Assessing Bioavailability and Bioperformance of Ingested Cellulose Fibrils Using a Novel Physiologically-Relevant *Ex Vivo* and *In Vitro* Integrated Methodology

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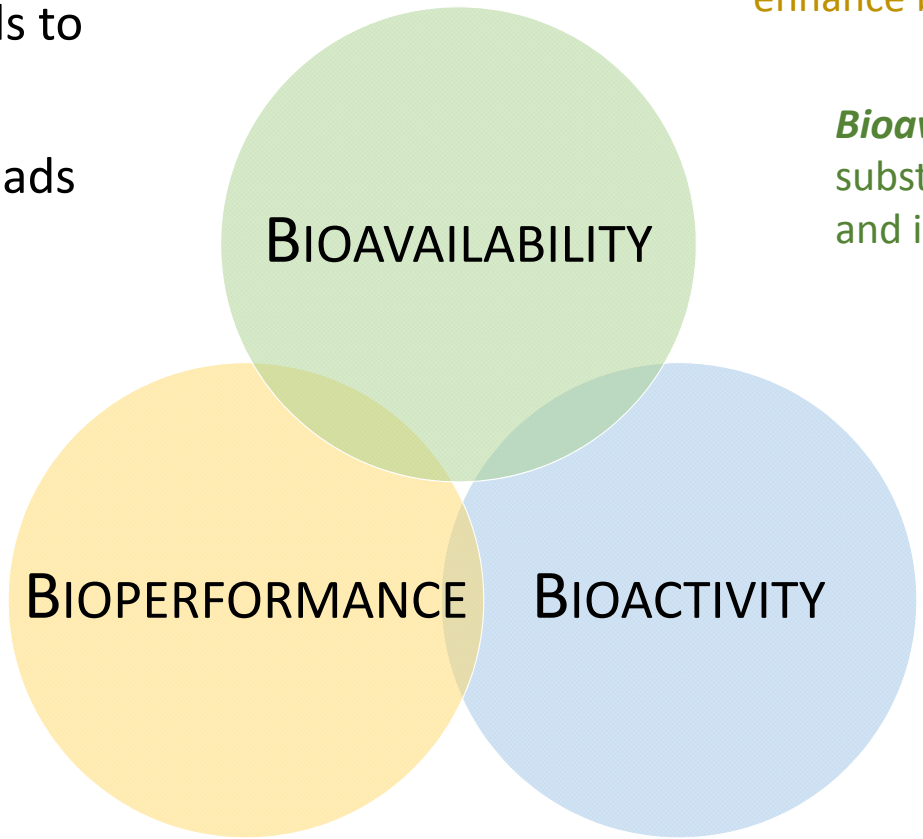
Bio-Bio-Bio

- Bioavailability leads to bioperformance
- Bioperformance leads to bioactivity
- Bioactivity is just another way to describe toxicity



Bioperformance is the assessment or measurement of the intended outcomes after a drug has been administered to enhance biological activity

Bioavailability is the proportion of a substance that enters an organism and is able to have an active effect.

Bioactivity is any effect on, interaction with, or response from living tissue.

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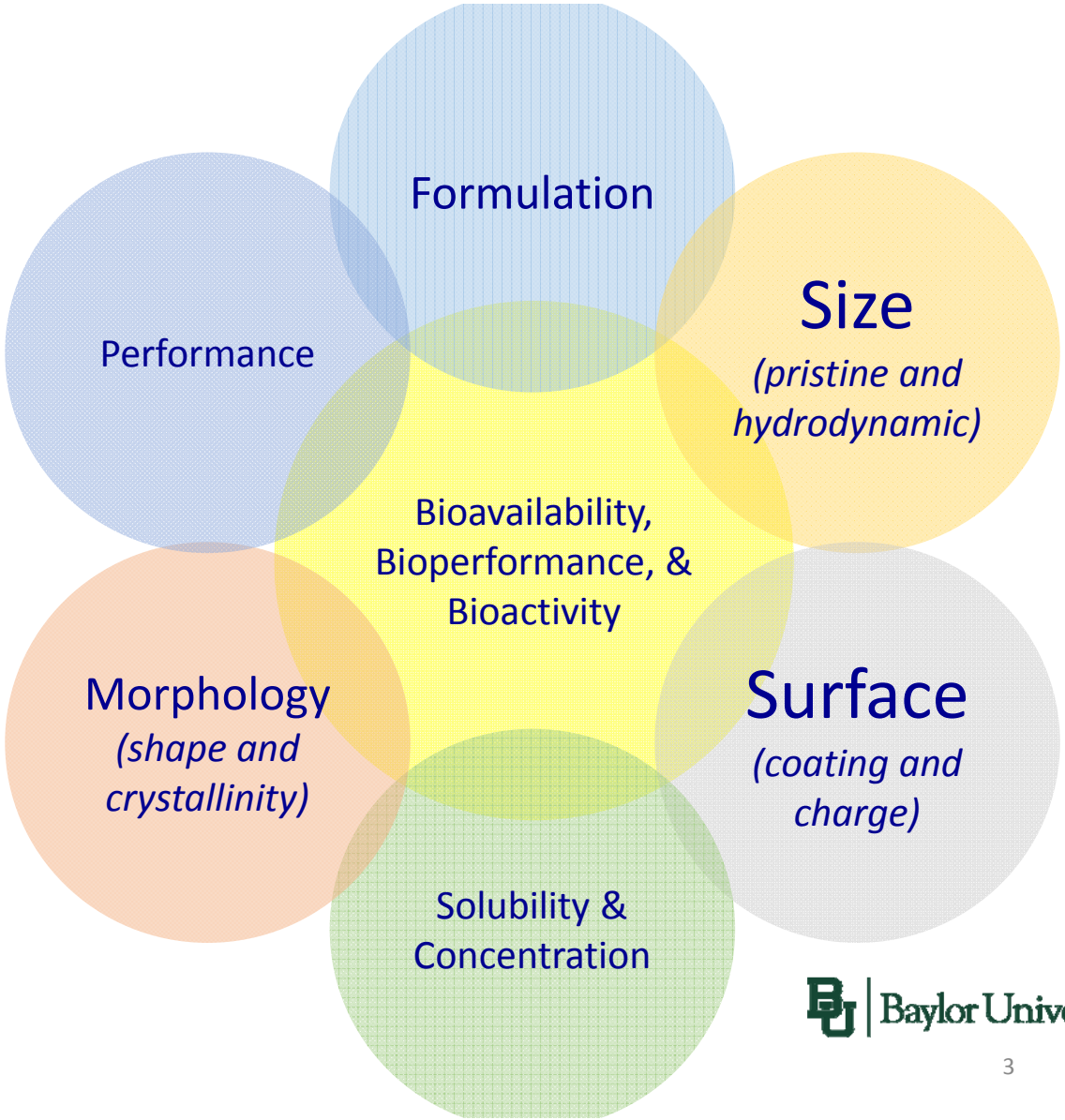
Advanced Material Physicochemical Properties

- There are plenty of material properties to measure
- The physicochemical properties of a material that potentially contribute to induced bioactivity are well understood



