han rats

Sangeeta Khare[a,1], Glen M. DeLoid[b,1], Ramon M. Molina[b], Kuppan Gokulan[a], Sneha P. Couvillion[c], Kent J. Bloodsworth[c], Elizabeth K. Eder[d], Allison R. Wong[d], David W. Hoyt[d], Lisa M. Bramer[e], Thomas O. Metz[c], Brian D. Thrall[c], Joseph D. Brain[b], Philip Demokritou[b,*]

Presentation Outline

- ❑ **Nature derived, nutrient modulating nanoplatforms (Functional Food)**

  Engineering interfacial processes in the gut to modulate absorption of nutrients using nature derived and non toxic nanoplatforms

- ❑ **Bio-polymer based functional materials for agri-chemical delivery and food packaging**
  - ➢ Abiotic and biotic triggered core-shell nanostructures for precise delivery of **agrichemicals** (agrichem)
  - ➢ Smart antimicrobial fibers for **food packaging** to enhance food safety and minimize spoilage (susPACK)

- ❑ **Engineered Water Nanostructures (EWNS):**
  - ➢ A “green”, antimicrobial, water-based nanocarrier platform for food safety and beyond

Enhancing agrichemical delivery and plant development with biopolymer-based stimuli responsive core-shell nanostructures

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Research fellow

Dr Tao Xu
Research fellow

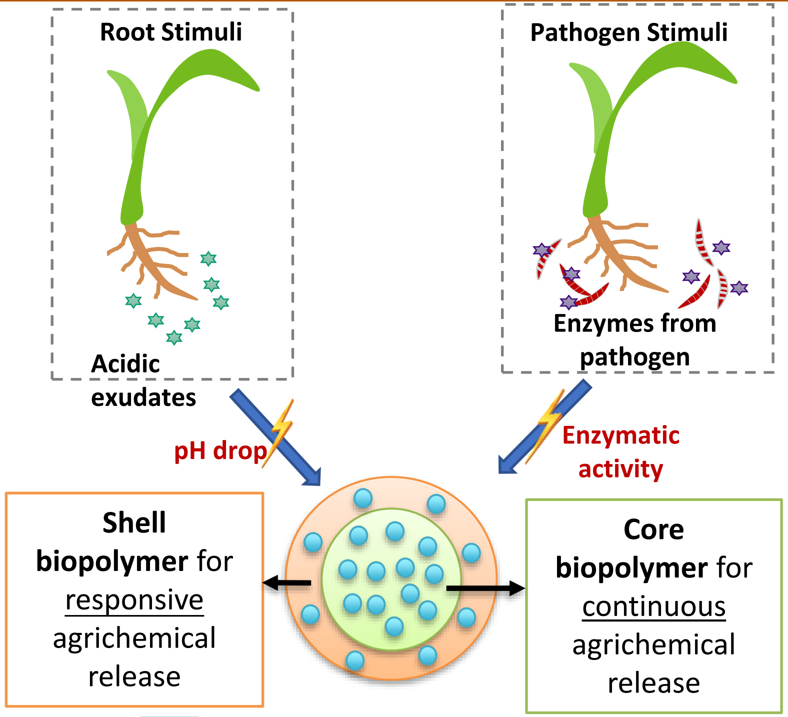
Dr. Jason White
Director

Dr. Yi Wang
(Research fellow)

CAES Team

Study design: pH and enzyme responsive biopolymer based SMART core/shell nanostructures for precise and targeted delivery of agrichemicals

- **Root environment:**
  - Plant roots release **acidic exudates** (e.g., organic acids, amino acids).
  - Plants take in cationic nutrients and **release H^+ ions** into the soil to balance charges.
  - **<u>Root-zone acidification</u> <u>(pH=5)</u> can be used** as an abiotic TRIGGER for agrichemical release to support Plant growth



Shell polymer for dual <u>responsive</u> release
- <u>Chitosan</u>: pH responsive (7 to 5)
- <u>Zein</u> and <u>Starch</u>: proteinase and amylase responsive
- <u>Cellulose acetate</u>: hydrophobic, avoid fast passive diffusion
- <u>PCL</u>: Improve the electrospray stability

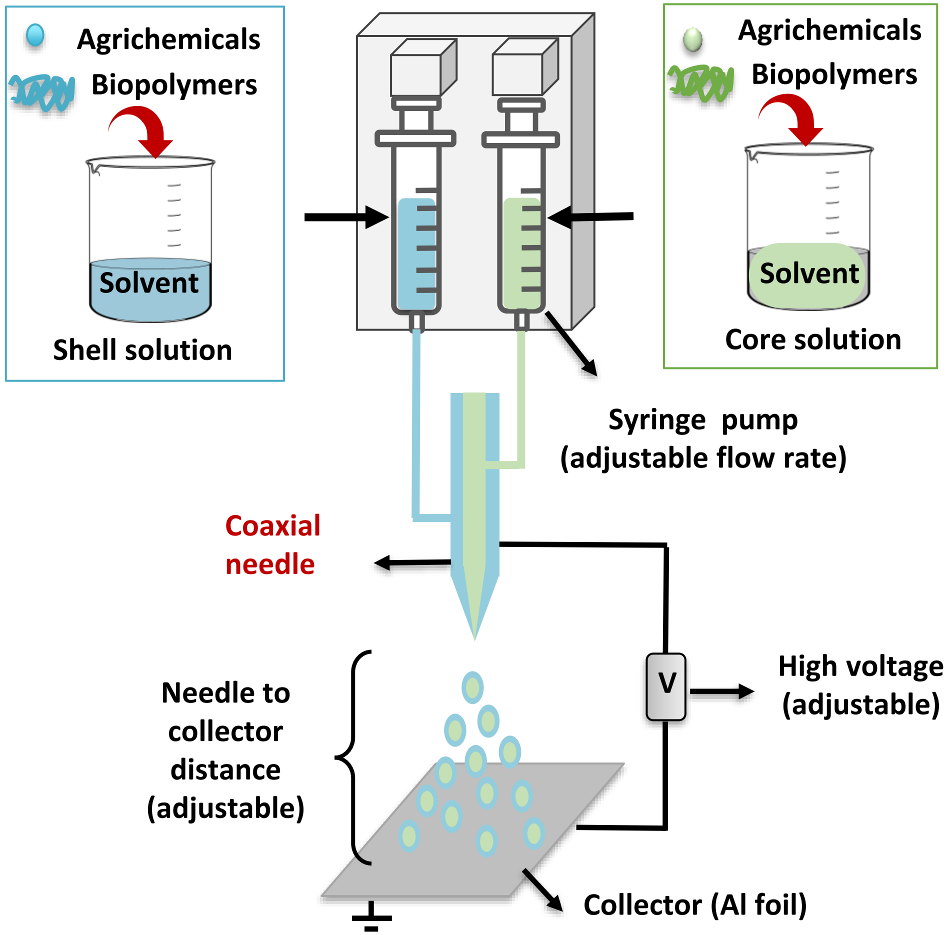
- **Core-shell nanostructures features:**
  - Biopolymer based
  - Stimuli responsive (i.e., pH and enzyme)
  - Ability to fine tune surface hydrophobicity and release kinetics to achieve the “3Rs”

- Phytopathogenic fungi release **enzymes:**
  - *Fusarium* species secretes a variety of **polysaccharide-degrading enzymes**, such as pectinase and amylase.
  - **The presence of enzymes can be used** as a biotic TRIGGER for the release of agrichemicals in order to **support plant growth** and **suppress pathogens.**

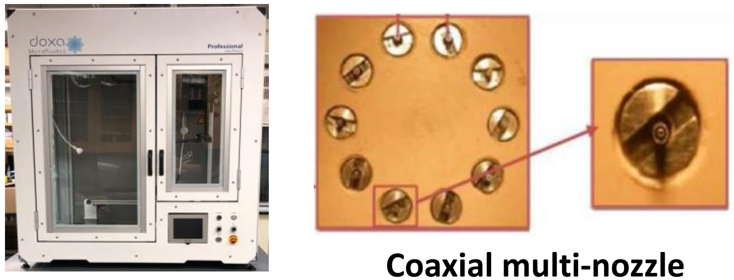
Core polymer Polycaprolactone (PCL)for continuous release
- <u>PCL</u>: hydrophobic polymer to achieve continuous release

Green Synthesis: Coaxial Electrospray

Coaxial Electrospray

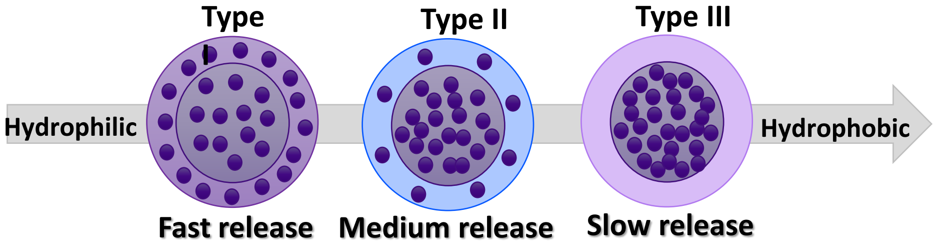


- **Highlight of electrospray synthesis**
  - Scalability (top-down synthesis)
  - Tunable particle size (down to 100 nm)
  - Simple and low cost
  - No chemical byproduct generation- (use of acetic acid as a solvent)

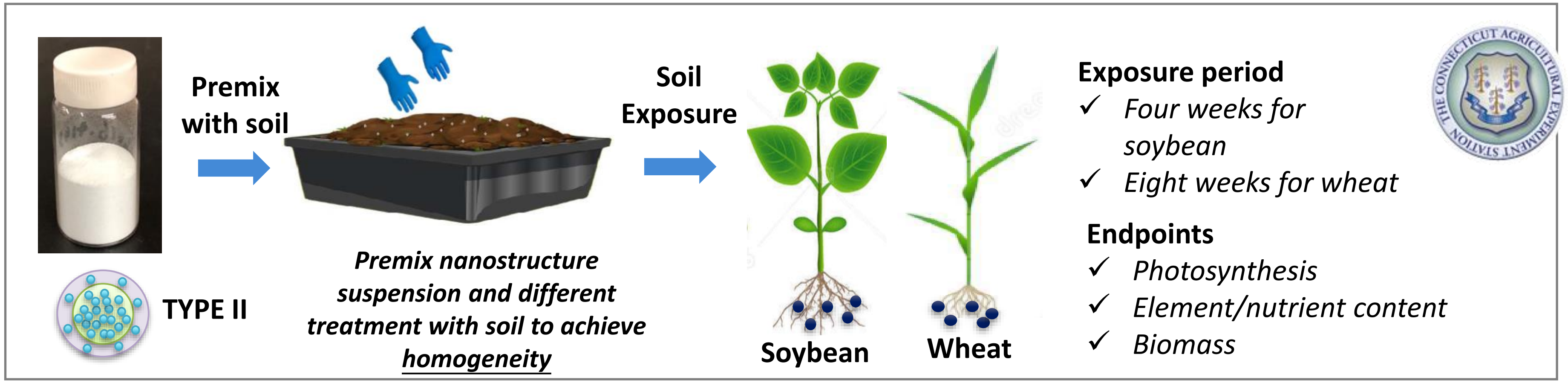


Coaxial multi-nozzle

- **Tuning the agrichemical release** by selecting shell polymer composition and agrichemical distribution in shell and core to achieve the “3Rs”.



Methods: Plant Growth



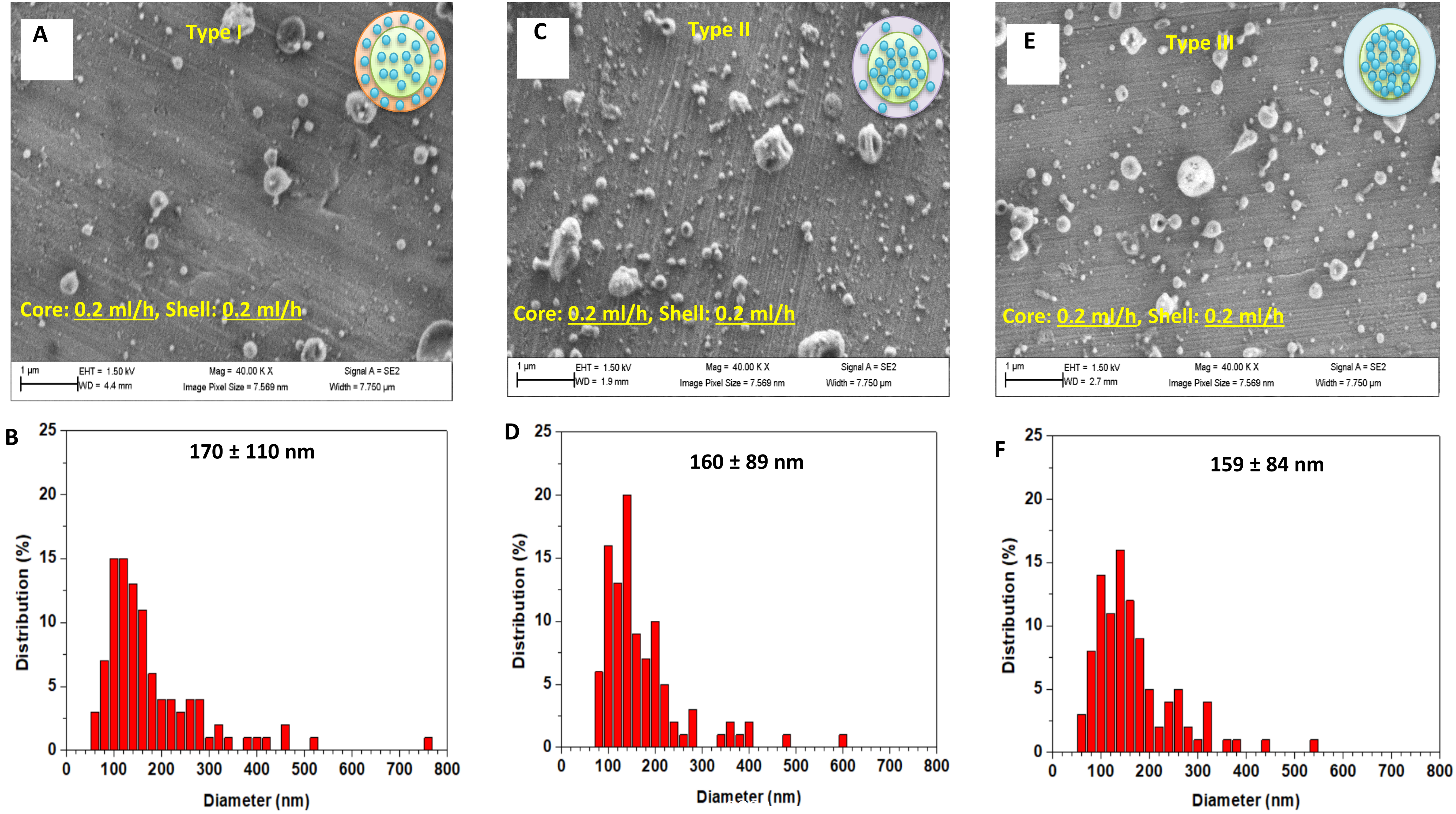
Soybean

Wheat

Treatment

- **Model agrichemical: NPK, Cu (ionic form)**
- **Nanostructure suspension (*131.58 mg, containing <u>25 mg NPK + 0.84 mg Cu</u>*)**
- **Ionic solution (equivalent agrichem)**
  - ***25 mg NPK + 0.84 mg Cu***
  - ***25 mg NPK***
- **Ionic solution (quadruple dose)**
  - ***100 mg NPK + 3.36 mg Cu***
  - ***100 mg NPK***
- **Water**

