

Supplemental Material:

Microplastic and Nanoplastic particle isolation from liquid and biological samples via mini-extruder filtration (MEF)

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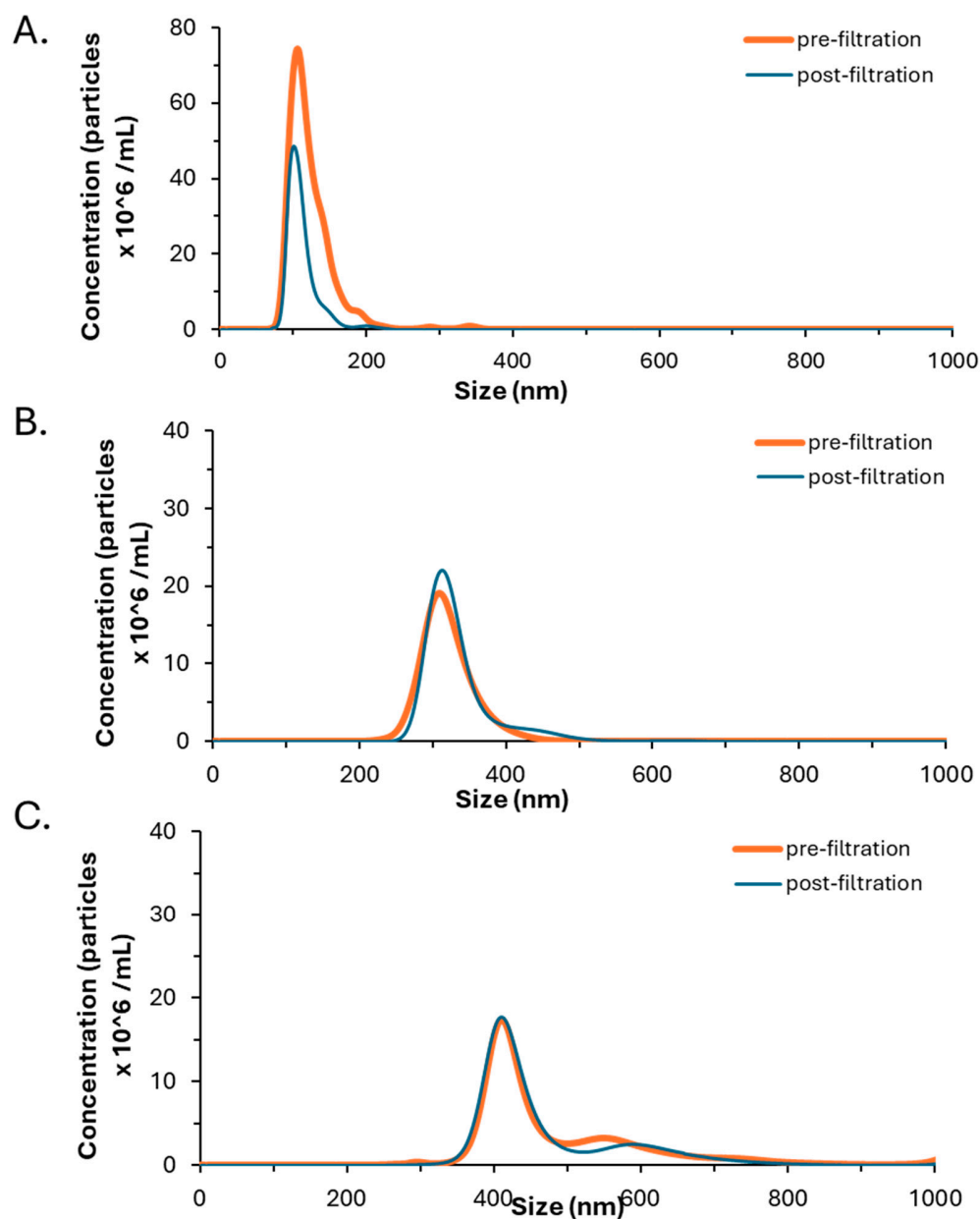
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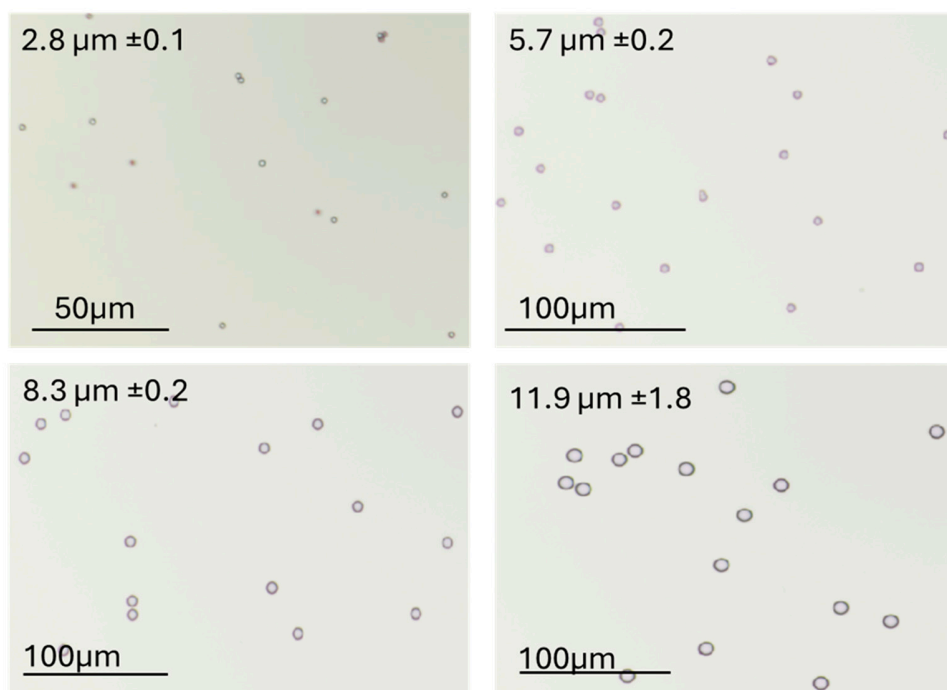