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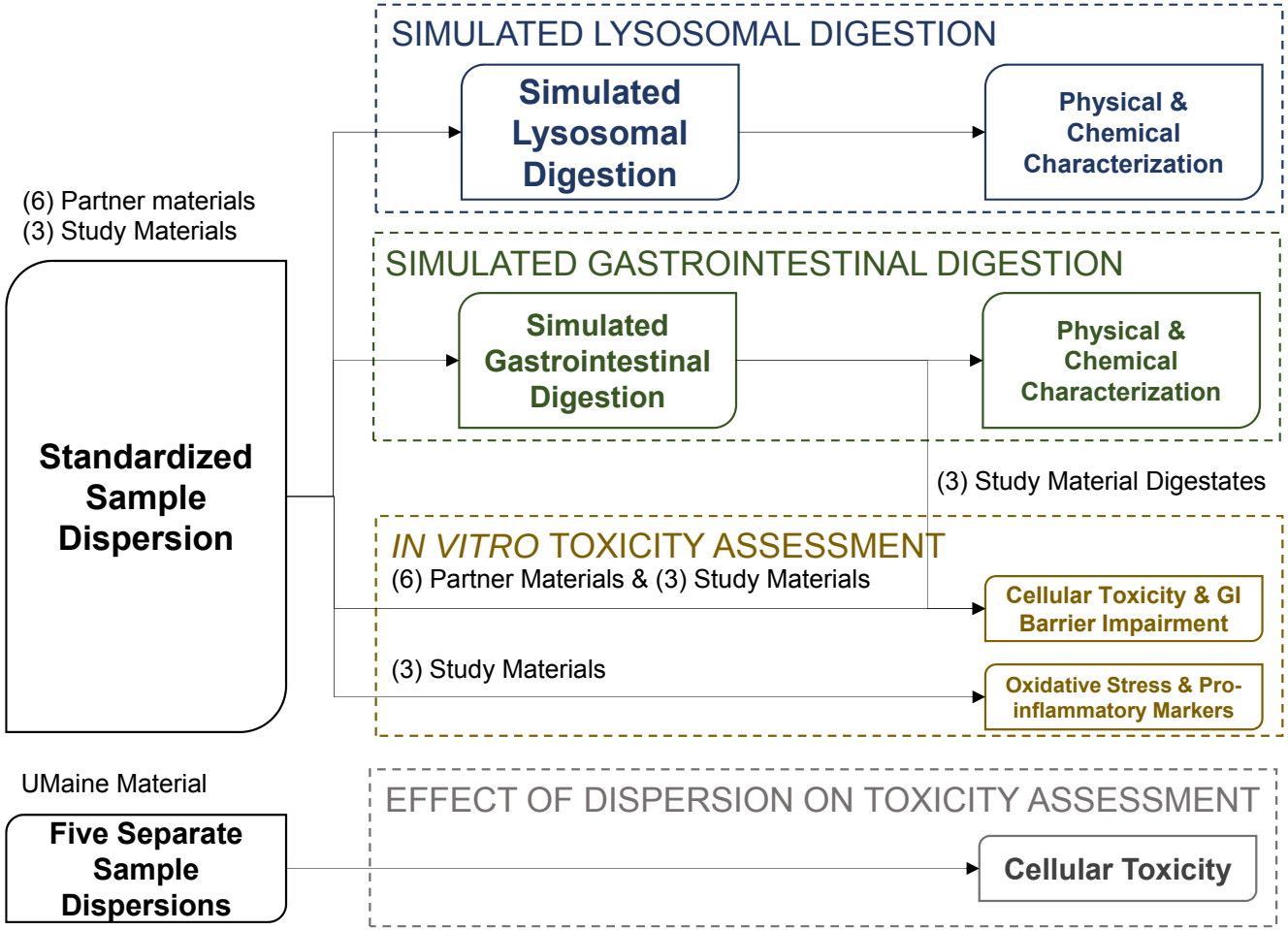
- Indicators of bio-bio-bio can be measured in *ex vivo* and *in vitro* test systems
- Results from material characterization, *ex vivo* studies, and *in vitro* toxicology can inform *in vivo* hazard assessments



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Step 1. Material Characterization

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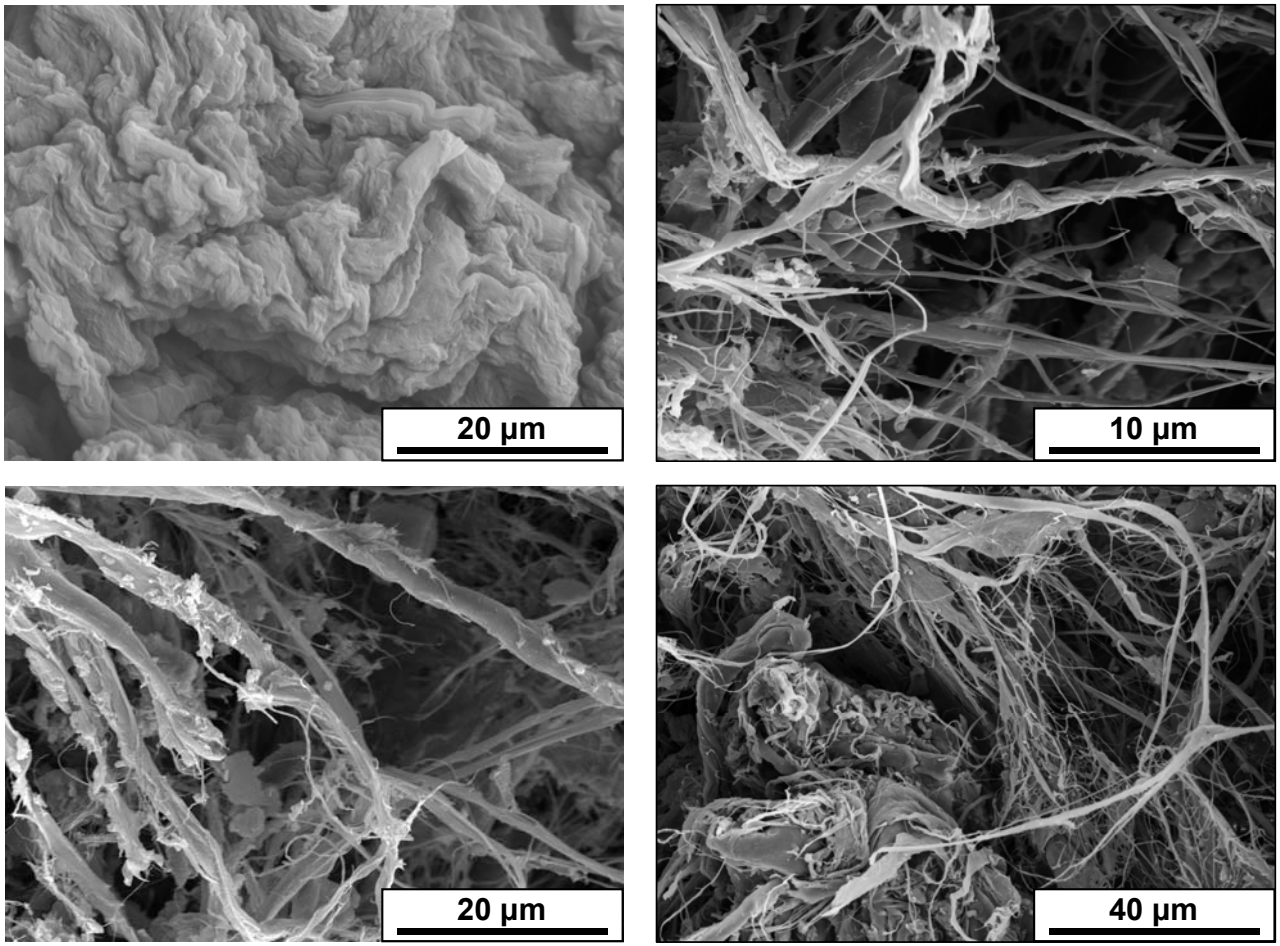
Step 1. Material Characterization – SEM of Fibrils

No preparation vs. embedded in epoxy



Fibrils are more easily characterized with SEM with sample preparation

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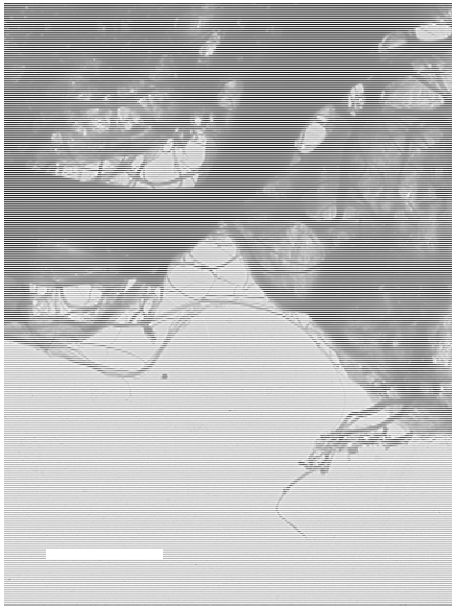
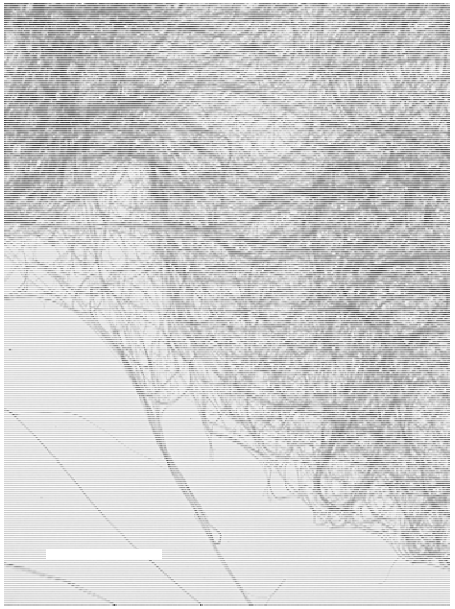
Step 1. Material Characterization – TEM of Fibrils

