Investigation of the Polyethylene Microparticle Protein Corona in a Simulated Human Gastric Microbiome

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ABSTRACT

When microparticles come into contact with biological systems, biomolecules such as proteins and lipids adsorb to the microparticle's surface. This "biocorona" or "protein corona" has implications on the particle within the body, influencing the surface properties of the particle and, thus, governing the fate and cellular interactions of the particle. The present study aimed at characterizing the protein biocorona of polyethylene microspheres within a simulated human gut microbiome using *Lactobacillus plantarum*. With growing evidence supporting the accumulation of plastic microparticles in the environment, the exposure to humans warrants further investigation of the health risks associated with plastic particle ingestion. After incubation of the particles, isolation of the particle-protein complex, and protein analysis, no protein was detected. These negative results suggest that protein corona formation may be a function of several different variables, some of which will be examined in this presentation.

METHODOLOGY

Clear polyethylene microspheres 0.96g/cc- 250-300 um

Incubation: 1x10^8 CFU/ml *Lactobacillus plantarum* in 50% MRS broth and 50% simulated Gastric fluid: 0.2 M potassium chloride and 0.2 M hydrochloric acid for a pH of 1.5, pepsin was added at a ratio of 10 units per milliliter of culture.
Exposure Time: Samples were incubated at varying lengths of time from 48 hours to 2 months.

Characterization of Particle: Examined Surface Characteristics via Stereo Optical Microscope. (Figures 2 and 3)

Isolation of Protein-Particle Complex: Centrifugation

Elution of Proteins: 1% SDS/TE buffer

Quantification of Protein: Bradford assay, DC Assay, BCA Assay. (SDS compatibility)

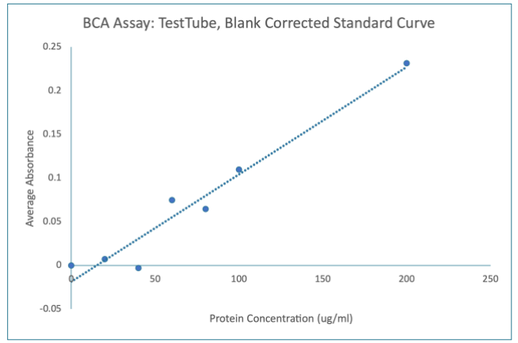


Figure 1: BCA Assay Blank Corrected Standard Curve, used to analyze a 48-hour incubation sample and a 10-day incubation sample. The best fit line is y = 0.0012x - 0.0186 and R^2 value is 0.9488.

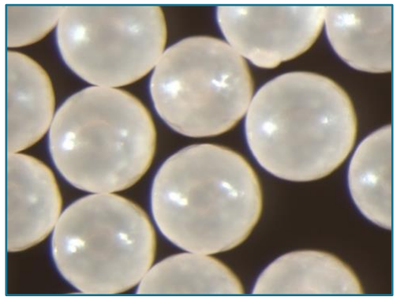


Figure 2: Pretreated Microplastics at an 85.5x Magnification via Stereo Microscopy.

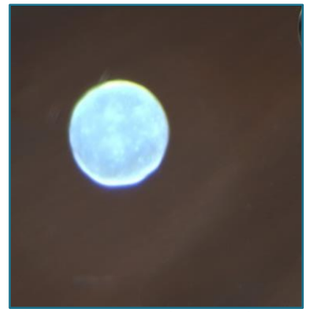


Figure 3: Treated Microplastic at an 85.5x Magnification via Stereo Microscopy.

BACKGROUND

Human Exposure to Microplastics

- ⇒ It is estimated that by 2025, **250 million tonnes of plastic** will have accumulated in the natural environment, making plastic pollution a critical environmental and potential health concern.[1]
- ⇒ Annually humans ingest 39,000 to 52,000 microplastic particles.[2]

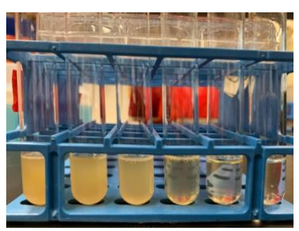
Protein Corona Formation

- ⇒ **Dynamic Process**, driven by the minimization of high surface free energy through the competitive binding of molecules for the particle surface area.[5]
- ⇒ The corona is evidenced to be a heterogeneous composition of molecules adsorbed to the particle surface influenced by particle properties (size of the particle, hydrophobicity, charge and surface chemistry, and the shape of the particle), the media (protein/biomolecule source and concentrations available for potential adsorption), and the exposure time.[5]
- ⇒ The biocorona becomes the interface between particle and its interactions with cells, conferring the particle with an alternate biological identity from its primary synthetic identity.[5]

RESULTS

- ⇒ Bacterial growth was evident with 50% MRS broth and 50% simulated gastric fluid with an OD_{600}= 0.271.

Figure 4: *L. plantarum* cultivated in varying percentages of MRS broth and simulated gastric fluid for 24 hours: From left to right, percentage of gastric fluid: 100%, 75%, 50%, 35%, 25%, 0%



- ⇒ There was **no detection of protein** from the incubated polyethylene microparticle. Each protein assay resulted in a negative sample protein concentration.

DISCUSSION

- ⇒ These results suggest that no protein corona was formed. However, literature evidences the presence of a protein corona complexes on plastic particles in *in vitro* GI studies.[4]
- ⇒ These results suggest that protein corona formation may be a function of several different variables like composition of the incubation media and protein-particle isolation techniques.
- ⇒ Future researchers of this topic should use clear and specific methodology in pursue of optimization and standardization of protein corona isolation techniques.
- ⇒ **Limitations**: The transfer of results to human situation due to differences in gastrointestinal pH, individual variations, food intake, and composition of gut.

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