RESULTS: Morphology and size distribution

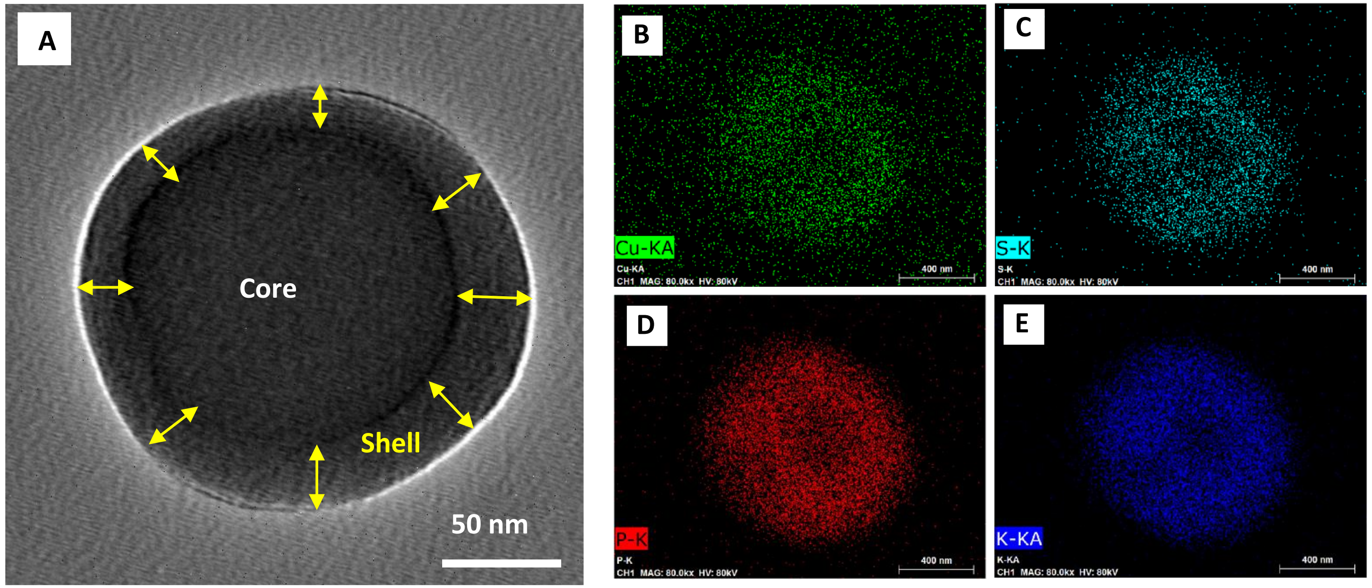


Core-shell structure

Results: Core shell structure

TEM (Type I as an example)

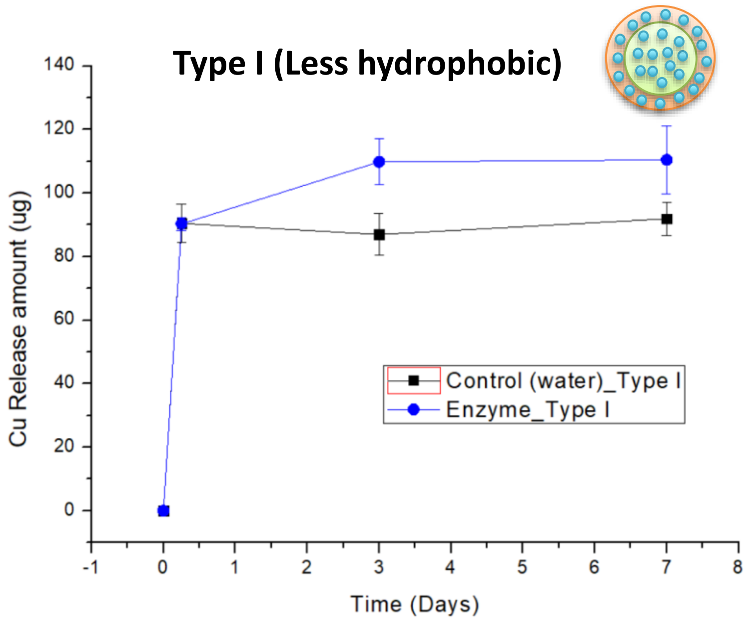
Element mapping of Cu, S, P and K

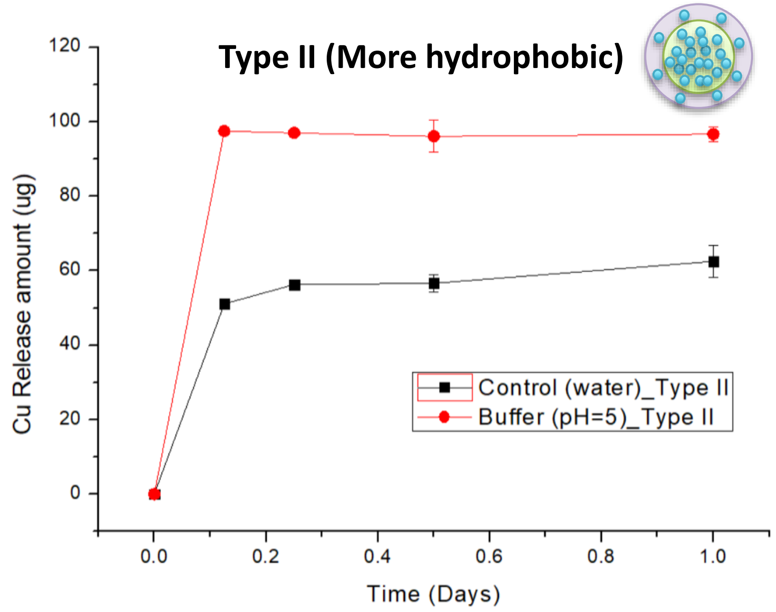


- The nanostructure showed a clear boundary between core and shell, which confirmed the successful synthesis of core/shell nanostructures.

- The element mapping of Cu, S, P, and K clearly showed the nanostructure morphology, indicating the uniform distribution of agrichemicals (i.e., $CuSO_4$ and NPK fertilizer) in the nanostructure.

pH and Enzyme responsive release

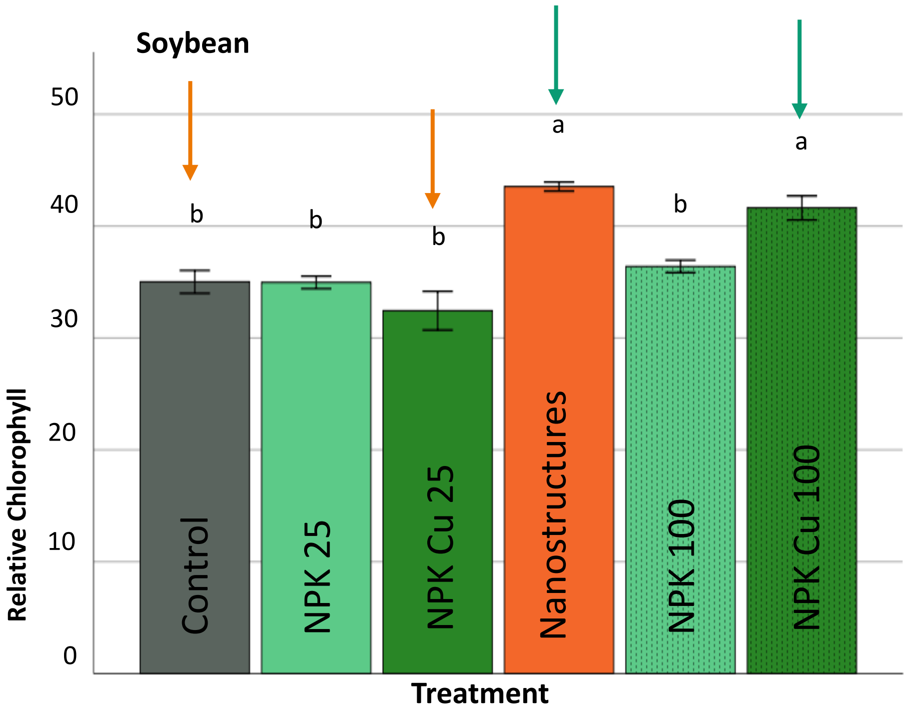




- Increasing the surface hydrophilicity and zein/starch ratio in the shell result to more enzyme sensitive nanostructures (20% increased release).
- For Type II nanostructure, the Cu^{2+} cumulative release amount at pH 5 was approximately 37 % higher than pH 7, indicating high pH responsiveness.

Results: Photosynthesis of soybean

- **<u>Photosynthesis:</u>** Plants convert CO2 and water into chemical energy (sugars) and O2 using sunlight
- **Chlorophyll content:** An indirect measure of photosynthesis and productivity.

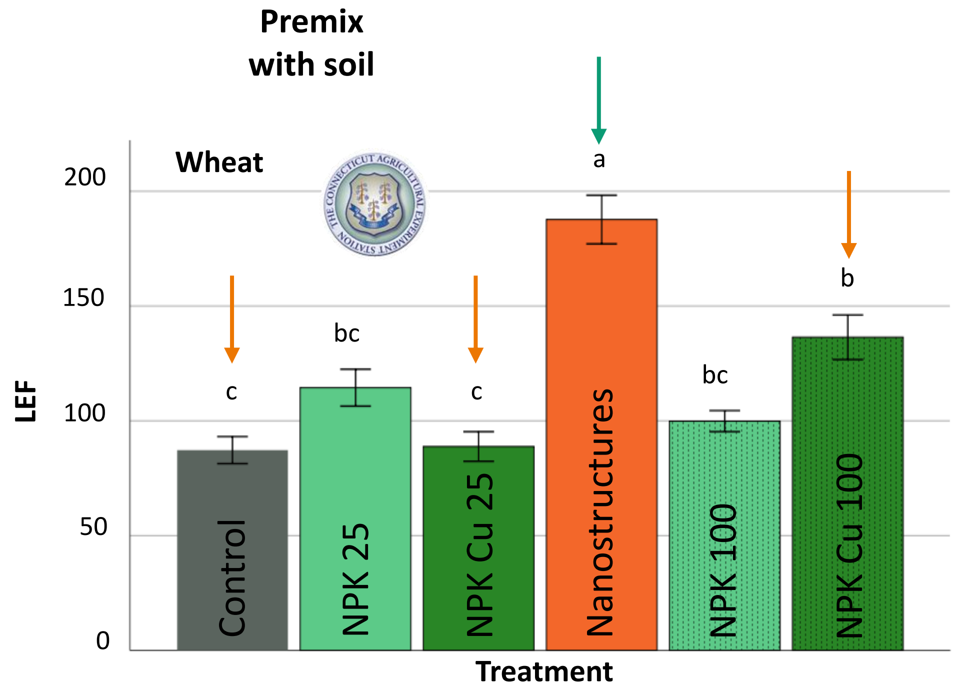


➢ The **relative chlorophyll content** of 4-week-old **soybean** seedlings

- The nanostructures exhibit significantly greater chlorophyll content (43.5) compared to control - 24 % increase
- Chlorophyl content was equivalent to that of 4 times higher agrichemical load in ionic form
- **Less agrichemical use** due to targeted and precise delivery

Results: Linear Electron Flow (LEF) for wheat

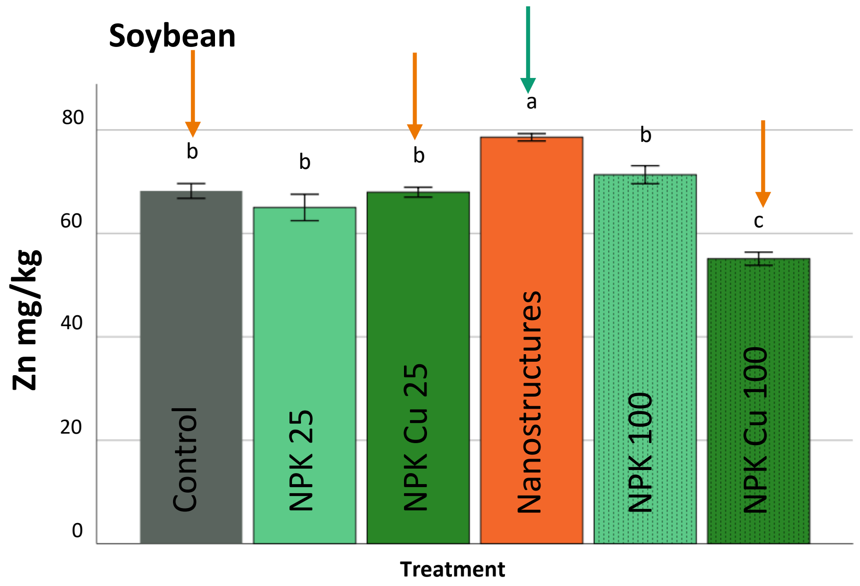
➢ The LEF of 8-week-old **wheat** seedlings.

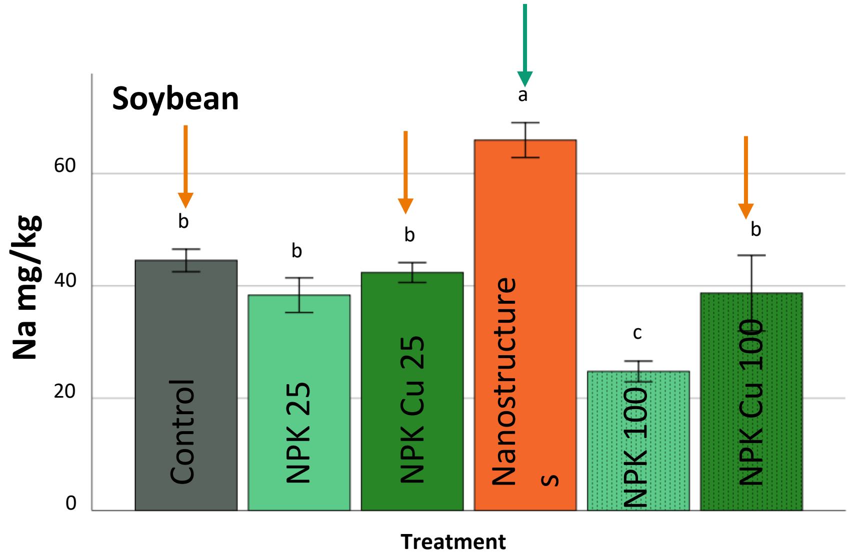


- **LEF** (linear electron flow) indicates the amount of energy that is being moved through the chloroplasts following exposure to light.
- **Chloroplasts** are plant cell organelles that produce chemical energy via the photosynthetic process.
- The CS nanostructures exhibited significantly greater value (187.7) than the control (87.2) and all other treatments (88.8-136.4), suggesting enhanced electron flow and carbon fixation ($p \leq 0.05$).

Results: Micronutrient content of soybean plants

➢ The Zn and Na content in the leaves of 4-week-old soybean seedlings





- **<u>Zn</u> and <u>Na</u> content** were significantly increased in soybean shoots with nanostructure amendment.
- Promising biofortification strategy for other micronutrients using the core-shell nanostructures.

Conclusions

- A scalable, biodegradable, sustainable (non-toxic), biopolymer-based multi stimuli responsive nanoplatform (i.e., core/shell nanostructure) was developed by a **“green” electrospray approach** for agrichemical delivery.

- The **pH and enzyme responsiveness** were demonstrated by the release kinetics of developed nanostructure as a function of nanostructure chemical composition.

- The responsive nanostructure exhibited **superior photosynthesis parameters** in both soybean and wheat, compared to conventional fertilizer controls.