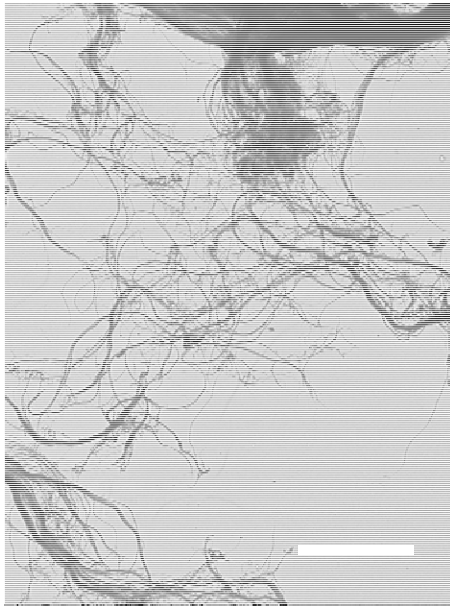
Transmission Electron Microscopy of cellulose fibrils after varying dispersion methods



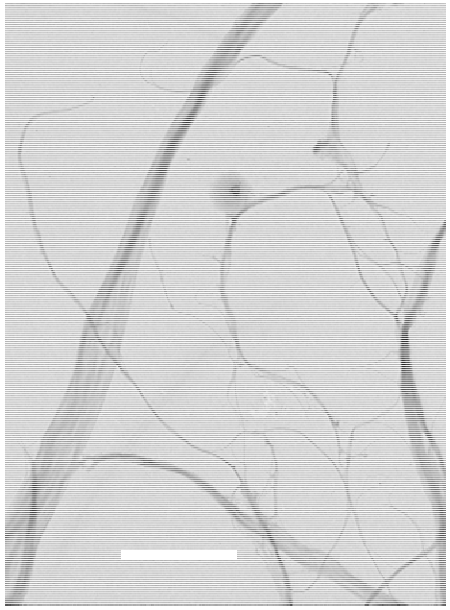
As-received
(no agitation)



Just Stirring
(stir, 12 hrs)



Disruptor Genie
(vortex, 10 min)



Ultrasonic Bath
(vibration, 10 min)

Fibrils are sensitive to sample dispersion methods

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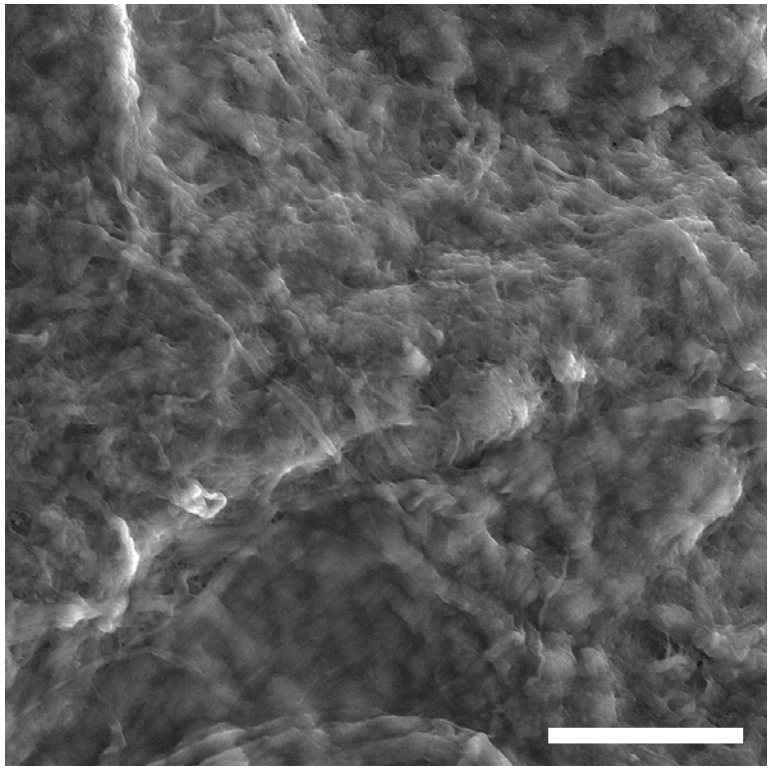
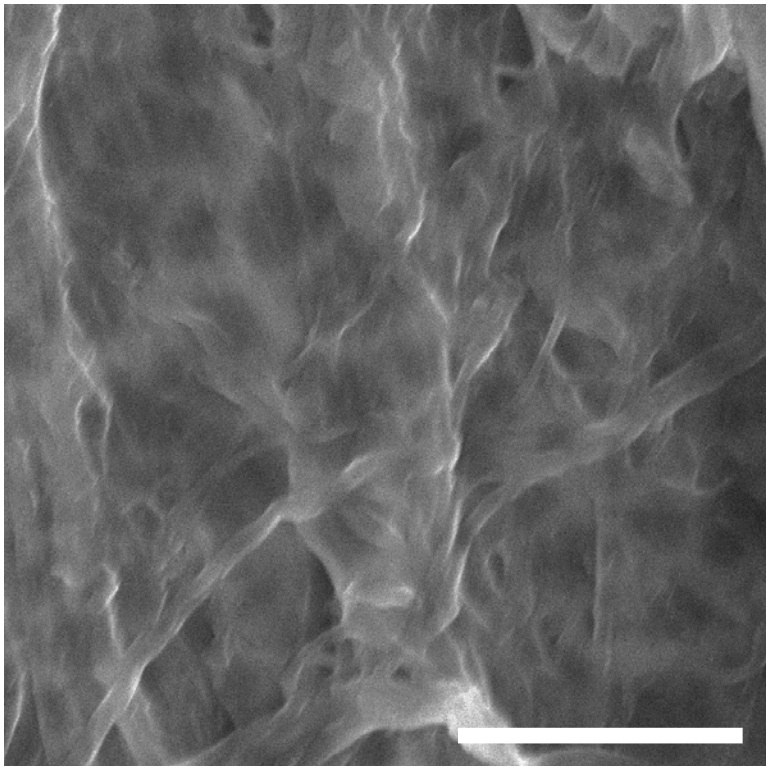
Step 1. Material Characterization – SEM of Crystals

Representative scanning electron micrographs of cellulose nanocrystals forming sheets when prepared for SEM imaging.

