Conflict of Interest Statement

There is no conflict of interest
Presentation Outlined

1) Initial Motivation
2) Background
3) Functionalization of Nanoparticles
4) Developing
Initial Motivation

- Clay nanocomposite for application in packaging material
- Main reason:
  - Improve barrier
  - Improve mechanical properties
  - Reduce the amount of polymer while maintaining optimum material performance
  - Low cost
Nano-Clay Added in Packaging Material

- Obtained from layered silicate minerals
- Common type: Montmorillonite (MMT)
- MMT structure: layered structure

Images from www.google.com 5-16-2015
Transport of Nanoclay and Exposure

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1. Importance of physicochemical characterization of ENPs prior to the investigation of the biological consequences (Haase et al 2012, Hristozov et al 2009, Seaton et al 2010)

2. Specifically for nanoclays

- **Size**: Nanosilica particles less than 100 nm penetrate and induced structural and functional abnormalities in mouse placenta and produce fetal growth restriction. (Yamashita et al 2011)
- **Shape**: platelet nanoclays were more cytotoxic than tubular ones (Lordan et al 2011)
- **Surface area** and the shape of nanoclays have an impact on cell viability (Verma et al 2012)
- **Aggregation** of nanoclays could be associated with cytotoxicity (Drescher et al 2011)
Our Concerns

- Are nanoclays released from nanocomposite into different environments?

- If nanoclays are released from nanocomposite,
  - How much is being released?
  - How are they released?

- What are the physical and chemical characteristics of the nanoclays before and after the release process?
Fluorescent Labeling and Tracking of Nanoclay

Diaz, Xia, Rubino, Auras, Jayaraman, Hotchkiss. Nanoscale 2013. 5:164-168
Label the Clay

Diaz, Xia, Rubino, Auras, Jayaraman, Hotchkiss. Nanoscale 2013. 5:164-168

**Step 1:**

1. Reaction of (3-Mercaptopropyl)-trimethoxysilane (4.5 g in 200 mL Sn) with HO-Si/Al (Nanoclay, substrate surface 15 g in 500 mL Sn). Sn = 80 wt% methanol + 20 wt% deionized water.

2. Constant stirring for 6 h at 23°C.

**Step 2:**

1. Reaction of mercaptosilane modified nanoclay (0.25 g in 100 mL ethanol) with fluorescein-5-maleimide (Abs: 492 Em: 515) and tetramethylrhodamine-5-maleimide (10 mg in 240 mL phosphate buffered saline solution).

2. Constant stirring for 1 h at 23°C.

**Final product:**

- Mercaptosilane modified nanoclay: Filtered and washed 3X with Sn; Dried (24 h at 80°C, 20” Hg vacuum) and powdered.
- Fluorescent labeled clay: Nanoclays precipitated via centrifugation; Unreacted dye washed out with ethanol.
Migration Study

- Nanocomposite production
- Full characterization of nanocomposite film
- Migration of Nano-clay
- Migration of surfactant
Nanocomposite Film Preparation

Melt compounding (Nanocomposite)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polymer</th>
<th>Compatibilizer</th>
<th>Nanoclay</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP-clay</td>
<td>PP, 85%</td>
<td>MAPP, 12%</td>
<td>I.44P, 3%</td>
</tr>
<tr>
<td>PA6-clay</td>
<td>PA6, 95%</td>
<td>None</td>
<td>Cloisite, 5%</td>
</tr>
</tbody>
</table>
Better compatibility between nanoclay and PA6 (exfoliation) than between nanoclay and PP (partial aggregation)
Experiment: Release of Nanoclay

- ASTM D4754-11
  - Film area: 100 cm$^2$
  - Simulant: ethanol
  - Solvent volume: 40 mL
  - Temperature: 22, 40, 70 °C
  - Migration cell: PP or glass
  - Multiple sampling points

Control samples applied:
- Film without nanoclay (assessment on nanoclay)
Internal validated by X-Ray Fluorescence (XRF)

Limit of quantification: 0.03 mg L\(^{-1}\) (Si) and 0.01 mg L\(^{-1}\) (Al)
At different temperatures:

- A small amount of nanoclay was released (0.1~0.2%)  
- More nanoclay release from PP-clay than from PA6-clay  
- Depending on the polymer-clay interaction
**Experiment: Release of Surfactant**