- The **Zn and Na content** in the leaves of 4-week old soybean seedlings were significantly increased with nanostructure amendment, which is a promising **biofortification strategy.**

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ARTICLE

Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures

Tao Xu,[#] Yi Wang,[#] Zeynep Aytac, Nubia Zuverza-Mena, Zhitong Zhao, Xiao Hu, Kee Woei Ng, Jason C. White,* and Philip Demokritou*

Cite This: https://doi.org/10.1021/acsnano.1c11490

ACS Sustainable Chemistry & Engineering

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Research Article

Enhancing Agrichemical Delivery and Seedling Development with Biodegradable, Tunable, Biopolymer-Based Nanofiber Seed Coatings

Tao Xu, Chuanxin Ma, Zeynep Aytac, Xiao Hu, Kee Woei Ng, Jason C. White,* and Philip Demokritou*

Enhancing food safety and quality:
Development of sustainable, biodegradable, stimuli responsive bio-polymer based antimicrobial nanofibers for food packaging materials

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Research fellow

Dr Runze Huang
Research fellow

Dr Tao Xu
Research fellow

Dr Zeynep Aytac
Research fellow

Background: Food Safety and Packaging

- Foodborne disease outbreaks have quadrupled the last decade
- Food waste: 30-50% of our food is wasted from farm to the fork
- **Food packaging**: Petroleum-based polymers, 9 billion Mt of plastics are introduced to the environment in the last 50 years creating the **micro-nanoplastic crisis**

Polyethylene (PE) bag

Synthetic polymers

- Mechanical/gas barrier properties
- Low cost (Siracusa et al., 2008)
- Non biodegradable
- Micro-nanoplastics: byproduct of mechanical and weathering over time of plastics - Environmental and Health implications

Biopolymers

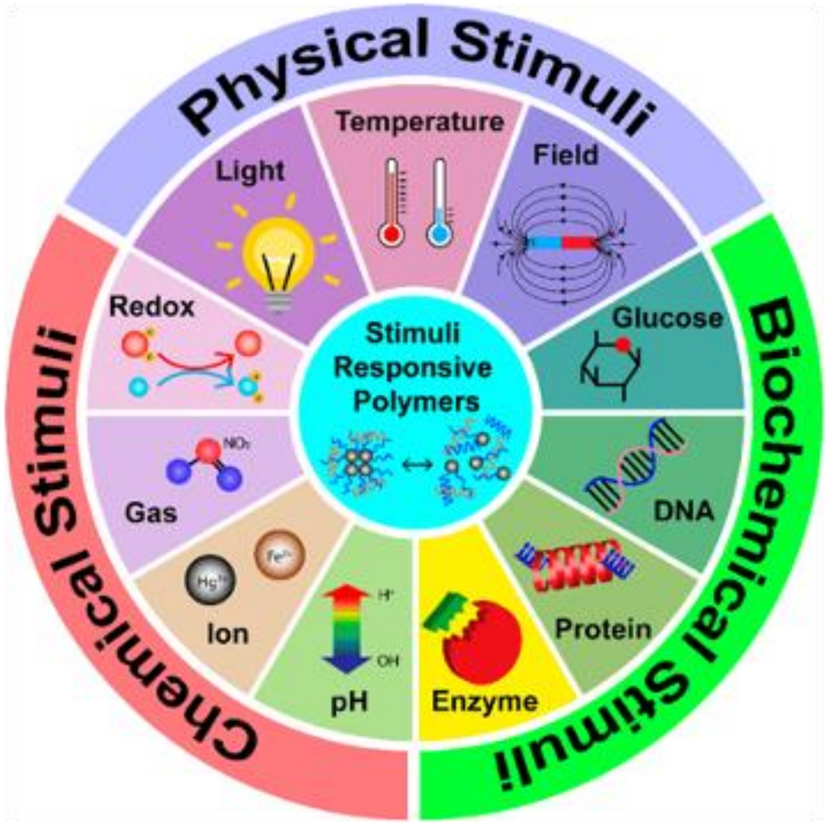
Polysaccharides

- ✓ Cellulose
- ✓ Chitosan
- ✓ Alginate
- ✓ Starch

Proteins

- ✓ Gelatin
- ✓ Zein

Sustainable, smart Food Packaging (SFP) to enhance food safety and quality



- **Biopolymer based, biodegradable non toxic materials**
- **In addition to mechanical and oxygen/moisture barrier SFP needs to have:**
  - Antimicrobial functionality
    - Responsive to abiotic and biotic stimuli and trigger the release of antimicrobials to enhance food safety and minimize spoilage
  - **Precision** in delivery of nature derived antimicrobials to minimize sensory effects and chemical risks
- **Abiotic and biotic triggers:**
  - **Digestive enzymes** from microrganisms- (i.e cellulase, amylase, protease)
  - **Relative humidity:** Microorganisms proliferate in high relative humidity environments

SusPACK Project

- **Development of biopolymer based, biodegradable “responsive” antimicrobial fibers suitable for SFP**

- **Fibrous materials due to their extensive specific surface area have an advantage over antimicrobial film-based approaches**

- **Fibers will be incorporated with nature-derived antimicrobials**

- **Fibers to be Responsive to abiotic and biotic stimuli**
    - **Digestive enzymes** from microrganisms- (i.e cellulase, amylase, protease)
    - **Relative humidity:** Microorganisms proliferate in high relative humidity environments

Nature-Derived Antimicrobial Agents

Antimicrobial Agents

Essential Oils

thyme oil

Organic acids

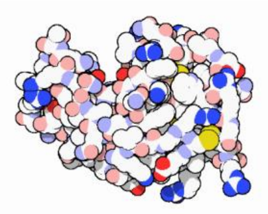
citric acid

Bacteriocins

nisin

Lysozyme

Present in many mucosal secretions (tears, saliva, and mucus)

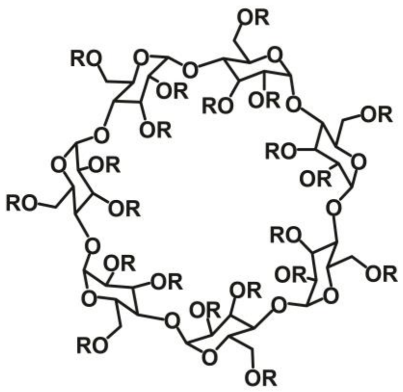


The First Antibiotic

(Discovered by A. Fleming)

RH responsive Cyclodextrin-Inclusion Complexes of AIs (CD-ICs) RH-responsive

- **Cyclodextrins** (CDs) are cyclic oligosaccharides produced by enzymatic degradation of starch.



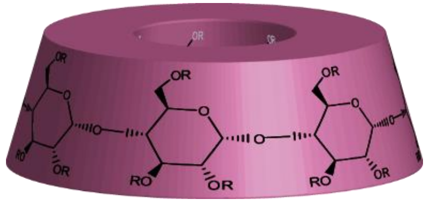
R: H

α-CD

R: H

β-CD

R: H



γ-CD

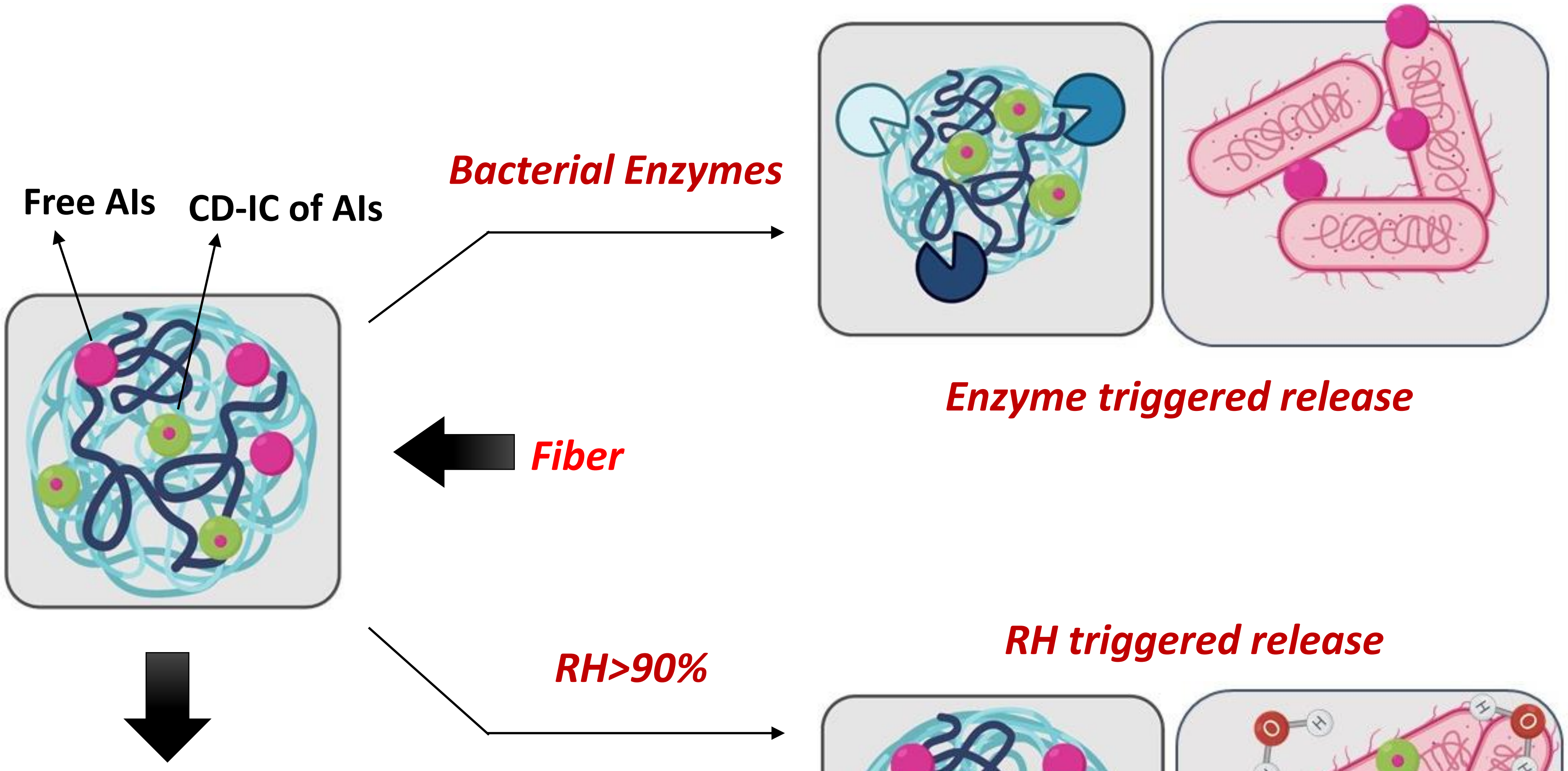
R: H

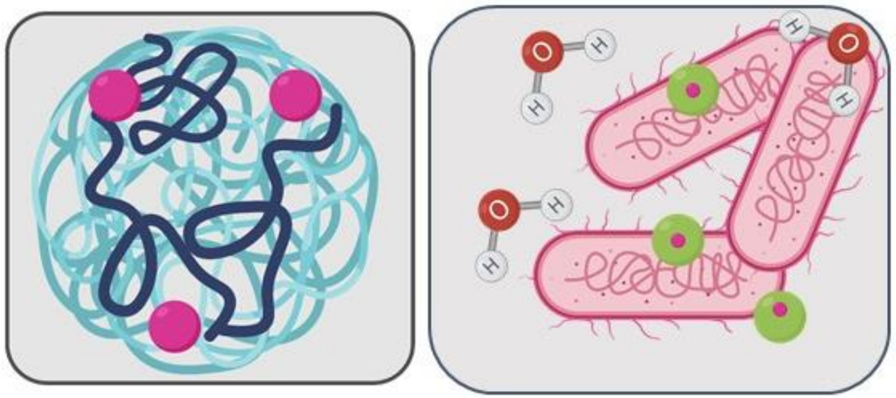
- The most interesting property of CDs is their ability **to form inclusion complexes (ICs)** with **hydrophobic molecules** by non-covalent interactions thanks to their relatively hydrophobic cavity.

- CD-ICs were used in **biomedical research to solubilize** hydrophobic molecules in drug delivery

- When RH exceeds 90%, the CD-ICs disassociate the hydrophobic AI load

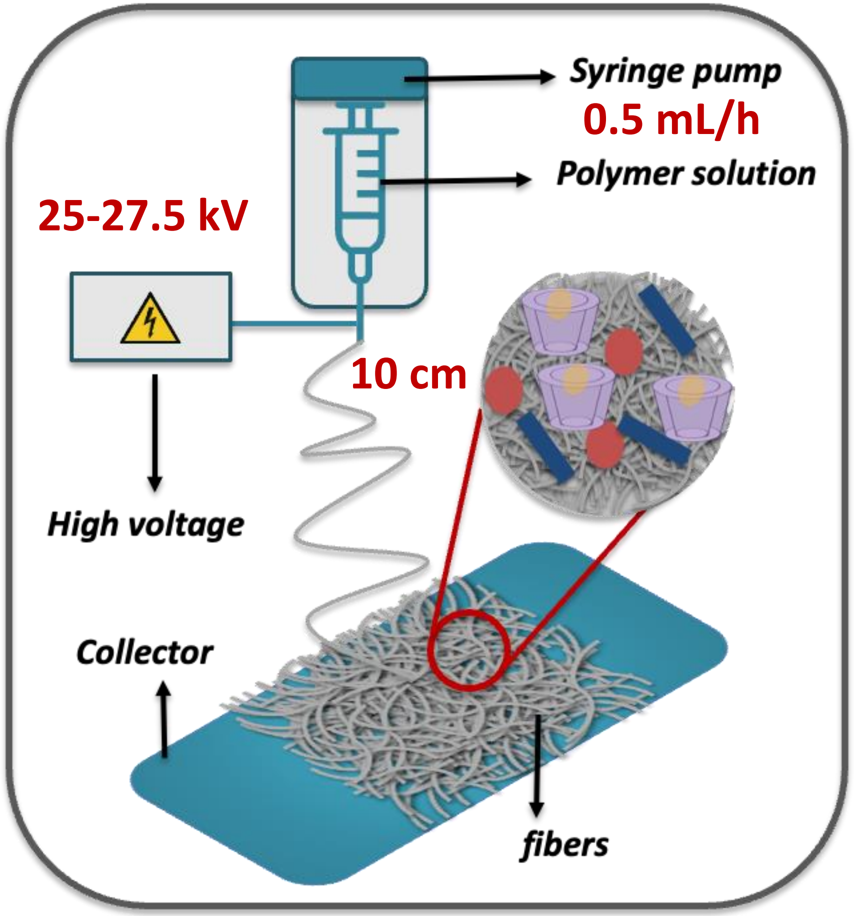
Green Synthesis of Multi-Responsive Fibers which can be used in food packaging





- The fiber is composed of **3 polymers**
  - **Zein** (to be degraded by protease)
  - **Starch** (to be degraded by amylase)
  - **Cellulose nanocrystals** (CNC) (to be degraded by cellulase)