Scale bar: 10 µm, 1 µm





Crystals are difficult to characterize via SEM

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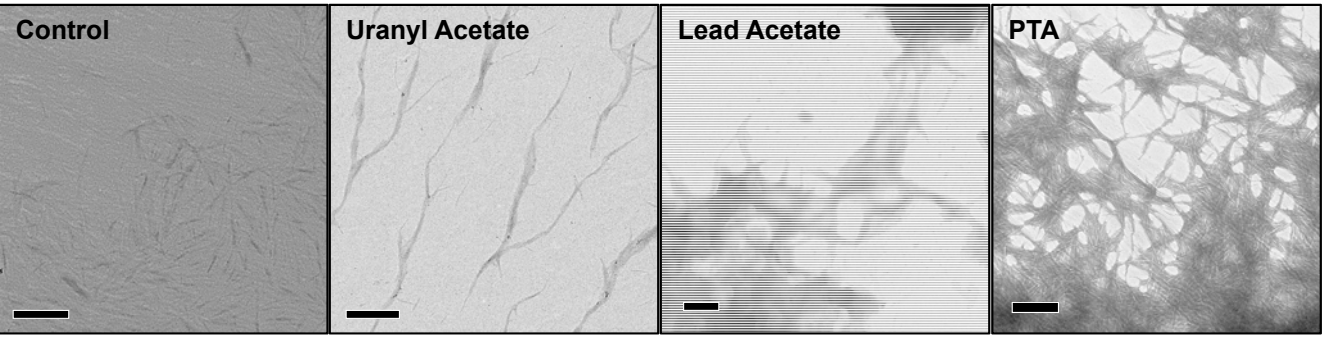
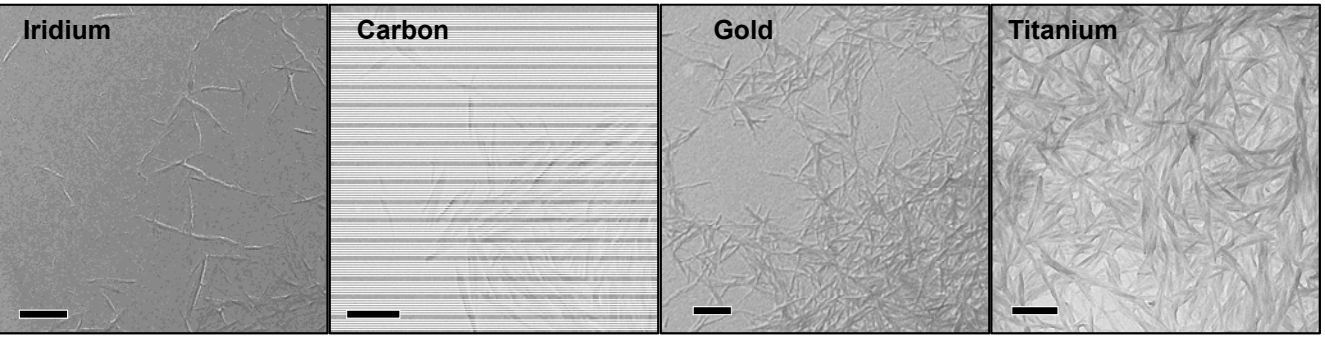
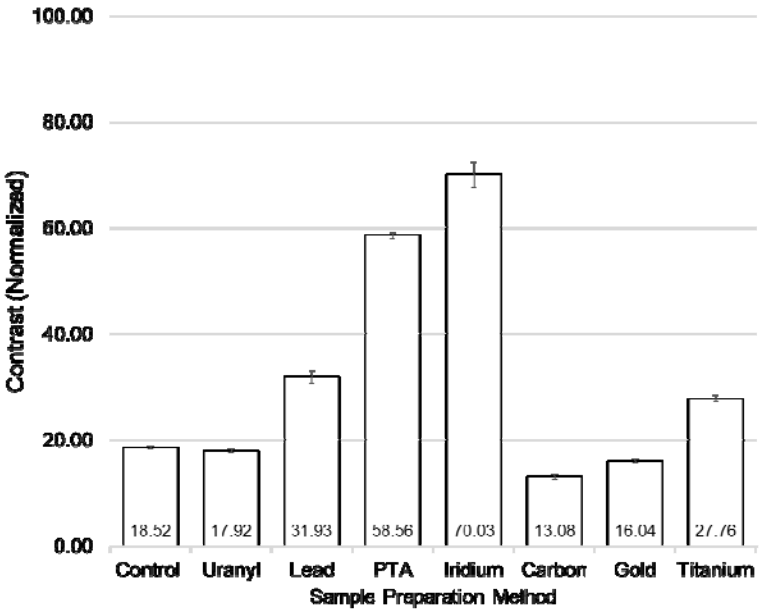
Step 1. Material Characterization – TEM of Crystals

Stains



Sputter





Crystals are more easily characterized via TEM with sample preparation

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Step 2. *Ex Vivo* Studies

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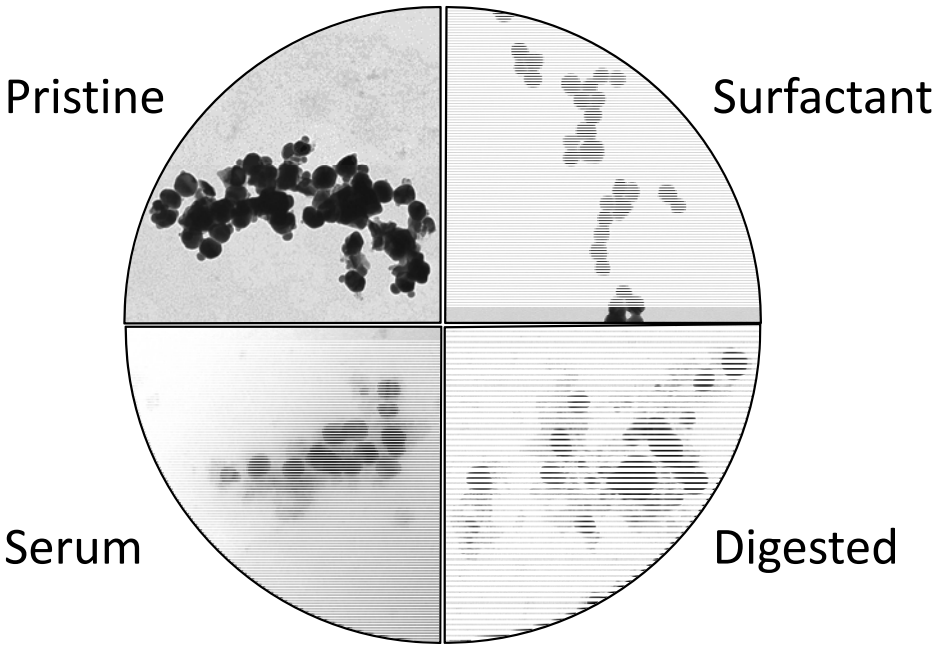
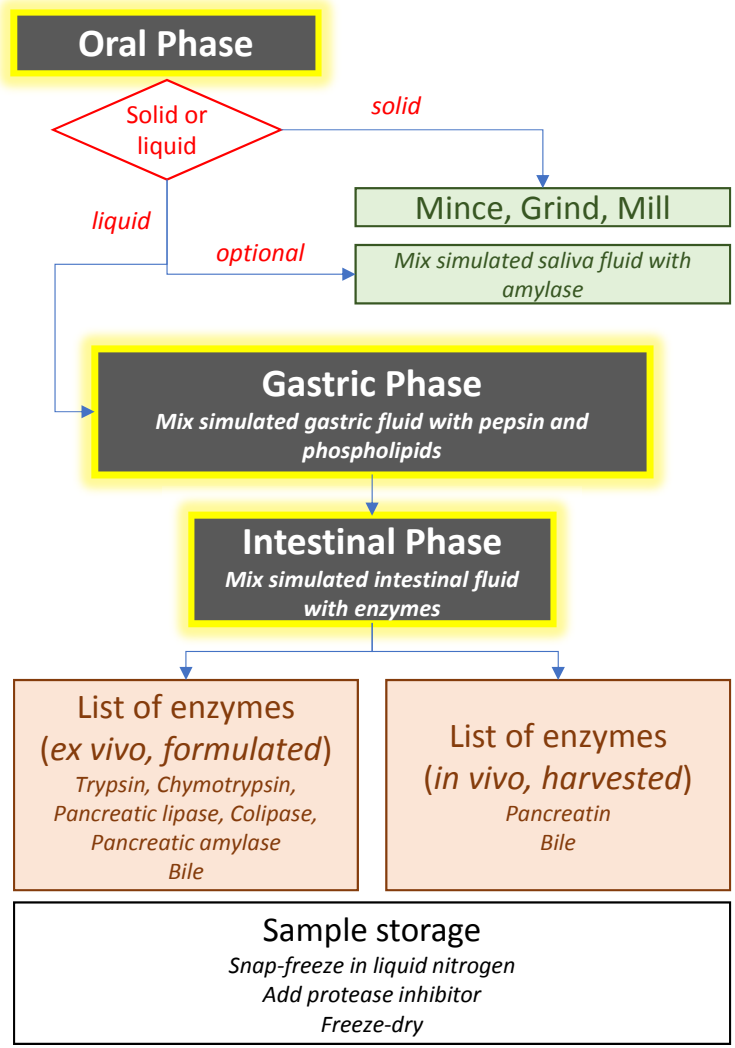
Step 2. *Ex Vivo* Studies