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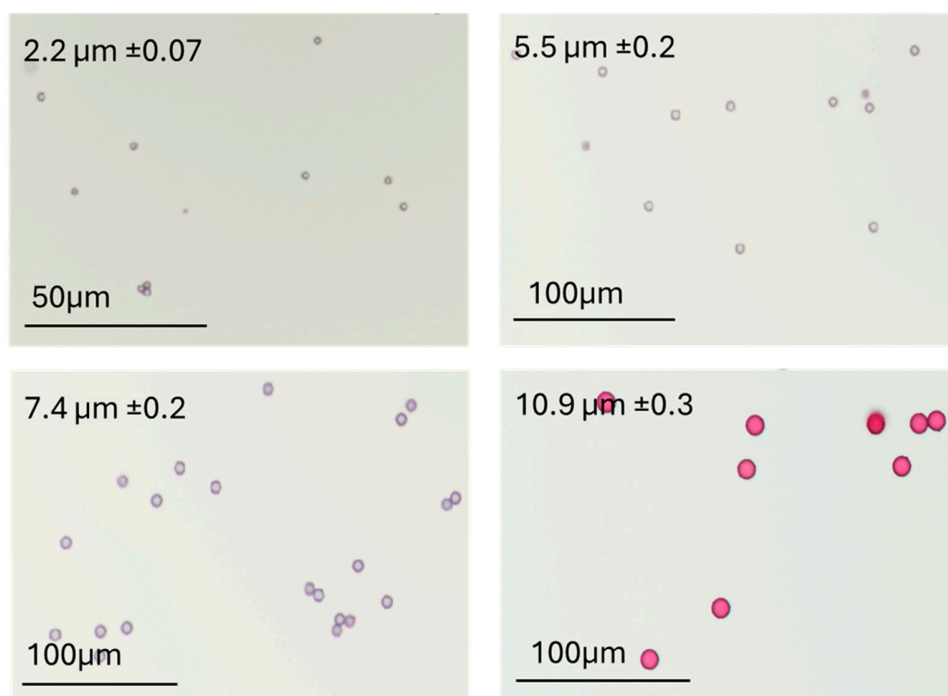


Suppl. Fig. S1: Representative average size distributions measured on Nanosight LM12 of a single sample before and after filtration on the mini extruder of submicron aPS particles. A. 100 nm aPS particles, B. 300 nm aPS particles, and C. 500 nm aPS particles.