Synthesis of Stimuli Responsive Fibers using electrospinning



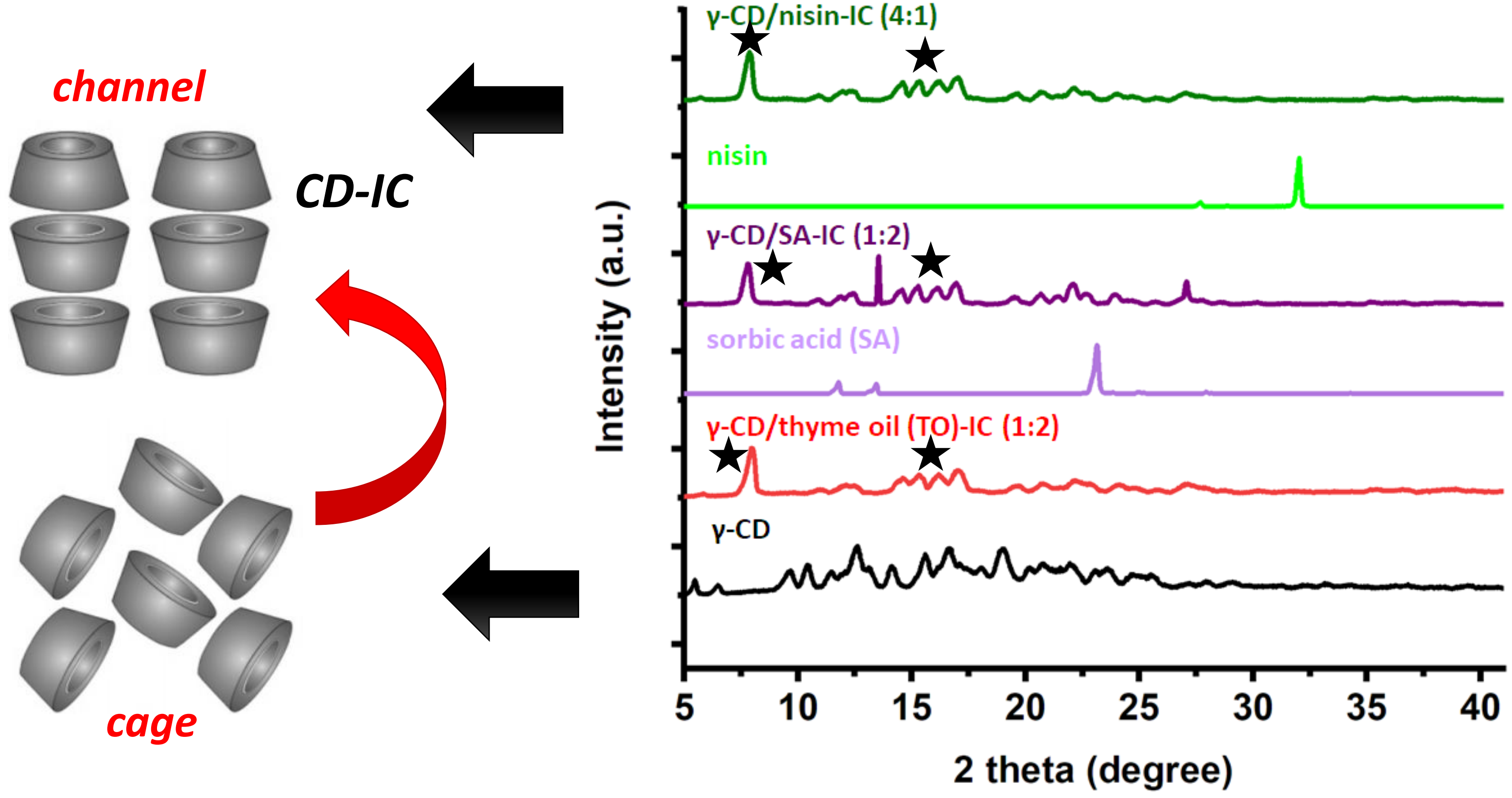
	Free AIs	CD-ICs of AIs
Pristine fibers	-	-
Enzyme responsive fibers	• thyme oil (1%, w/v) • citric acid (5%, w/v) • nisin (0.2%, w/v)	-
RH responsive fibers	-	• thyme oil (1%, w/v) • sorbic acid (0.5%, w/v) • nisin (0.2%, w/v)
Multi stimuli responsive fibers	• thyme oil (1%, w/v) • citric acid (5%, w/v) • nisin (0.2%, w/v)	• thyme oil (1%, w/v) • sorbic acid (0.5%, w/v) • nisin (0.2%, w/v)

Injector: Single-needle Injector
Substrate: Aluminum foil
Fiber mass per surface area: 1.25 and 2.5 mg/cm2

RESULTS

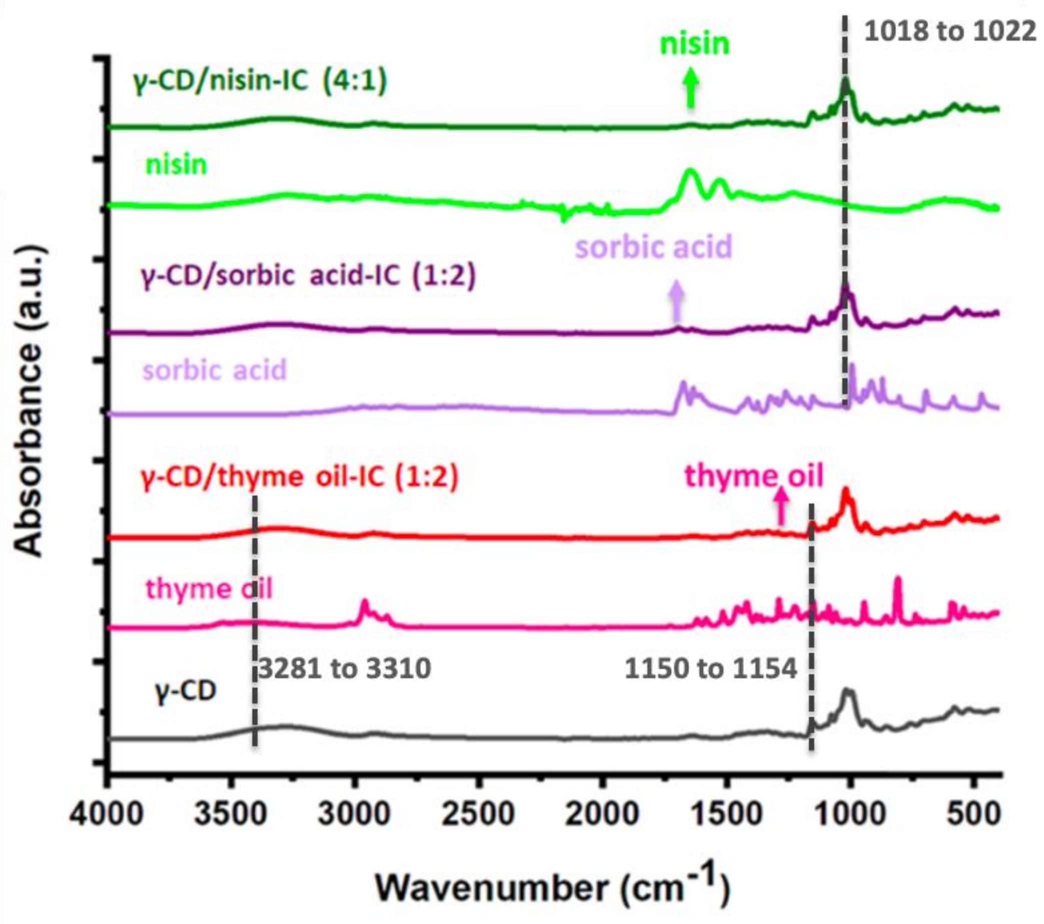
Results: Physicochemical Characterization of CD-ICs-XRD

- The **presence** of channel type **crystalline peaks** of γ-CD in CD-ICs confirm the synthesis of CD-ICs with Sorbic Acid, thyme oil and nisin
- The **absence** of crystalline peaks of AIs further support the IC formation.



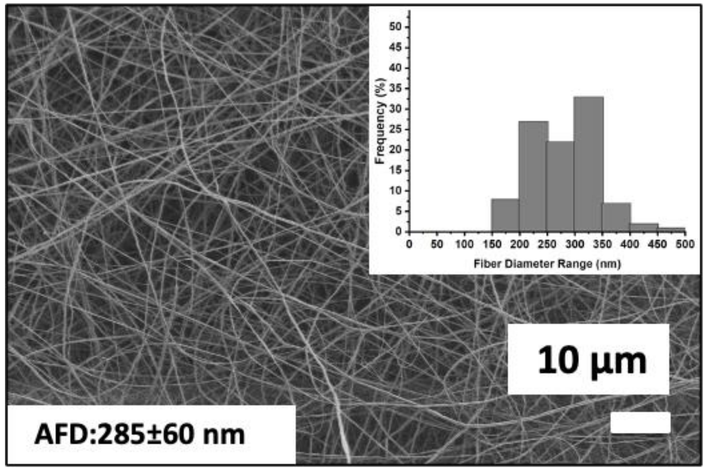
Results: Physicochemical Characterization of CD-ICs-FTIR

- The **presence** of the peaks of AIs in CD-ICs confirm the presence of AIs in CD-ICs.
- The shifts observed in the peaks shows the interaction and IC formation between CDs and AIs.

