Isolation and Quantification of Microplastics from Pediatric Tonsils

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Background:

Microplastics have been identified in nearly every organ system, posing an increasing and poorly characterized threat to human health. This project aims to isolate and quantify environmentally acquired microplastics in pediatric tonsil samples. Tonsils provide a unique opportunity for the isolation of microplastics. Not only are they highly vascularized, but they are also exposed to microplastics through eating, drinking, and breathing.

Methods:

Pediatric tonsils, removed as standard of care, were obtained from a Stanford University collaborator on an approved IRB protocol. Samples were digested in 10% KOH at 50C for 48hrs with 30mins of ultrasonication at 24hrs (42kHz). Enzymatic digestion followed using collagenase (1mg/mL), DNase (100µg/mL), and proteinase K (250µg/mL) for 24hrs. Post-digestion, samples were stained with Nile Red (30µg/mL) to selectively visualize microplastics and filtered via 0.2µm aluminum oxide filters in an all-glass vacuum apparatus. Filters were imaged under brightfield, Texas Red (ex-560nm), and CY3 (ex-540nm) fluorescence channels. Image quantification was performed using R-statistical software to assess particle count, diameter, and area.

Results:

The digestion protocol effectively degraded tissue while preserving suspected microplastics. Filters captured fluorescently labeled particles larger than 0.2µm. Preliminary analyses revealed fluorescently labeled particles in every sample (n=6). Across samples there was a mean particle count of 268±274 and a median particle diameter ranging from 13.0µm to 15.6µm. No observed correlation between tonsil mass (78.3±39.4mg) and particle number. In the tissue free negative control, 39 particles were identified.

Conclusion/Impact:

Despite growing evidence of systemic microplastic contamination and an ever-increasing exposure to plastics, reliable methods for isolating and characterizing environmentally acquired microplastics remain limited. This work establishes an optimized protocol for non-destructive isolation and quantification of microplastics from human tissue. The tonsil-isolated microplastics will undergo further particle typing and be used in subsequent tissue culture models to better understand microplastic exposure implications to human health.