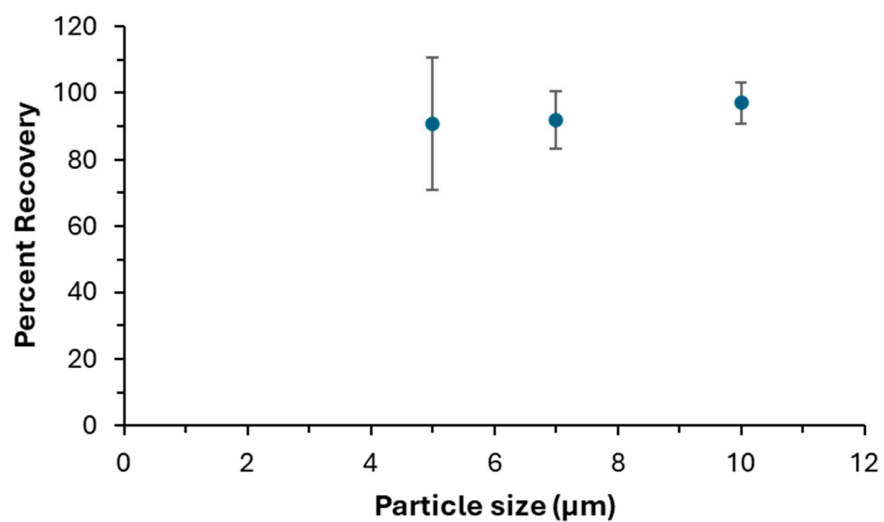
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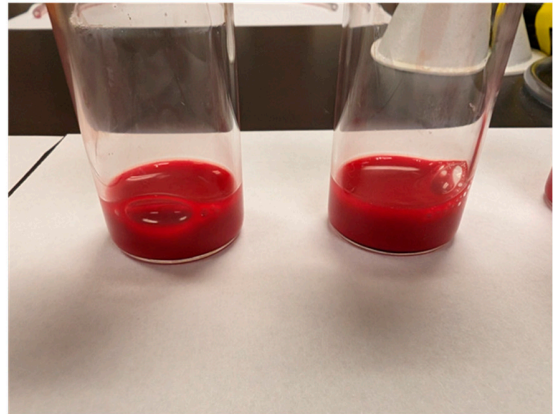
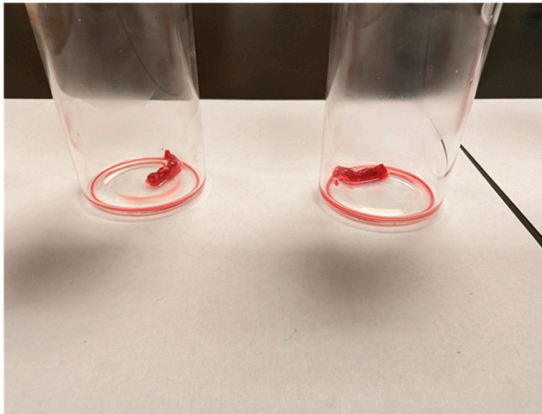


Suppl. Fig. S2: Representative brightfield images of micron particles used for particle size analysis, A. aPS micron particles, B. cPS micron particles.

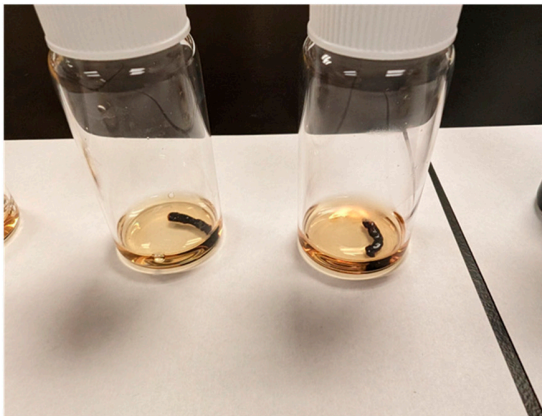


Suppl. Fig. S3: Percent recovery of equal particle/mL concentration of 5, 7, and 10μm particles after mini-extruder filtration. 2 μm particles were also present in the sample, but recovery could not be assessed due to aggregation of 2 μm particles onto the larger particles.