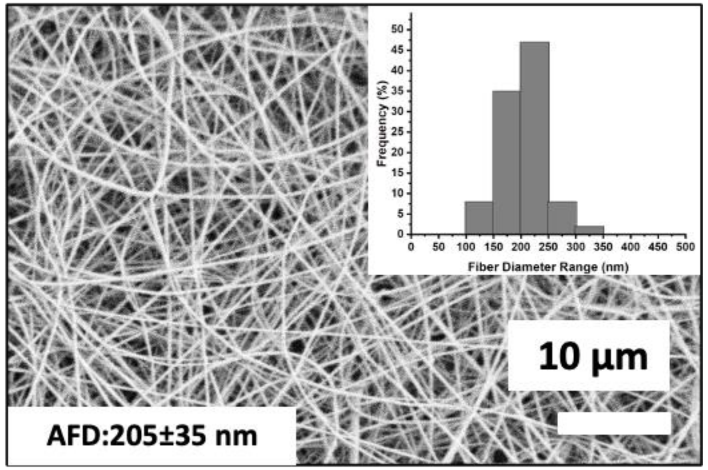


Results: Morphological Characterization of Fibers-SEM

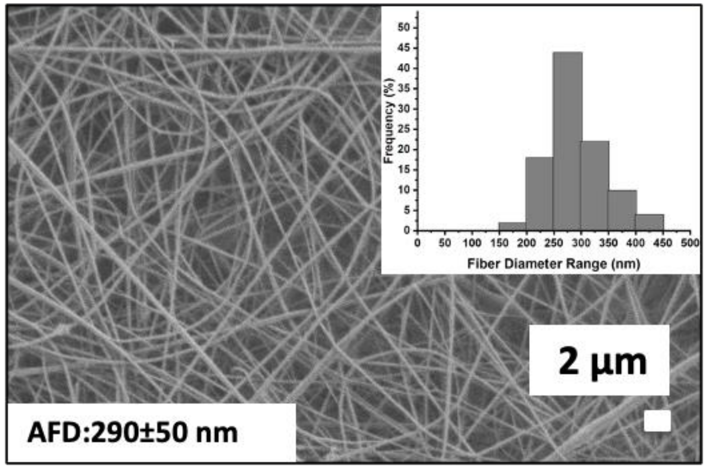
pristine fibers



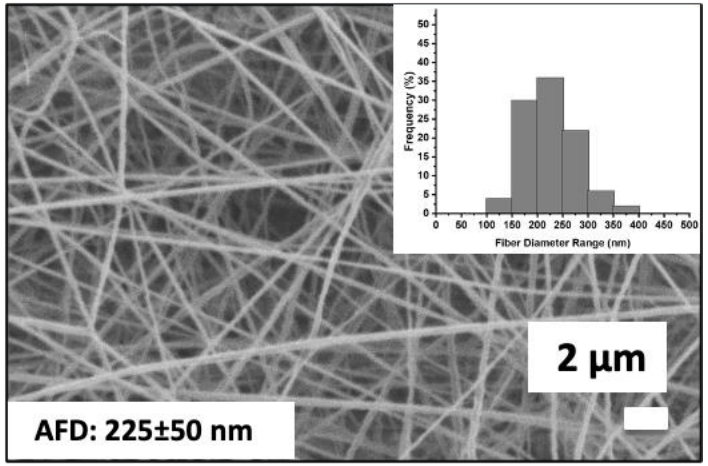
enzyme responsive fibers



RH responsive fibers

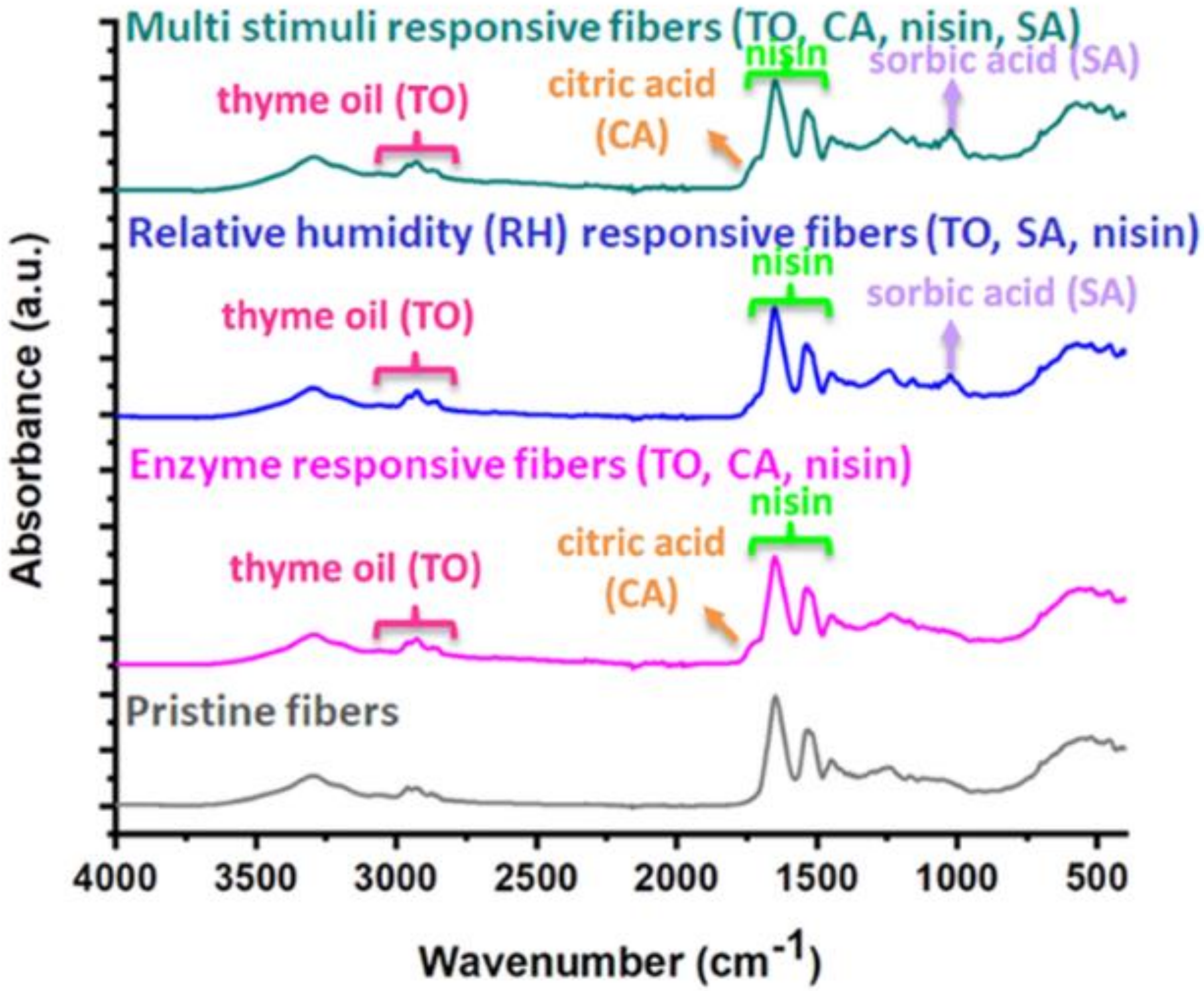


Multi-stimuli responsive fibers



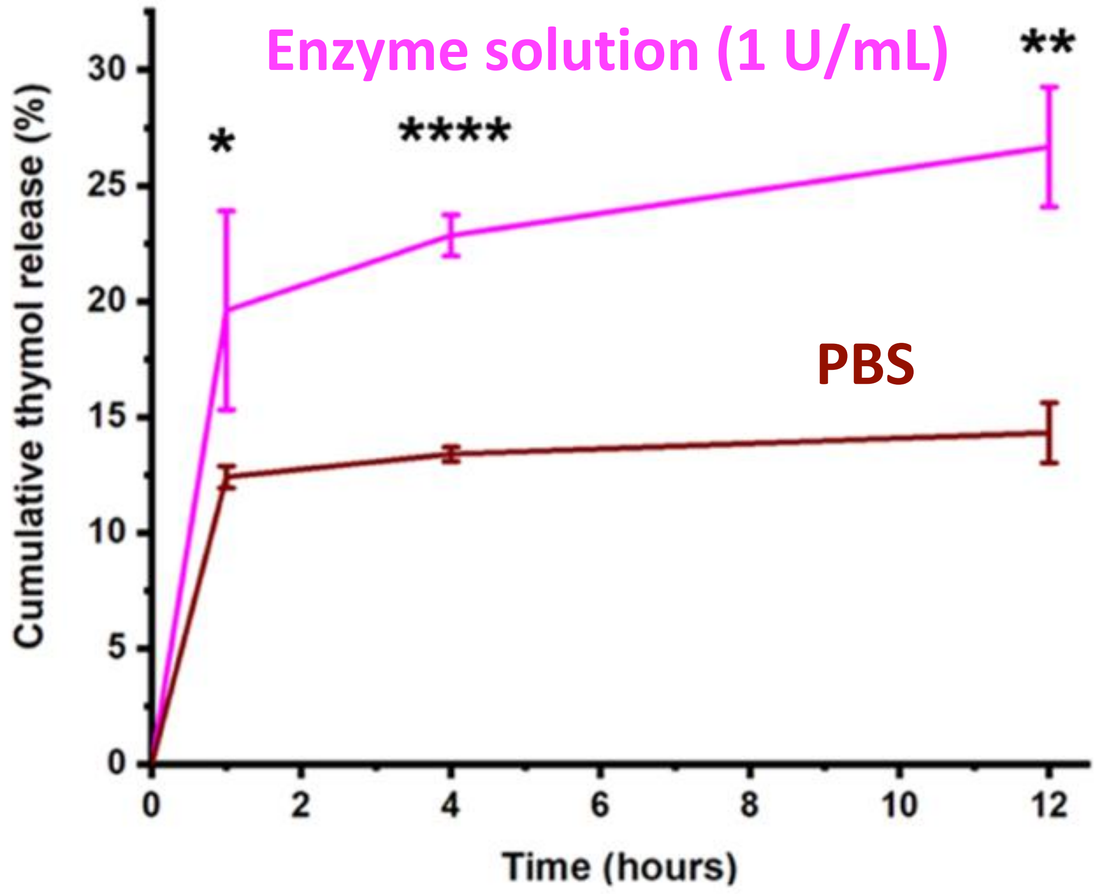
Results: Physicochemical Characterization of Fibers-FTIR

- The spectra confirms the successful incorporation of AIs in the fibers.



Results: Enzyme Triggered Release of Thyme Oil from Fibers

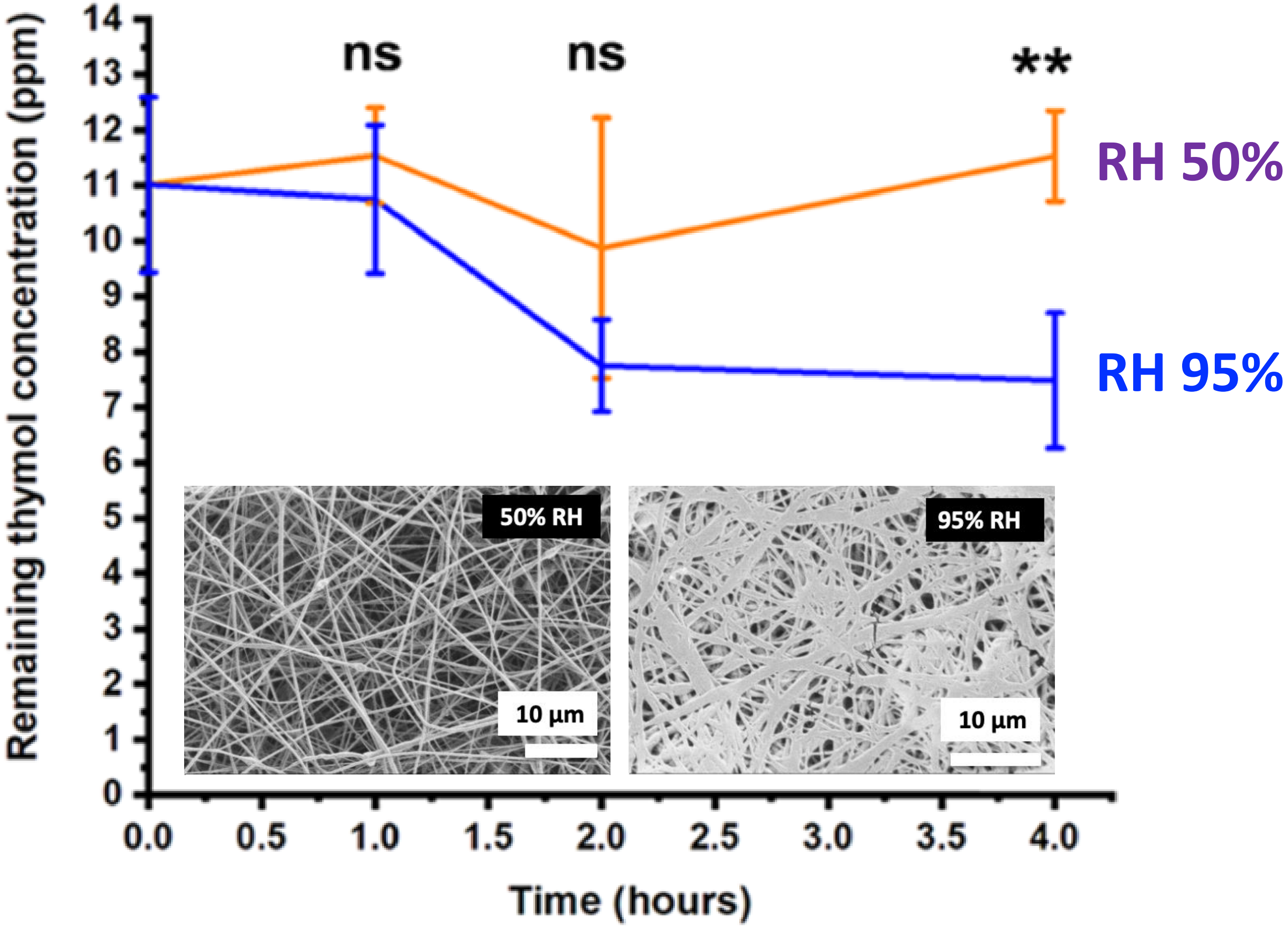
- Higher quantity of thyme oil was released in the presence of enzymes, highlighting the responsiveness of the fibers to enzymes.



The significant difference among data in the same contact time was labeled with nonsignificant (ns): P > 0.05, * P ≤ 0.05, ** P ≤ 0.01, or **** P ≤ 0.0001.

Triggered Release of Thyme Oil from Fibers

- At 95% RH, thymol concentration remained in the fibers were significantly lower than 50% RH.

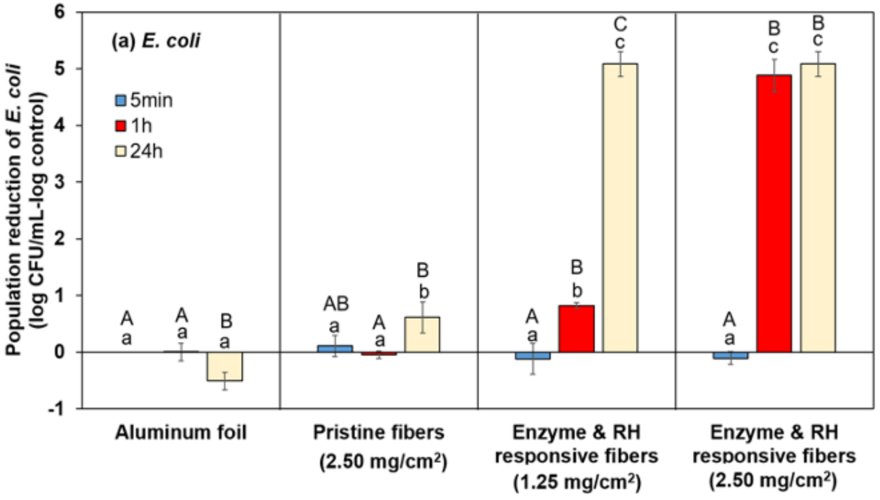


The significant difference among data in the same contact time was labeled with nonsignificant (ns): $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, or **** $P \leq 0.0001$.

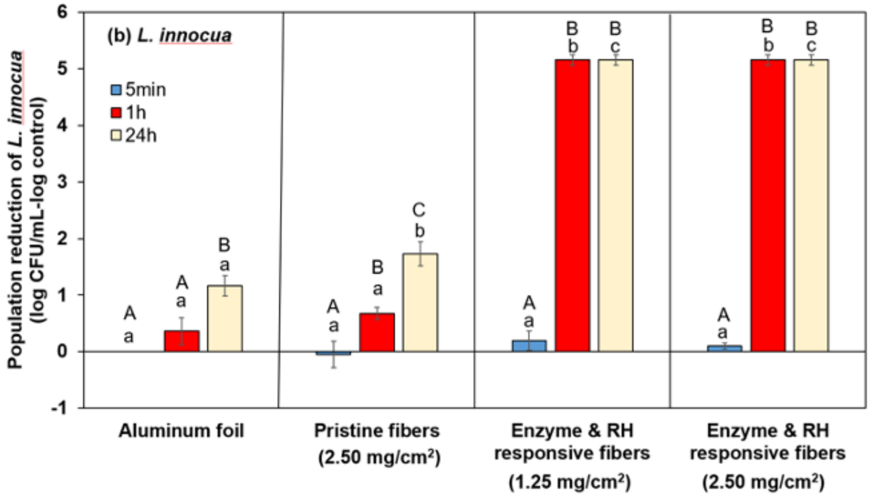
Results: Antimicrobial Activity of Fibers (1/2)

- 5 logs of reduction for both food pathogens in 1 hour exposure.
- Antimicrobial activity at miniscule mass per surface area (1.25 mg/cm2)

E. coli



L. innocua

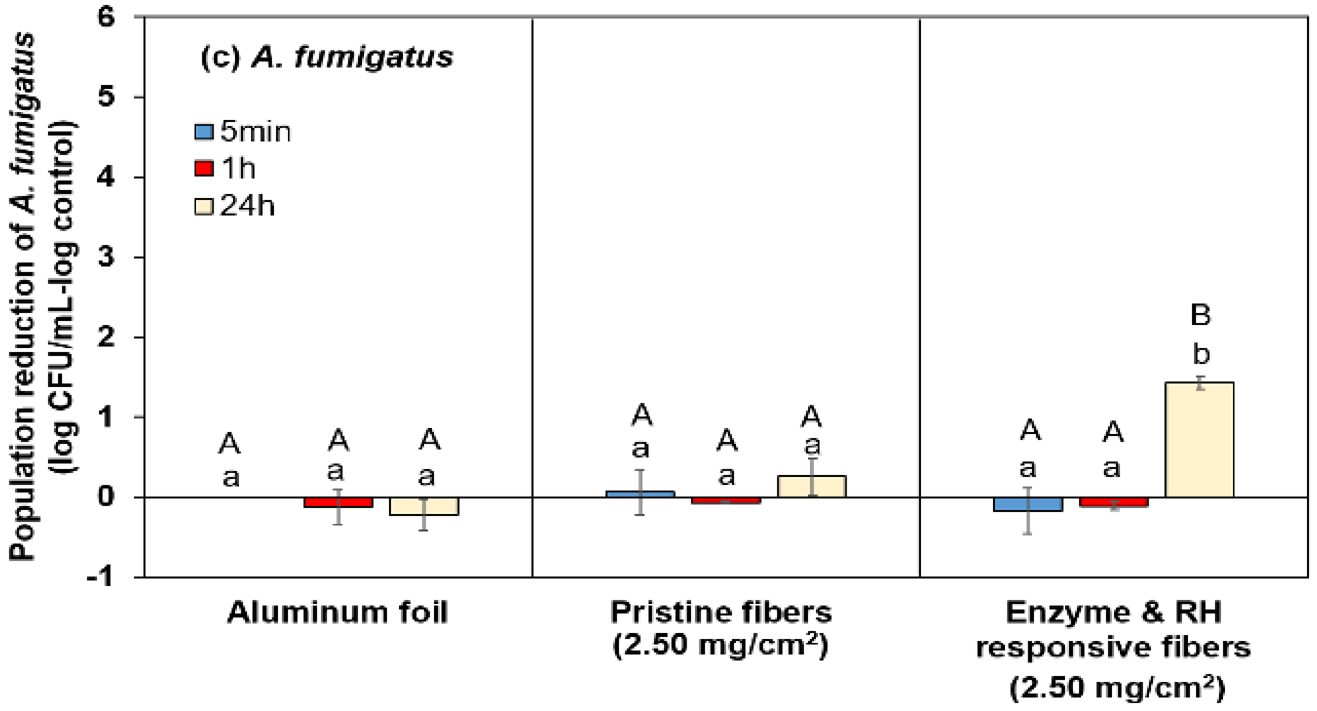


Data in the same material labeled with different uppercase letters is significantly different ($P < 0.05$). Data in the same treatment time group labeled with different lowercase letters is significantly different ($P < 0.05$).

Results: Antimicrobial Activity of Fibers (2/2)

- Significant population reduction was evident against *A. fumigatus* as well.