Simulated digestion protocol
Assessment of potential physical and chemical changes in conditions representative of the human gastrointestinal tract is considered a key first step in the stepwise approach

Example: Citrate-stabilized silver nanoparticles





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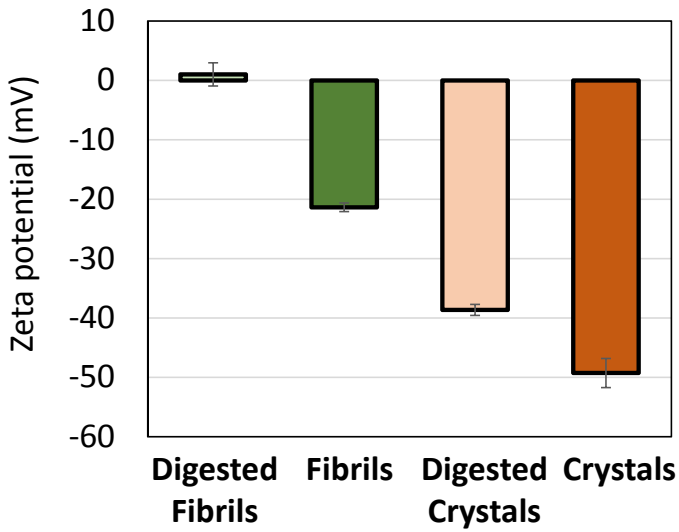
Step 2. *Ex Vivo* Studies. Properties before vs. after digestion

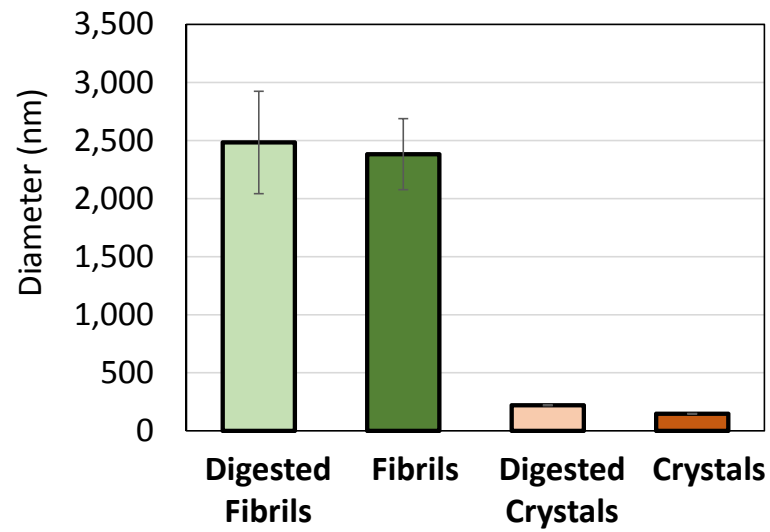
Polydispersity Index

Sample ID	PdI
Digested Fibrils	0.72 ± 0.17
Fibrils	**0.80 ± 0.19**
Digested Crystals	0.49 ± 0.02
Crystals	**0.53 ± 0.08**

Surface Charge



Hydrodynamic Diameter



Observations

- Crystals have lower PdI indicating more monodispersity
- Fibrils have higher PdI indicating more polydisperity
- Digested materials have lower PdI than the pre-digested materials

- Crystals' zeta potential is strongly negative indicating the surface charge is negative and suspension is stable
- The zeta potential of the digested fibrils is neutrally charged

- Crystals have lower overall hydrodynamic diameter
- The HDD does not change significant either material from before vs. after digestion

The digestion process changes some of the fibril and crystal properties

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Step 3. *In Vitro* Toxicology

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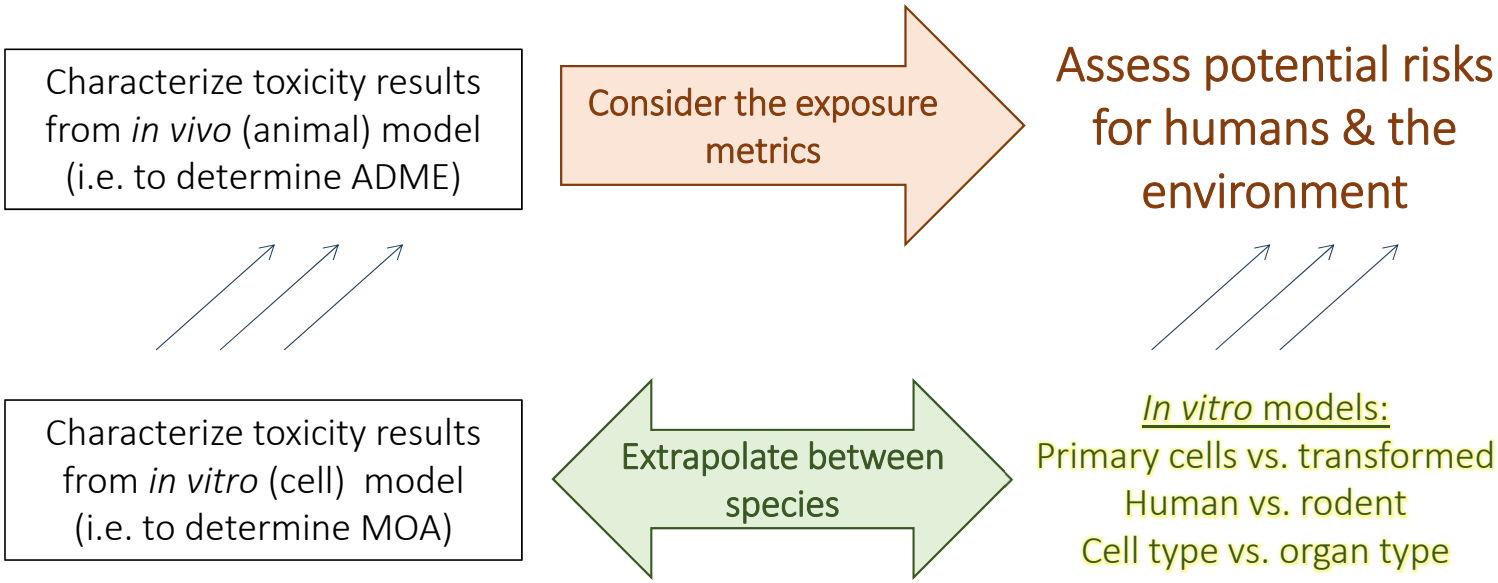
Step 3. *In Vitro* Toxicology

Components of Toxicological Characterization



***In vitro* methods are useful in hazard assessments**

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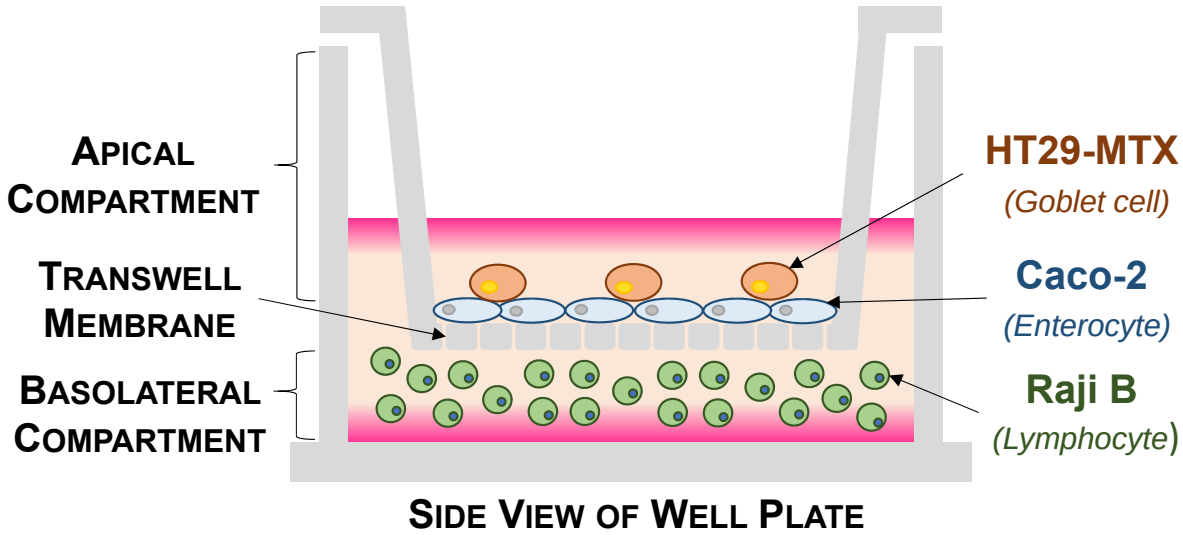
Step 3. *In Vitro* Toxicology – Model Development