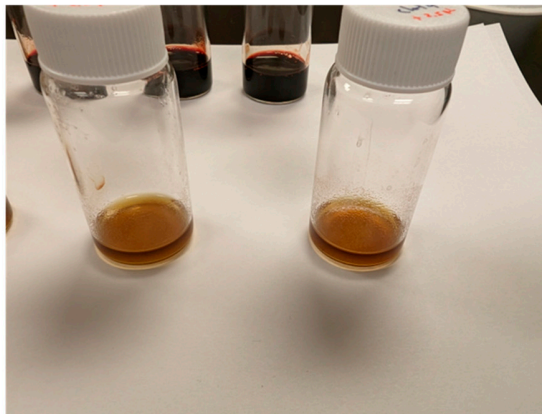
A.



B.



C.



Suppl. Fig. S4: A. Clot samples (left) and serum samples (right) in glass vials before digestion, B. clots and serums directly after addition of 10% KOH, C. Clot and serum after incubation with KOH at 50°C overnight.

Supplementary Protocol S1 – Mini Extruder Filtration

1. Prepare sample or particle stock solution in a 2mg/mL BSA/0.01% tween 20 solution, let a freshly diluted particle stock solution sit for at least 4 hours prior to use.
2. Sonicate sample for 5 minutes just prior to beginning filtration.
3. Prewarm 2 mL of DI H₂O and 0.8 mL of 0.01% Tween 20
4. Assemble mini extruder apparatus by wetting a filter support in DI water and then placing it in the center of the O-ring of the Teflon support in the extruder housing.
5. Carefully place the polycarbonate membrane on top of the filter support.
6. Place the other Teflon support with the O ring facing towards the membrane and then place the extruder housing nut on top and tighten firmly.
7. Insert extruder housing into the holding block and then insert the two glass syringes. The syringe inserted into the housing closest to the housing nut is referred to in this protocol as the loading syringe, the syringe opposite it is the waste syringe.
8. Obtain one 20mL disposable glass vial and label
9. Prewet pc membrane by adding 0.5mL of warm DI H₂O into the loading syringe, insert into the Teflon support and pushing the sample through the filter to the waste syringe gently to avoid rupturing the membrane.
10. Remove waste syringe, empty to it waste, and reassemble apparatus
11. Prepare sample and gently load it into apparatus while applying constant pressure
12. Remove waste syringe, empty to waste, and reassemble apparatus
13. To rinse the filter flow 1mL of warm DI H₂O from the loading syringe through the membrane and into the waste syringe. Remove waste syringe and empty to waste and reassemble.
14. Remove loading syringe and ensure all fluid possible is removed from the apparatus by loading 300 μ L of air into apparatus and pushing through.
15. Disassemble apparatus while avoiding touching the membrane.
16. Dispense 400 μ L of warm 0.01% Tween 20 buffer into the pre-labeled vial
17. Remove filter from apparatus with caution while avoiding touching the center of the filter. If any fluid is on the membrane or in the housing pipette into the vial and note as this can indicate a loss in sample recovery.
18. Discard filter support.
19. Place membrane into the labeled vial with the side containing particles facing the solution
20. Sonicate vial in a bath sonicator for 3 minutes and then incubate on orbital shaker for 7 minutes.
21. Dispense 400 μ L of warm tween into labeled vial (vial will now have 800 μ L total and bath sonicate for an additional 3 minutes and then incubate for 7 minutes.
22. Use tweezers to grab the membrane, expose the side containing particles and stick it to the sidewall of the vial
23. Using a clean collect 200 μ L of the vial solution and rinse the filter on the sidewall of the vial 4 times.