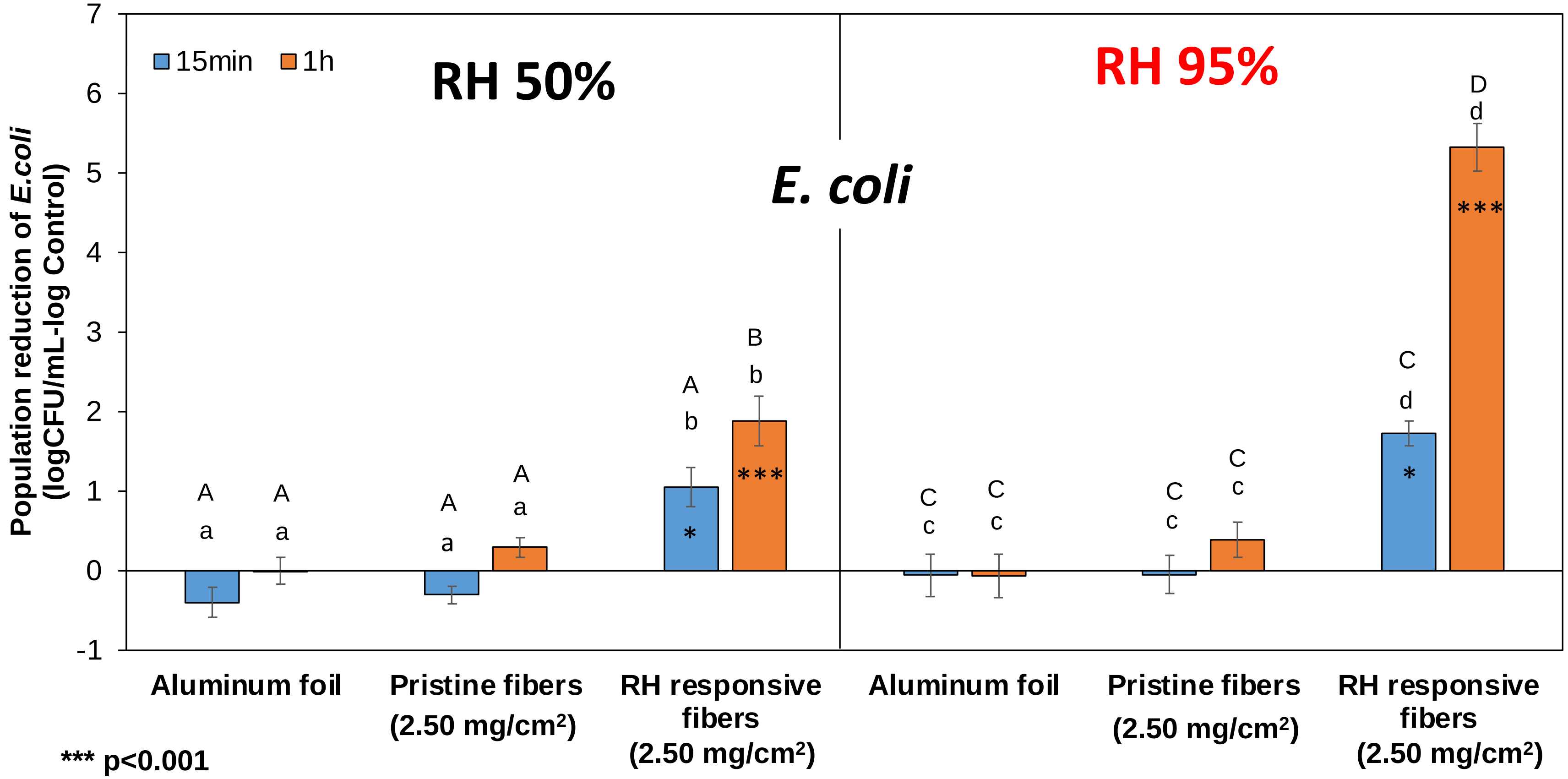
A. fumigatus



Data in the same material labeled with different uppercase letters is significantly different ($P < 0.05$). Data in the same treatment time group labeled with different lowercase letters is significantly different ($P < 0.05$).

Results: RH Triggered Antimicrobial Activity of Fibers

- For 1 h contact time, fibers show 5 logs of reduction after being conditioned at 95% RH, whereas only 2 logs of reduction was seen for fibers conditioned at 50% RH.

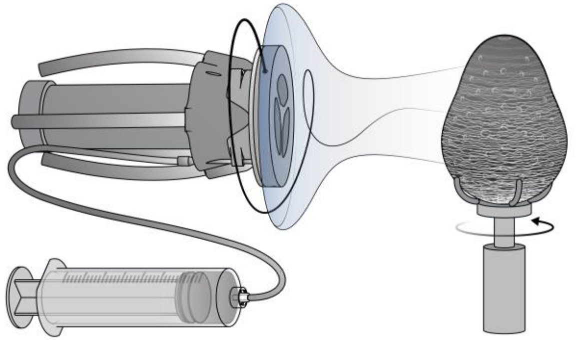


*** p<0.001
* p<0.05

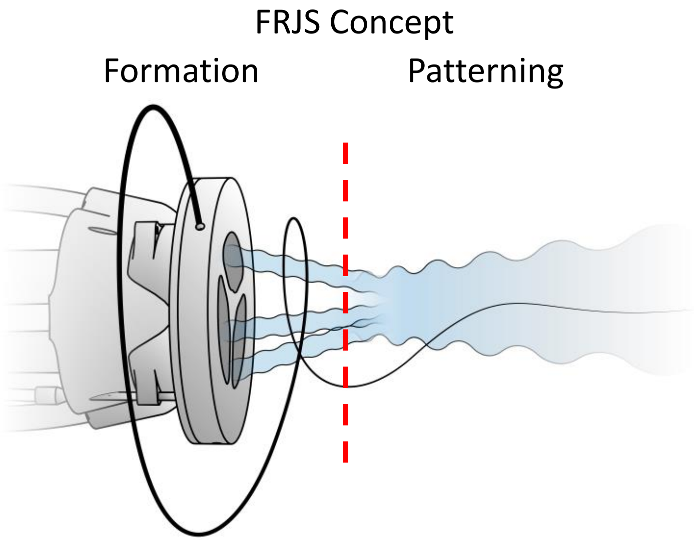
At the same RH level, data in the same material labeled with different uppercase letters is significantly different ($P < 0.05$). Data in the same contact time labeled with different lowercase letters is significantly different ($P < 0.05$).
At different RH levels, significant difference among data in the same contact time of RH-responsive fibers was labeled with *: *** $P \leq 0.001$, * $P \leq 0.05$. The error bars in the figure represent the standard deviation (SD).

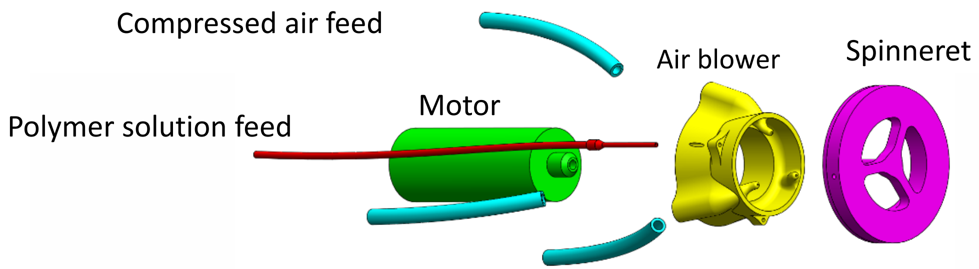
High yield fiber synthesis: Rotary Jet Spinning (RJS)

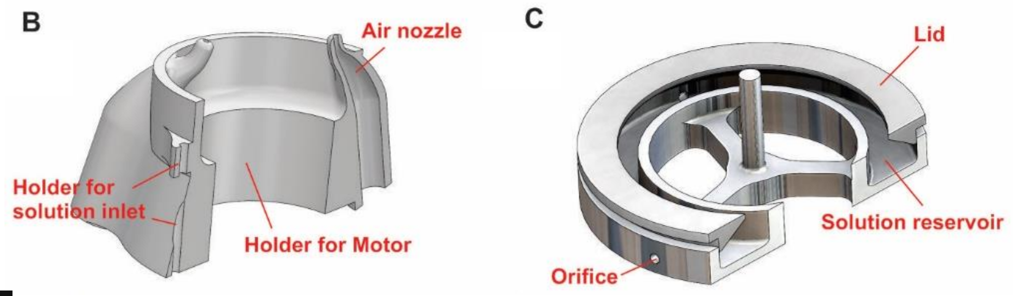
Biodegradable, Antimicrobial Food Packaging Using Focused Rotary Jet Spinning-produced Pullulan Fibers

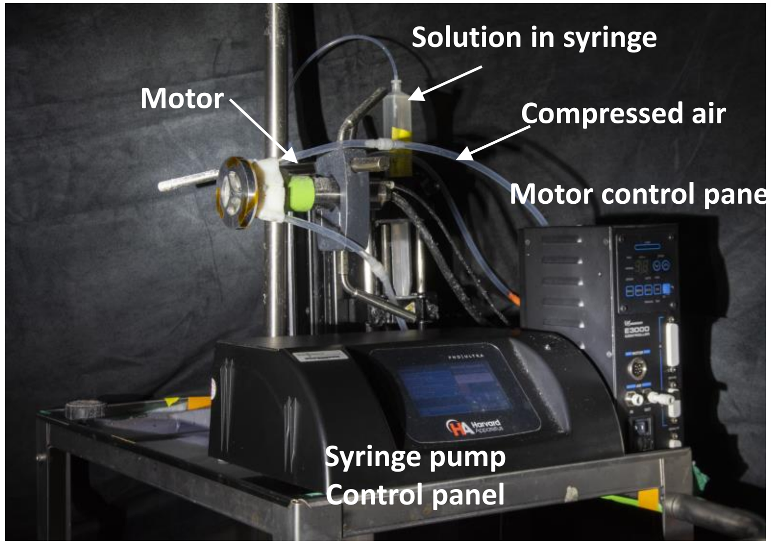


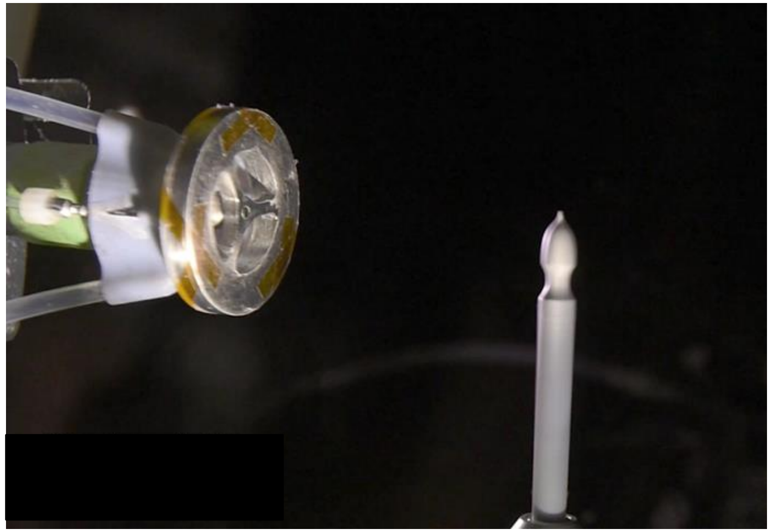
Focused Rotary Jet Spinning : Air Jet + Rotary Jet Spinning





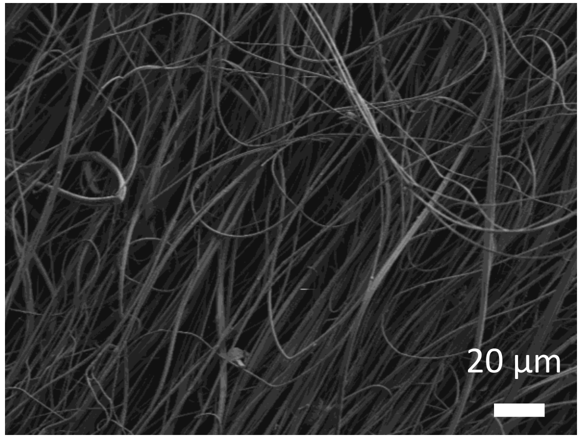


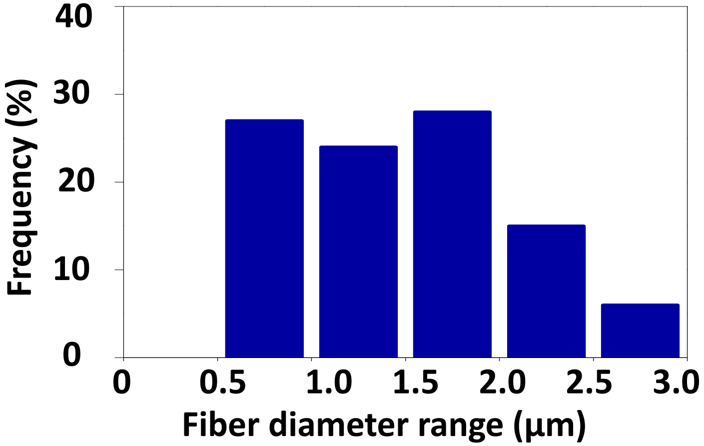




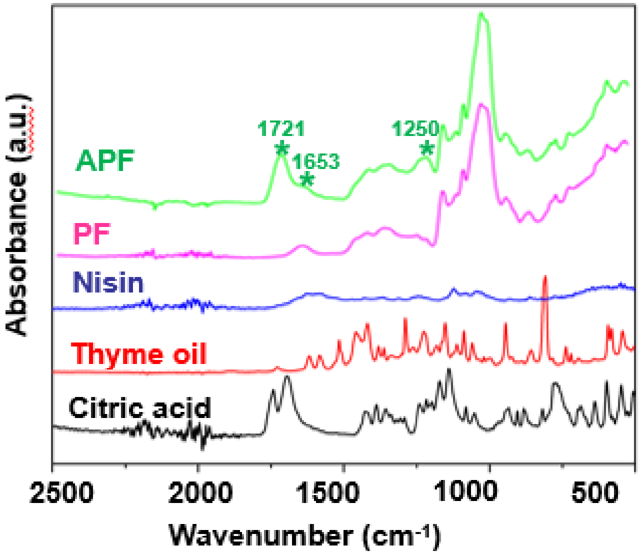
Antimicrobial Pullulan Fibers (APFs) Containing Nature-derived antimicrobial Agents

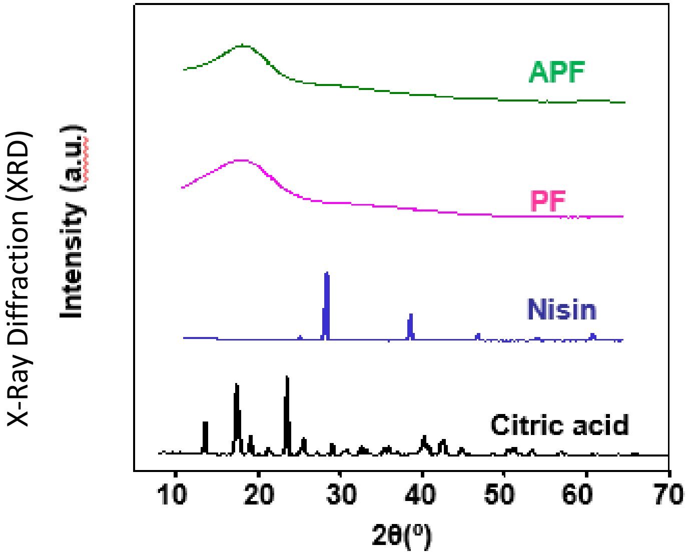
Scanning electron microscope (SEM) of antimicrobial pullulan fibers





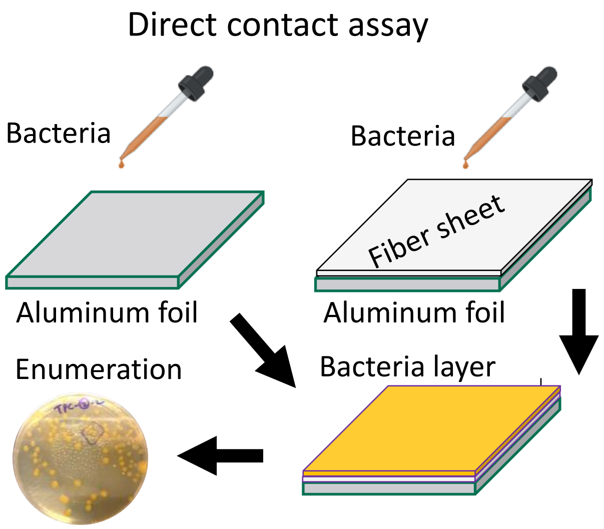
Fourier-transform infrared spectroscopy (FTIR)