Most common methodology is to use single cell-type cultures

Tri-culture Assembly:

- HT29-MTX cells are human mucus secreting intestinal cells
- Caco-2 cells are immortal human colon cells
- Raji B cells are human immune cells derived from the B-lymphocytes

Hypothesis

Caco-2/HT29-MTX/Raji B tri-culture models can better predict intestinal and colonic permeability compared to Caco-2 monoculture

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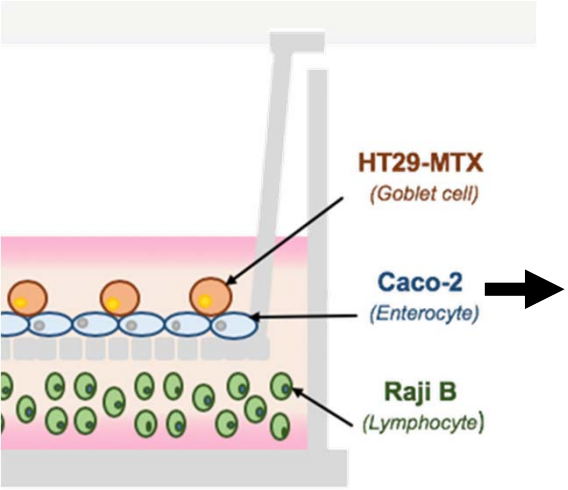
Step 3. *In Vitro* Toxicology – Cell Health

Caco-2 cell characterization before exposure

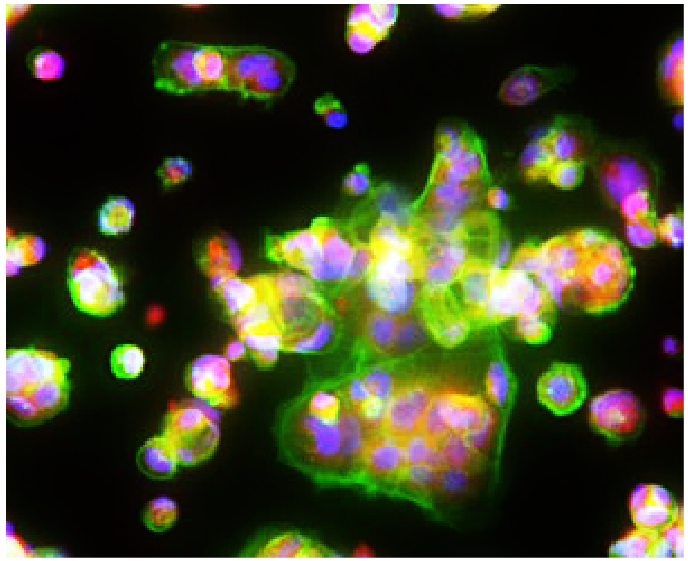
RED: MitoTracker (oxidative stress); BLUE: DAPI (nucleus); GREEN: F-actin (cytoskeleton)

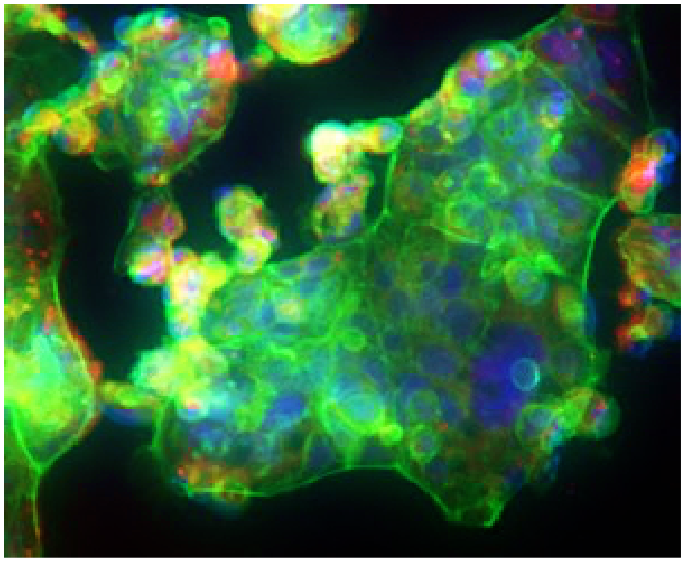
PINK indicates cellular stress, while **GREEN** indicates healthy cell population



Tri-Culture Model *(side view)*

Fluorescence Microscopy (60x)





Caco-2 confluency must be optimized before exposure

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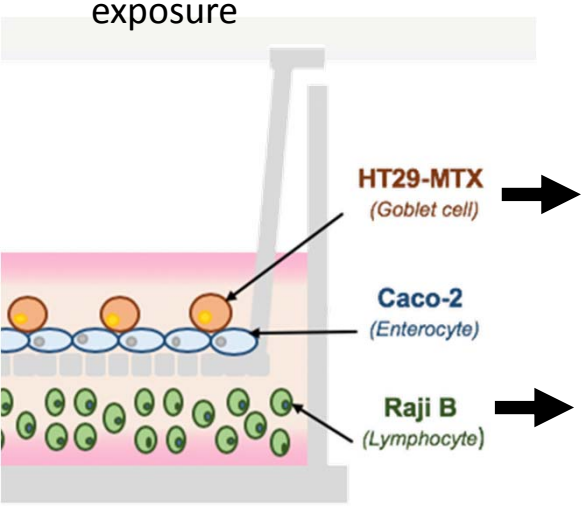
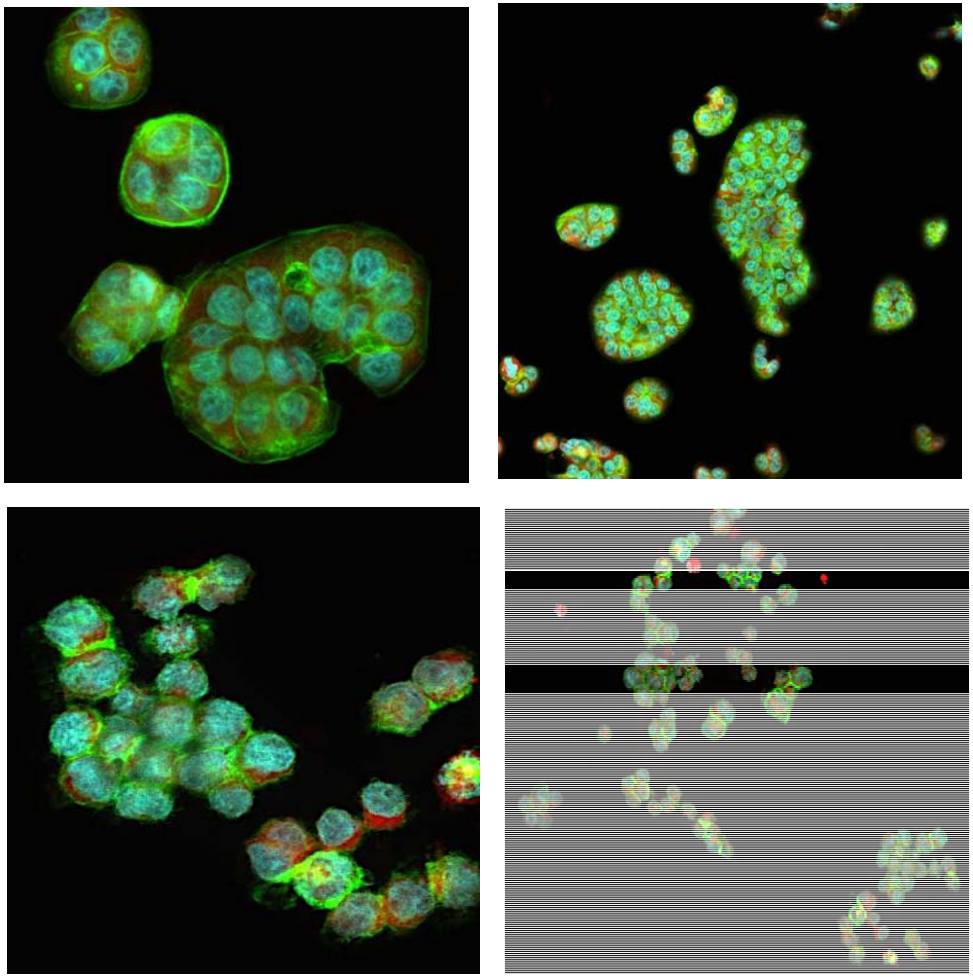
HT29-MTX and Raji B cell characterization before exposure