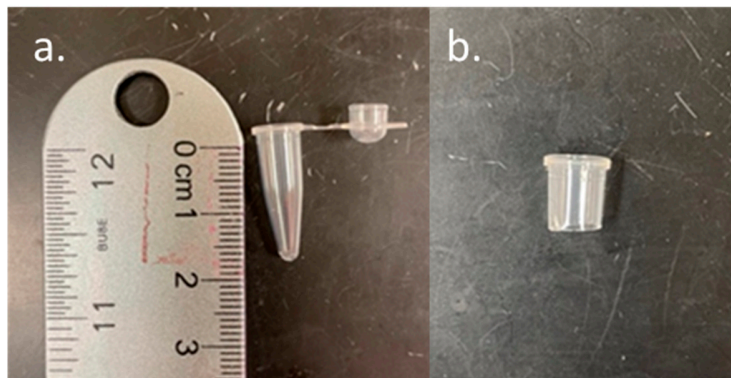
Supplementary Protocol S2 – Chandler loop clot formation

(Ideally complete material preparation before the citrated blood samples are collected)

1. Turn on water bath and allow it to begin coming up to temperature while the other materials are prepped.
2. Cut an appropriate number of tubing loops for the number of chandler loop clots being formed for that day's experiment. Typically, the length will be 37cm for the 7/32" OD tubing on a 60 radial diameter drum, but it is best to test the fit of each loop on the drum to ensure fit before beginning experiment.

(Note: the tubing has some stretching capacity, so do not stretch or put tension as you wrap the tubing on the drum or the tubing will be slightly short and more likely to rupture or leak during the clotting process)

3. Using a straight razorblade **carefully** cut the conical portion (~5mm below the lip of the tube) and lid off of 0.2mL PCR tubes to form the loop connections (see Figure).



Supp. Protocol 2 Figure 1. a) The starting PCR tube, the cut is made at approximately 5mm below the lip, b) the completed connector.

4. Now that loop components are complete, begin thawing the FITC-Fb, and plasma (if using plasma) or whole blood (if using chilled whole blood, do not use frozen whole blood)
5. Double check that loops, connectors, and parafilm is ready and the waterbath for the chandler loop system is ready, once clotting is initiated the loops need to be formed and placed on the drum quickly.
6. To begin clotting add CaCl₂ in the following proportion to the sample
 - a. Whole blood (20 μ L CaCl₂/ 340 μ L Whole blood)
 - b. Plasma (30 μ L CaCl₂/340 μ L Plasma)After adding the calcium mix well by inverting the tube several times

7. Add sample to the loop in one of two methods by connecting the tubing to a 3 or 5 mL syringe and place the tubing in the tube with the blood/plasma sample. Carefully draw back 2mL of sample and disconnect the tubing. Check for bubbles, if there are any bubbles in the middle of the loop, with both ends pointing up, gently shake the loop to release the bubble.
8. Once the tube is filled, use the connector to close the loop.
9. Place the tube on the drum, start the motor to rotate the sample. If you have additional clots to form, make sure the loops that are already filled are rotating on the drum before you form the next loop. Once the sample is in the loop the goal is to have it rotating as soon as possible so the clot forms under the shear force.
10. Shear calculation should refer to the equation below, the example is calculated using the recommended tubing on a 60 radial diameter rotating drum.

$$\dot{\gamma} = \frac{2\pi R_0 \omega}{15R}$$

e.g. R0 (drum's loop curvature radius) = 60 mm; R (tubing inner radius) = 1.985 mm; w (rotational rate) = 20 RPM; These values gives a $\dot{\gamma}$ of 253 s⁻¹ by the equation above.

Note: The loop curvature radius gives a good approximation of laminar flow. When other loop curvature radius is used, numerical solution is needed to calculate a reliable shear rate.

11. Form all the clots required for the experiment (invert the sample tube between each sample to ensure homogeneity of the sample used for each loop)
12. Run the Chandler loop for 60 mins for whole blood samples or 90 minutes for plasma samples.
13. Once clotting is complete, separate the ends of the Chandler loop and invert into an appropriate tube to capture the serum and the clot.
14. The clot should be imaged and weighed to get a baseline before being incorporated into other assays