**ASTM D4754-11**

- Film area: 100 cm²
- Simulant: ethanol
- Solvent volume: 40 mL
- Temperature: 22, 40, 70° C
- Migration cell: PP or glass
- Multiple sampling points

Control samples applied:
- Nanoclay suspension (assessment on surfactant)
Release of Surfactant at 22, 40 & 70°C

UV/VIS spectroscopy methodology was developed and utilized for the in-situ nano-scale measurement of the size of mineral clay agglomerates in solvents.
We have developed metrology tools for:

- Quantifying nanoparticles
- Characterize nanoparticles *in situ*
- Characterize nanocomposites
- Quantify and model release of nanoparticles from different components from different matrices (one publication in progress)
Rethinking Our Path Forward

- Release of nanoclay is in the low ppb levels
- Release of surfactant is in the ppm levels could be designed and model
- New powerful metrology tools can be used to simulate and model nanoparticles and nanocomposite
- Use nanoclays for the development of active and safe nanocomposite
Rationale for the New Approach

- Functionalization of nanoclay with different active ingredients
- Application of functionalized nanoclay on surfaces
- Considering different active ingredient such as bactericide, oxygen absorber, etc....
1 Approach

Functionalize nano-clay by **grafting** an active ingredient.

Incorporation of functionalize nanoparticles in a **coatings** for the developing of active surfaces.
Other Examples of Functionalized Engineering Nanoparticles (ENPs)

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Functionalization</th>
<th>Mechanism</th>
<th>Application</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-wall carbon nanotube</td>
<td>poly(L-lysine) and poly(L-glutamic acid)</td>
<td>adsorption, self-assemble adsorption</td>
<td><em>E. coli</em> and <em>S. epidermidis</em></td>
<td>Aslan et al. 2012</td>
</tr>
<tr>
<td>Multi-wall carbon nanotube</td>
<td>amoxicillin</td>
<td>adsorption</td>
<td><em>E. coli</em>, <em>S. aureus</em> and <em>B. subtilis</em></td>
<td>Kumar et al., 2010</td>
</tr>
<tr>
<td>Multi-wall carbon nanotube</td>
<td>epilson-polylysine</td>
<td>covalent grafting</td>
<td><em>E. coli</em>, <em>P. aeruginosa</em> and <em>S. aureus</em></td>
<td>Zhou and Qi, 2011</td>
</tr>
<tr>
<td>Graphene oxide</td>
<td>para amino benzoic acid</td>
<td>covalent grafting</td>
<td><em>E. coli</em></td>
<td>Ghosh et al., 2010</td>
</tr>
<tr>
<td>Graphene oxide</td>
<td>chitosan</td>
<td>adsorption</td>
<td><em>E. coli</em></td>
<td>Ko et al., 2013</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>cationic surfactants</td>
<td>ion exchange</td>
<td><em>S. aureus</em> and <em>E. coli</em></td>
<td>Nigmatulin et al., 2008</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>polyphenolic compounds</td>
<td>adsorption</td>
<td><em>S. aureus</em></td>
<td>Barbosa-Pereira et al., 2014</td>
</tr>
<tr>
<td>Montmorillonite, Kaolin and Laponite</td>
<td>ciprofloxacin</td>
<td>adsorption</td>
<td><em>S. epidermidis</em> and <em>P. acnes</em></td>
<td>Hamilton et al., 2014</td>
</tr>
</tbody>
</table>
What is the Innovation?

- Develop a surface with specific mode of actions
  - Immobilize an active ingredient such as bactericide on nanoparticles
  - Identify a coating where the functionalized clay was dispersed and finally applied on PP
  - UV curing coating was considered

Functionalization of Nanoparticle

**Step 1**
- Reagents: 3-Glycidyloxypropyl Trimethoxysilane + Ampicillin
- Medium: Water, pH≈4
- Conditions: Stir, 40°C

**Step 2**
- Reagents: HO–Si/Al + Nanoclay
- Medium: Water:MeOH=2:1
- Conditions: Stir, 40°C
Characterization of Functionalize MMT-g-AMP

FTIR spectra of the pristine (MMT) and Amp-functionalized (MMT-g-Amp) clays. The inset has the same axis units as the larger graph. Test used to evaluate the wash solution in order to determine that not free Amp was left in the functionalize clay.
Characterization of Functionalize MMT-g-AMP

TGA profiles of the pristine (MMT) and functionalized clays without (MMT-g-AMP) and with (OMMT-g-Amp) surfactant
### Composition in wt% of the Pristine and Functionalized Clays