Tri-Culture Model *(side view)*

Fluorescence Microscopy (60x) **Fluorescence Microscopy** (20x)



RED: MitoTracker (oxidative stress); BLUE: DAPI (nucleus); GREEN: F-actin (cytoskeleton)

PINK indicates cellular stress, while **GREEN** indicates healthy cell population

HT29 and Raji B are less sensitive to confluency

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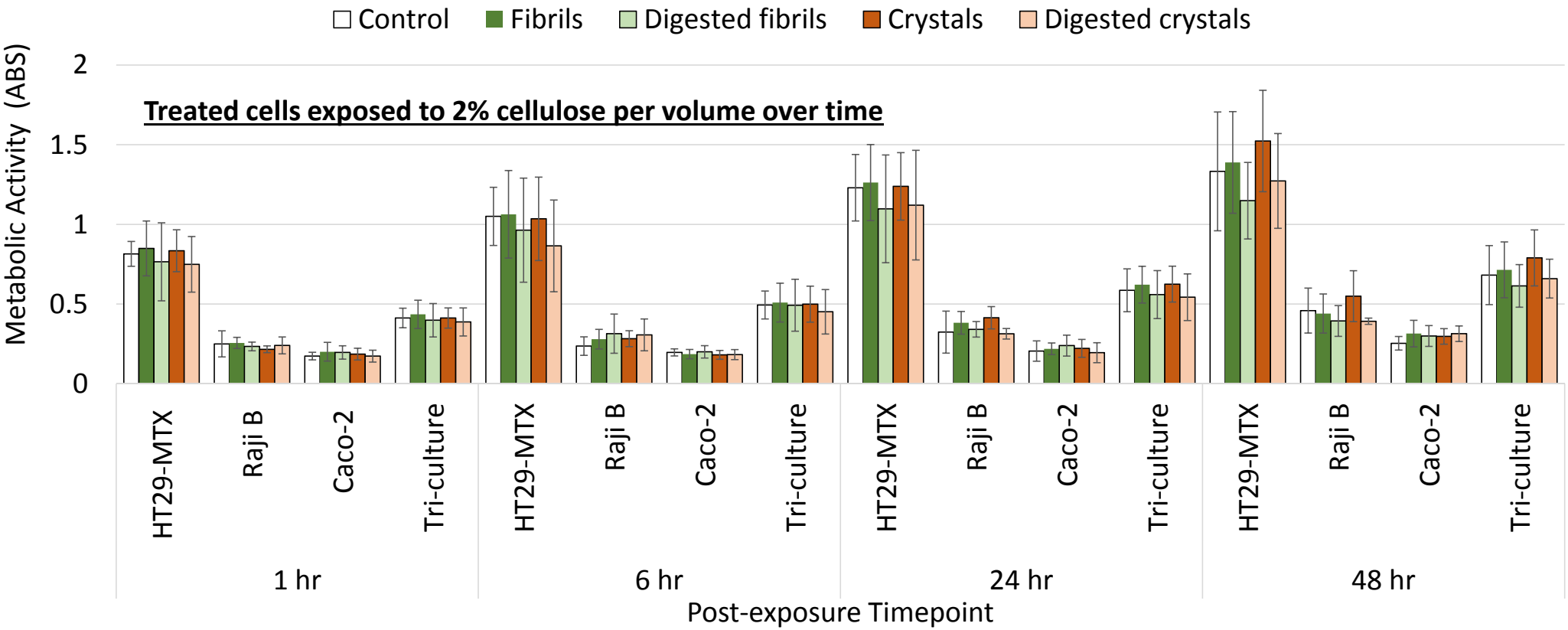
Step 3. *In Vitro* Toxicology – Metabolic Activity



A 50% decrease in metabolic activity wasn't observed at even the highest dosing concentrations

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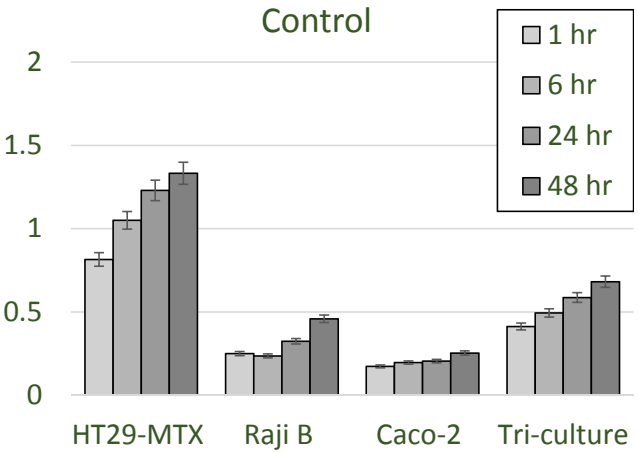
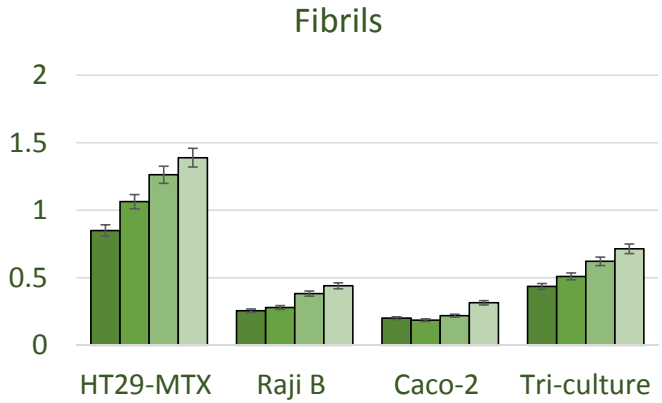
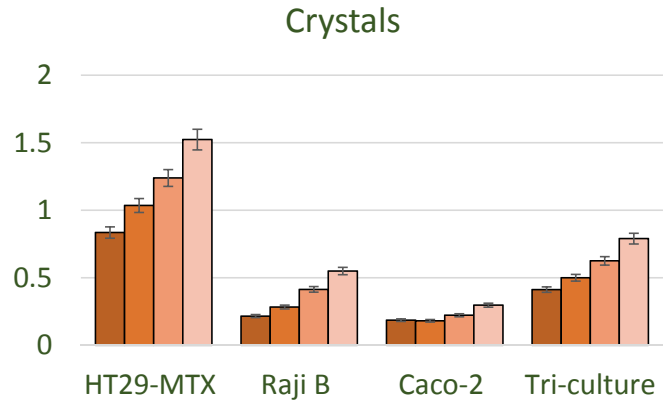
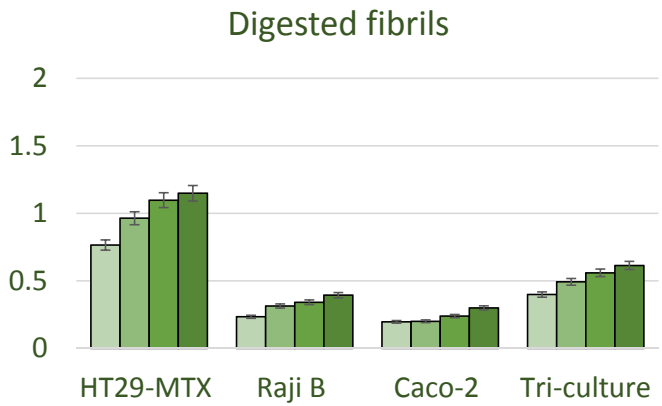
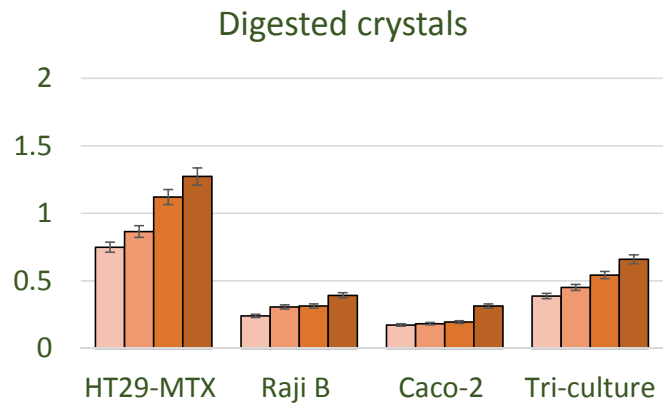
Step 3. *In Vitro* Toxicology – Metabolic Activity (2% cellulose per volume)