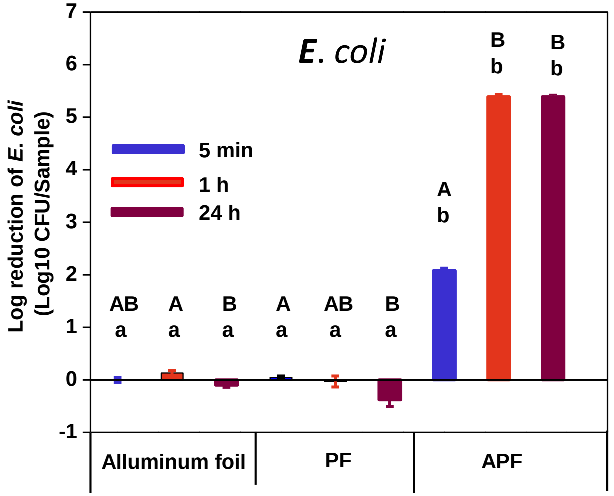




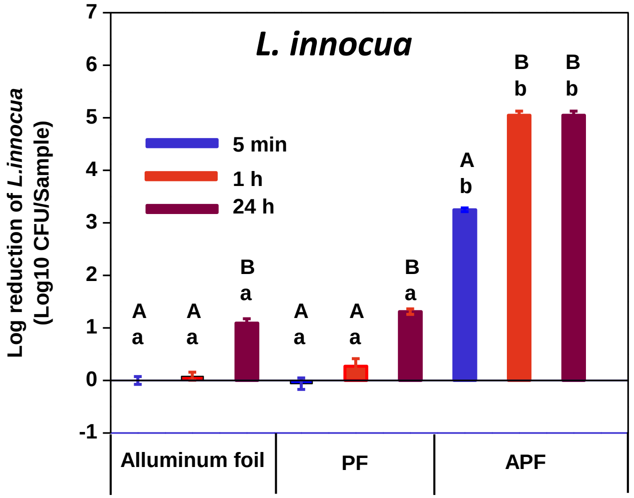
Individual antimicrobial pullulan fiber were successfully spun using FRJS

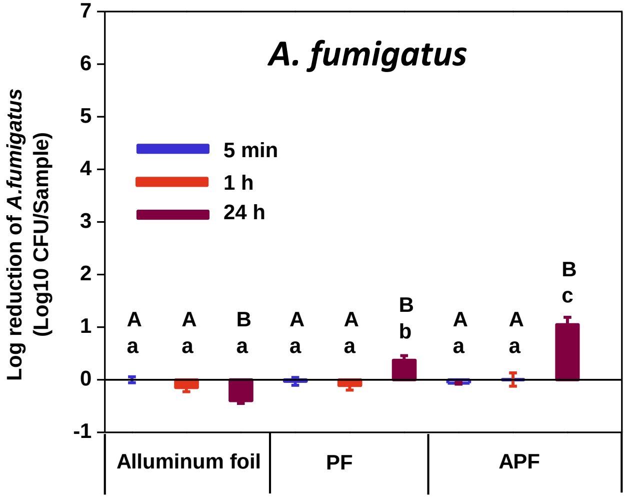
Antimicrobial Activity of APFs





PF: pullulan fiber,
APF; antimicrobial pullulan fiber



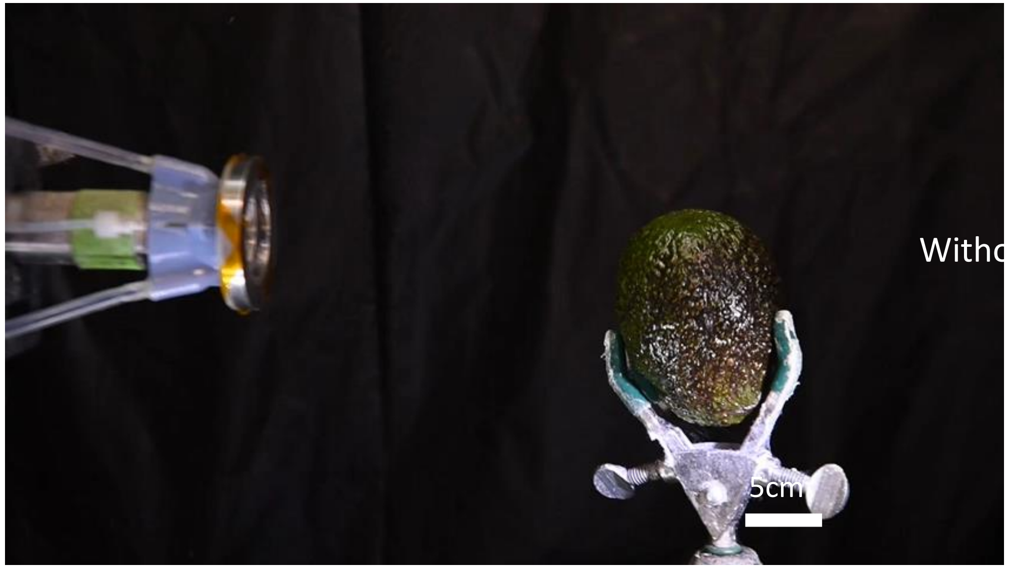


- APFs achieved an ~5 log population reduction of *E. coli and L. innocua* after 1 hour of contact time
- APFs achieved an ~1 log population reduction of *A. fumigatus* after 24 hours of contact time

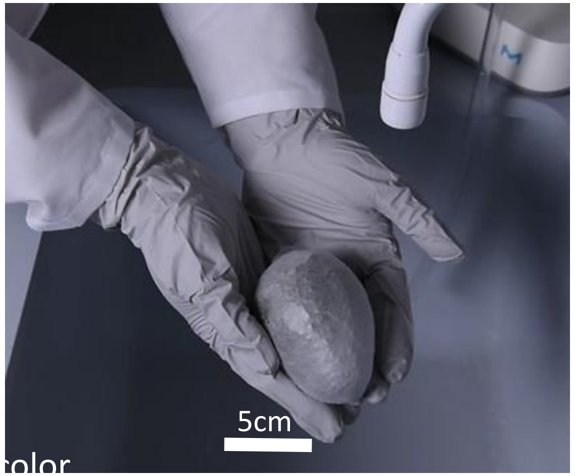
APF shows improved antimicrobial and antifungal activity as compared to pure pullulan fiber or aluminum foil

World’s first “washable” antimicrobial biopolymer based food packaging

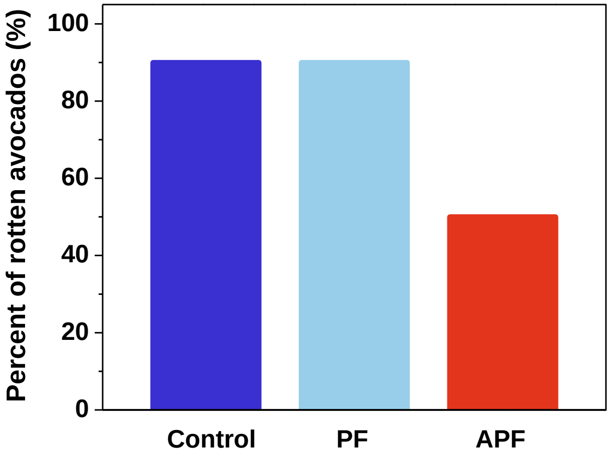
A food dye can also be included into pullulan fiber to enable fiber colors of consumer interest.



APF–based food packaging can be easily removed by hand washing in water



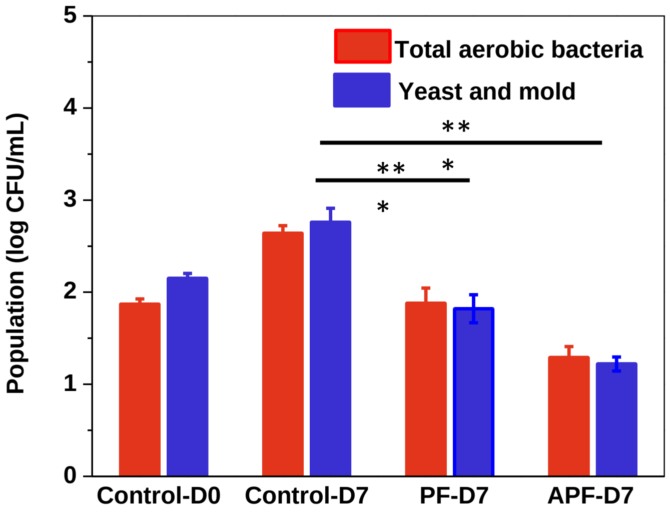
APF Effect on Avocado Rotting rate in one week



Storage condition: **7 days at 22℃**   * Indicates rotted mesocarp areas (n=10)

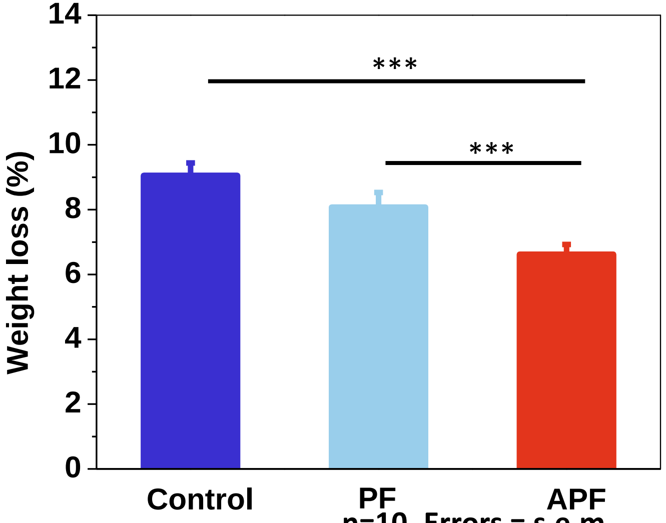
APF coating was able to reduce the rotten avocado from 90% to 50% after 7 days storage

Natural Microflora on the Exocarp of Avocados



n=3, Errors = s.e.m.

- APF coated avocados displayed the least amount of natural microflora after 7 days
- Control avocados showed an increase in microflora
- PF coated avocados displayed similar levels to those initially observed on day 0.



n=10, Errors = s.e.m.

- Both PF and APF coated avocados displayed decreased weight loss after 7 days as compared to control samples
- APFs coated samples show even less weight loss as compared to PF coated samples

APF inhibits the microflora population and weight loss, thus improving the shelf life of avocados

nature food
ARTICLES
https://doi.org/10.1038/s43016-022-00523-w
Check for updates

High-throughput coating with biodegradable antimicrobial pullulan fibres extends shelf life and reduces weight loss in an avocado model

Huibin Chang[1,4], Jie Xu[2,4], Luke A. Macqueen[1], Zeynep Aytac[2], Michael M. Peters[1], John F. Zimmerman[1], Tao Xu[2], Philip Demokritou[2,3] ✉ and Kevin Kit Parker[1] ✉

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Research Article

Enzyme- and Relative Humidity-Responsive Antimicrobial Fibers for Active Food Packaging

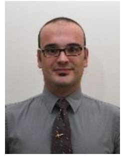
Zeynep Aytac,[▽] Jie Xu,[▽] Suresh Kumar Raman Pillai, Brian D. Eitzer, Tao Xu, Nachiket Vaze, Kee Woei Ng, Jason C. White, Mary B. Chan-Park, Yaguang Luo, and Philip Demokritou*

Outline of Presentation

- ❑ **Nature derived nano-modulating platforms to modulate digestion and absorption of unwanted substances in the gut**:
  - ➢ Engineering interfacial processes in the gut to modulate absorption of nutrients using nature derived and non toxic bio-polymers
- ❑ **Bio-polymer based fibers for agri-food systems:**
  - ➢ Nanofibers for targeted and precise delivery of agrichemicals
  - ➢ Antimicrobial fibers for food package materials
- ❑ **Engineered Water Nanostructures (EWNS):**
  - ➢ A nature inspired, green, antimicrobial platform for food safety and beyond

Engineered Water Nanostructures: A “green”, nanotechnology based antimicrobial platform for food safety and beyond

Dr Nachiket Vaze
Research fellow



Georgios Pyrgiotakis,
Research Scientist

Dr Runze Huang
Research fellow

Engineered Water Nanostructures (EWNS): Turning Water into a "Bug Killer"


The Economist
World politics | Business & finance | Economics | Science & technology | Culture

Technology Quarterly: Q2 2014

Monitor

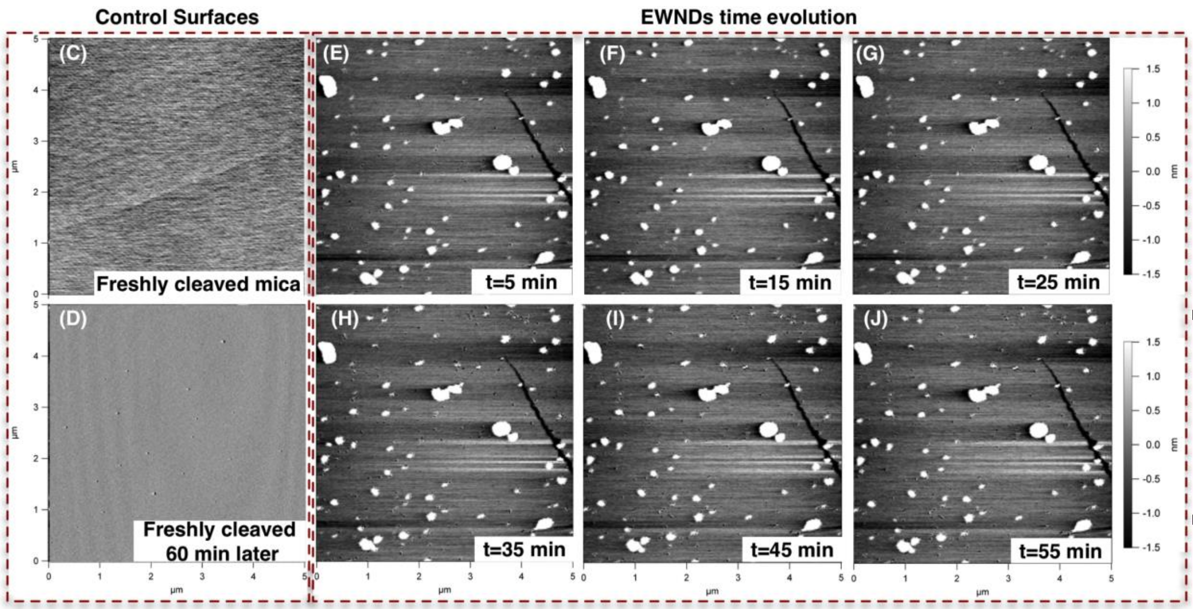
A new bug killer

Water particles could provide a powerful airborne shield against nasty bacteria

Jun 7th 2014 | From the print edition

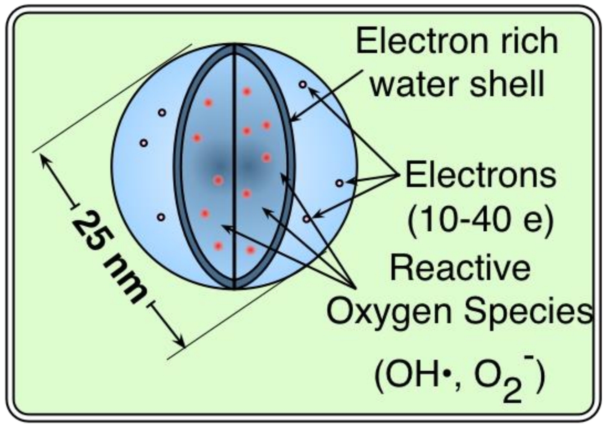
THE use and overuse of antibiotics have led to bacteria evolving resistance to many