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clay components, wt%</th>
<th>Water</th>
<th>3GTS-Amp</th>
<th>3GTS</th>
<th>Amp</th>
<th>Surfactant</th>
<th>MMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMT</td>
<td>8.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>91.7</td>
</tr>
<tr>
<td>MMT-g-Amp</td>
<td>9.4</td>
<td>8.2</td>
<td>3.3</td>
<td>4.9</td>
<td>0</td>
<td>82.4</td>
<td></td>
</tr>
<tr>
<td>OMMT-g-Amp</td>
<td>1.9</td>
<td>6.1</td>
<td>2.5</td>
<td>3.6</td>
<td>31.4</td>
<td>60.6</td>
<td></td>
</tr>
</tbody>
</table>

Note: The 3GTS-Amp content in MMT-g-Amp was estimated based on the weight loss at 100 to 550 °C, then 3GTS and Amp contents were calculated based on their molecular weight ratio (3GTS:Amp = 236:349). The 3GTS-Amp content in OMMT-g-Amp was calculated based on the mass ratio of 3GTS-Amp to MMT for MMT-g-Amp (8.2:82.4).
Characterization of Functionalize Coating

XRD patterns of nanoclay before and after ctionalization

TEM image of functionalized clay in the coating
Efficacy of Coating/Surface

Direct colony count (ASTM E2180 and JIS Z2801)

Coated Film

Film covering the coated film

Incubation at 37C for 24 h
Colonies of *L. monocytogenes* on MOX agar and *S. Typhimurium* on TSA after 24-h Contact with the Coating Surface Containing a Different Amount (wt%) of Functionalized Clay
Populations of *L. monocytogenes* and *S. Typhimurium* after 24-h contact with active acrylate coating containing various levels (wt%) of functionalized clay.

<table>
<thead>
<tr>
<th>Clay content</th>
<th>Listeria</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU mL⁻¹</td>
<td>Log CFU mL⁻¹</td>
</tr>
<tr>
<td>Controla</td>
<td>(3.07 ± 0.10)×10⁸</td>
<td>8.49 ± 0.01bA</td>
</tr>
<tr>
<td>1 wt%</td>
<td>(2.40 ± 0.11)×10⁸</td>
<td>8.38 ± 0.02A</td>
</tr>
<tr>
<td>3 wt%</td>
<td>(2.06 ± 0.17)×10⁷</td>
<td>7.31 ± 0.04B</td>
</tr>
<tr>
<td>5 wt%</td>
<td>(5.57 ± 1.12)×10⁶</td>
<td>6.74 ± 0.09C</td>
</tr>
</tbody>
</table>

a) Control refers to the coating containing 5 wt% OMMT; b) values are expressed as mean ± standard deviation, and means within each column with different uppercase letters are significantly different (p < 0.05, n=3).
Agar Diffusion Test was Conducted Confirming Not Free

Agar diffusion test of the coating (as indicated by the arrows) containing 5 wt% functionalized clay against a) *L. monocytogenes* and b) *S. Typhimurium*. 
Conclusion

- Novel approach to develop active surfaces

- Two essential features in the design of the active surface were the:
  - use of nanoclay
  - UV-curable coating

- The immobilization of Amp to the nanoclay was verified by instrumental analysis and microbiological assessment

- The antimicrobial activity of Amp was not adversely affected by its grafting
Advantages and Application

- Traditionally, antimicrobials, antioxidants and other chemical compounds are added in the polymer bulk/matrix their actions depend on the eventual sustained release.

- Few bactericides are fully qualified for this method because they need to be heated at high pressure or the payload is too low.

- Adding a bactericide in a coating that solidify without heat provide significant advantages. Research in coatings based on epoxy amine formulation for food contact application is essential.
The active ingredients will be located near and/or in direct contact with the product improving the availability and efficiency.

A great variety of active ingredients with diverse chemistries can be used since no heat or pressure is required for the fabrication of the coating.

Active packages can be designed by selecting the best active ingredients (bactericide, antioxidant, etc.) and modes of action for different application and food products.

The polymer matrix is unaltered, so that the recovering route (i.e., recycling) of the polymer is not deteriorated.

The scale up of the coating will follow a well-established packaging coating process.


5) Alin J., Rubino M*., Auras R 2015 In situ characterization of organo-modified and unmodified montmorillonite aqueous suspensions by UV–visible spectroscopy

Acknowledgements