Observations

- Slight inhibited growth of cells exposed to digested cellulose
- 2-way ANOVA confirms statistical significance between
  - Fibrils vs. Digested fibrils in HT29 & Tri-culture cells @ 24 & 48 hr pe
  - Crystals vs. Digested crystals in HT29 & Tri-culture cells @ 24 & 48 hr pe

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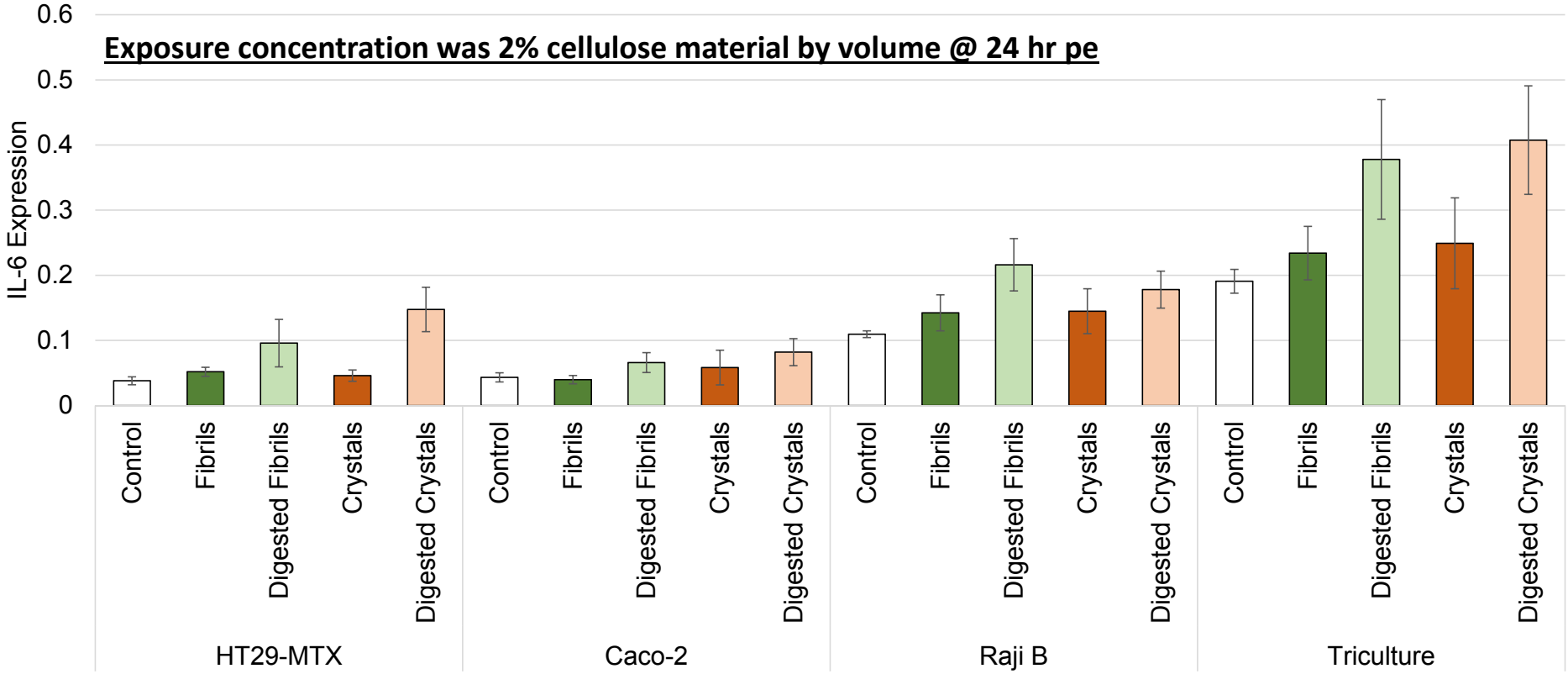
Step 3. *In Vitro* Toxicology – Cytokine Response



In general, digested versions of cellulose material produced more inflammatory response than untreated and pristine version

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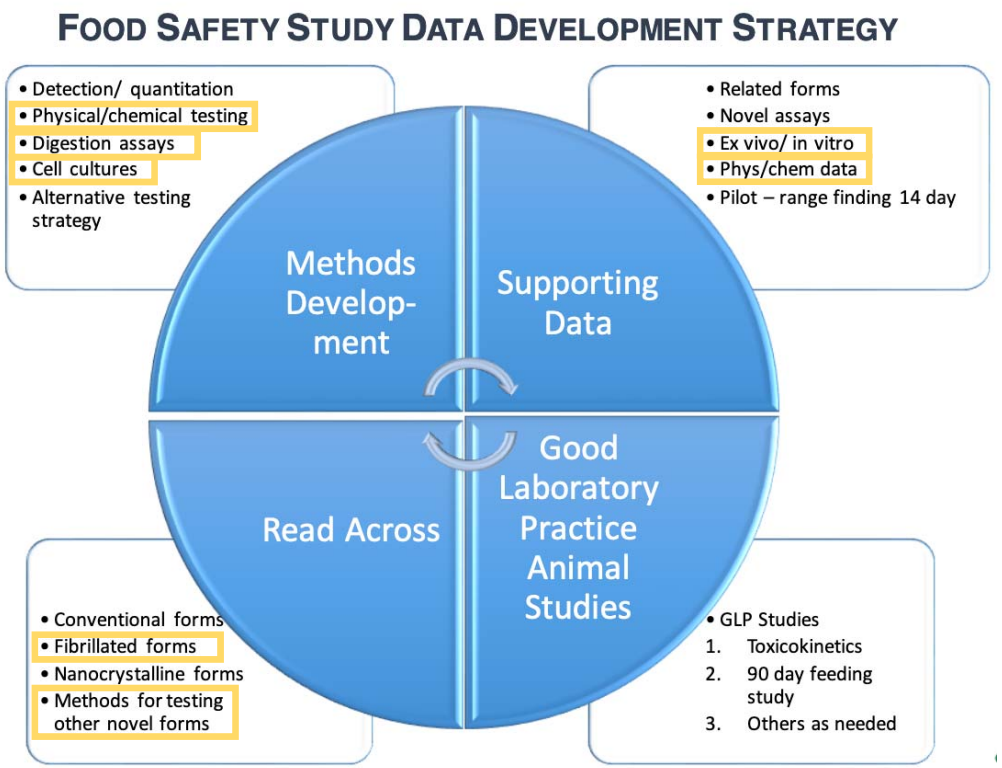
Conclusions and Future Work



Slide courtesy of Dr. Jo Anne Shatkin

This data shows significant progress made in the overall FOOD SAFETY STUDY

- Material characterization offers useful information in safety testing
- Appropriate controls should be included in all aspects of study design
- Data sets should be measured at least 2 different ways
- Tri-culture toxicity models result in different responses as compared to single cell monolayers

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Thank you

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