EWNS Synthesis at a glance: Electrospray+Ionization



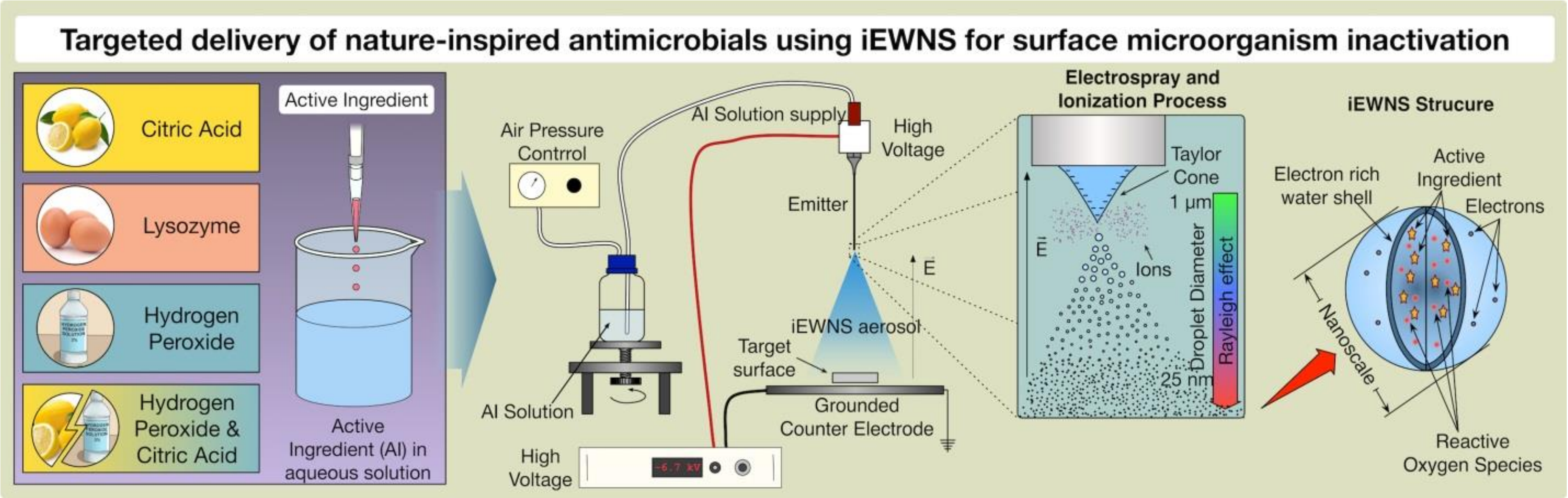
Magnetic Field Strength (G)

C. EWNS Structure



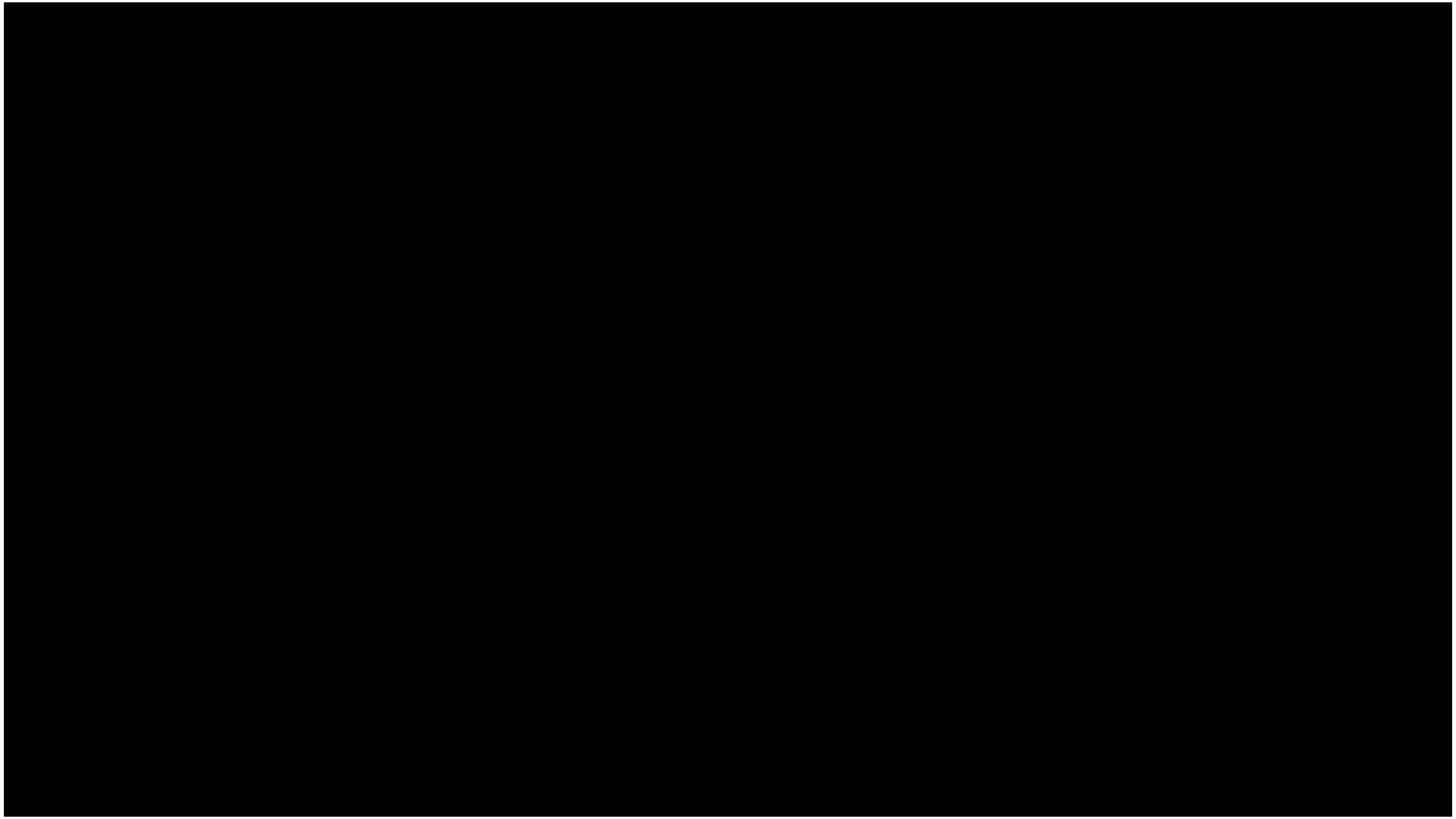
- **Electrospray:** A capillary with a polar liquid (water) is held to a high voltage against a grounded electrode.
- Electric charges are accumulated at the air water interface.
- The strong electric field is causing the water to form an oscillating cone/meniscus from where unstable water droplets are detaching.
- Classic electrospray Taylor theory and phenomena do not apply in this case
- **Ionization:** Water molecules can also split and form ROS under specific electric field conditions.
- **EWNS pcm properties:**
  - **Size:** 10-100 nm, tunable
  - **Electric charge:** 10-40 e, tunable
  - **Electric Charge:** increases surface energy and tension and reduces evaporation
  - **EWNS lifespan**: hours in room conditions
  - **ROS: hydroxyl radical, superoxides and H2O2** are incorporated in the EWNS structure

EWNS: A nanocarrier platform for the targeted delivery of antimicrobials



- EWNS can be used as a nanocarrier platform to deliver AIs in a targeted and precise manner on surfaces and in the air

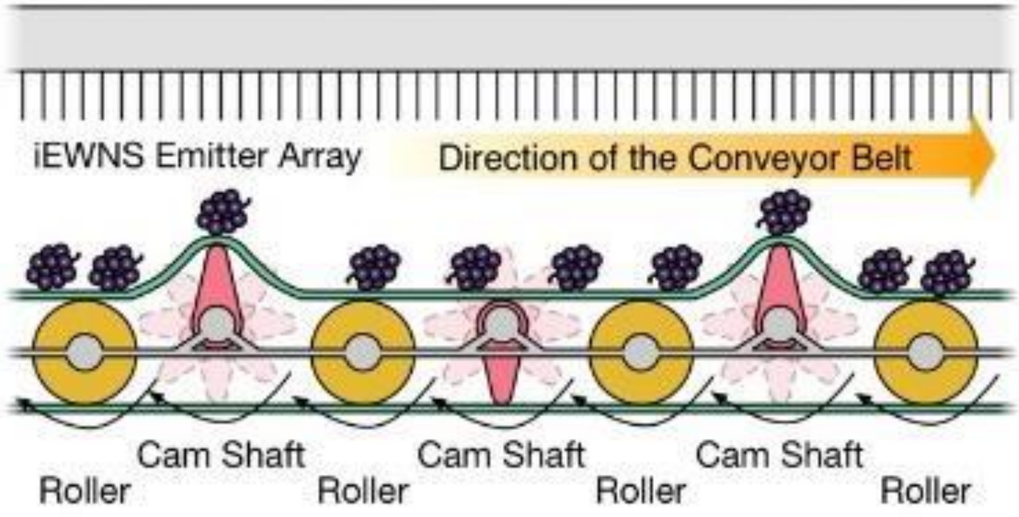
Inactivating Foodborne Pathogens Using EWNS

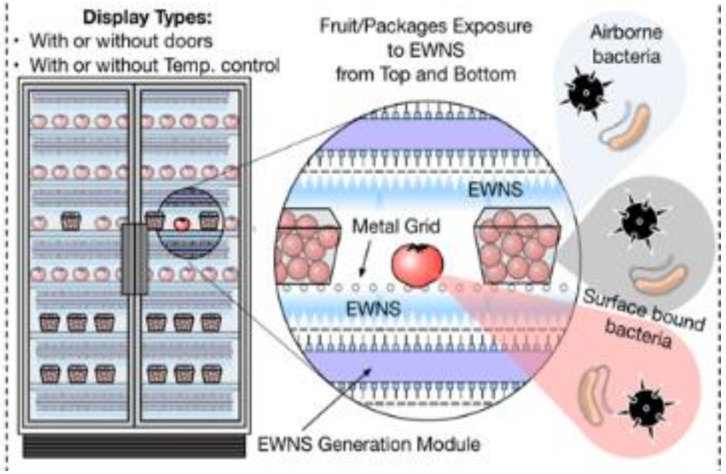


Applications across farm to the fork

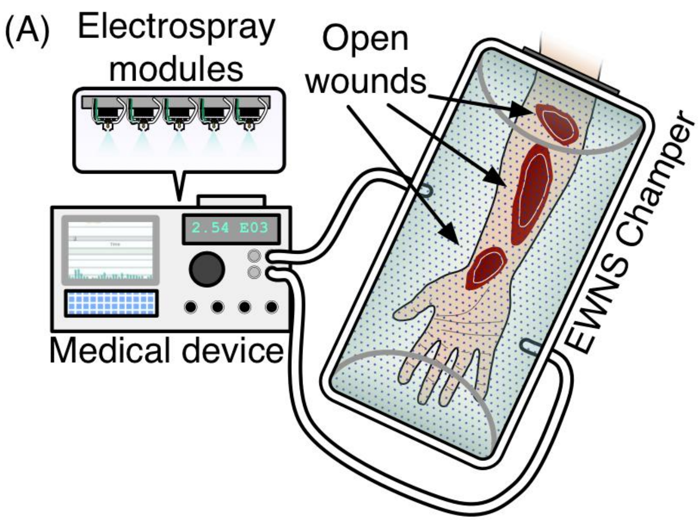
- ❖ iEWNS are effective in inactivating food related patogens including bacteria and viruses such as norovirus, hepatitis, etc

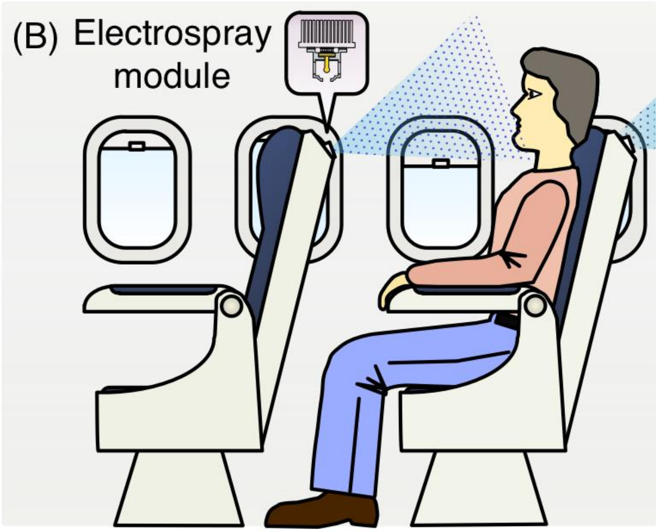
- ❖ Develop high volume, commercial grade apparatus for synthesis and delivery of iEWNS Nano-sanitizers for produce disinfection at various CCPs





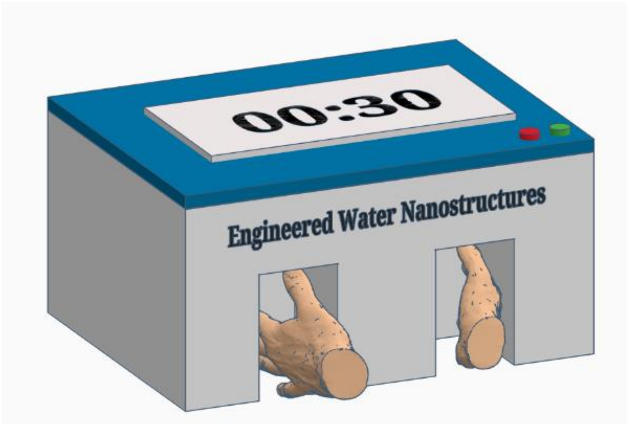
Other Applications:
Would Healing, Air Disinfection, Hand Hygiene





Wound Healing
Environmental Chamber for wound healing management

Air disinfection in Transportation
EWNS to be used to provide a local shield against pathogens.



Hand Hygiene:
A “green”, airless and waterless, hand disinfection technology using non toxic, nature inspired active ingredients

ACS Sustainable Chemistry & Engineering
Research Article
Cite This: *ACS Sustainable Chem. Eng.* XXXX, XXX, XXX–XXX
pubs.acs.org/journal/ascecg

Inactivation of Hand Hygiene-Related Pathogens Using Engineered Water Nanostructures

Runze Huang,[†] Nachiket Vaze,[†] Anand Soorneedi,[‡] Matthew D. Moore,[‡] Yalong Xue,[§] Dhimiter Bello,[†,§] and Philip Demokritou*,[†]

nanomaterials
MDPI

Article

Inactivating SARS-CoV-2 Surrogates on Surfaces Using Engineered Water Nanostructures Incorporated with Nature Derived Antimicrobials

Nachiket Vaze [1,†], Anand R. Soorneedi [2,†], Matthew D. Moore [2] and Philip Demokritou [1,3,*]

The Harvard Gazette
HEALTH & MEDICINE

Disinfecting your hands with 'magic'

Nanostructures can provide an alternative for hand hygiene that is airless and waterless. "... this is like magic. You don't see; you don't feel; you don't smell; but your hands are sanitized," says Associate Professor of Aerosol Physics Philip Demokritou.

Photos by Kris Snibbe/Harvard Staff Photographer

ELSEVIER
Nanomedicine: Nanotechnology, Biology, and Medicine 42 (2022) 102537
nanomedicine Nanotechnology, Biology, and Medicine
nanomedjournal.com

Using engineered water nanostructures (EWNS) for wound disinfection: Case study of *Acinetobacter baumannii* inactivation on skin and the inhibition of biofilm formation

Nachiket Vaze, PhD[b], Philip Demokritou, PhD[a,*]

[a]Henry Rutgers Chair in Nanoscience and Environmental Bioengineering at the Rutgers School of Public Health and Environmental and Occupational Health Sciences Institute, Piscataway, NJ
[b]Center for Nanotechnology and Nanotoxicology, Harvard T.H. Chan School of Public Health, Boston, MA

Revised 1 December 2021

Thank You!! Questions??