Supplementary Materials

Circulating Microplastics in Routine Peripheral Blood: Bedside-Accessible Raman Screening in Myocardial Infarction and Controls

Tables S1 and S2b were transcribed from the primary Open Specy output. The analysis dataset and the point-by-point spectral record from which every value in this document and in the main manuscript is derived are available from the corresponding authors on reasonable request.

Text S1. Per-Sample Processing Record and Raw Spectral Assignments

Table S1 lists every clinical sample processed in this study, together with the procedural blanks and positive controls run alongside them (Table S2), with the volume of blood analysed and every spectral assignment returned by Open Specy together with its Pearson match quality. The digestion protocol is identical for every sample and is given in ref. 10 of the main text, so the per-sample processing conditions are not reproduced here. Assignments below the 0.80 threshold are listed but were not counted; assignments matched to a procedural blank are marked and were excluded.

Two features of this record warrant explicit comment. First, sub-threshold assignments are listed in full, and where the untargeted search returned a non-polymeric reference at a higher correlation than the polymer on the same point, that competing match is given in square brackets; this is the carbonaceous background described in Section 3.1 of the main text. Second, control subject C3 was processed in three independent analytical runs, one of which returned no particles; all three are listed and all three contribute to that subject's volume denominator. All other subjects were processed once.

Provenance of two assignments. The EVA particle in A5 was identified during the methodological validation of this workflow and is described there (ref. 10 of the main text); its Open Specy top match was returned as polyethylene at 0.94, and it was assigned EVA on the acetate carbonyl band near 1740 cm^{-1}, which the polyethylene reference does not carry. The PU particle in A4 is reported for the first time here; its competing graphite match is discussed in Text S2b and shown in Figure S1.

Scope of the point-by-point record. Thirteen clinical samples were measured point by point and the complete Open Specy output was retained for each; these are the samples whose sub-threshold assignments are itemised in Table S1 and counted in Table S2b. For samples C1, C2 and A1 only the assigned particle was retained, and in C7 and C8 no particle was located on the filter. Particle counts, including the 0.70 sensitivity analysis, are based on particles, i.e. objects located on the filter and registered as candidate particles in the laboratory record; the point-by-point record comprises every measurement point, most of which were not registered as candidate particles. Procedural blanks were run repeatedly through the study; the two listed here are those run alongside the samples from which the reported particles derive. No other blank returned a polymer assignment at or above the threshold.

Summary. Across the 16 pre-procedural analytical runs, 11 assignments met the 0.80 threshold; two of these (both PBT, in patient A1) matched a procedural blank and were excluded, leaving **9 particles** in the clinical dataset — polyethylene (n = 4), polystyrene (n = 3), ethylene–vinyl acetate (n = 1) and polyurethane (n = 1) — recovered from 147.0 mL of blood (93.0 mL in controls across 10 runs, 54.0 mL in AMI patients across 6 runs). Subject-level prevalence was 2/8 (25.0%) in controls and 4/6 (66.7%) in AMI patients.

Table S1. Complete per-sample processing record and raw spectral assignments. Match qualities are Pearson correlation coefficients returned by Open Specy; where a polymer was assigned to more than one particle in a sample, the qualities of each are given. Shaded rows are samples excluded from the primary analysis: post-procedural patient samples and control materials. In sample A1, two polybutylene terephthalate (PBT) particles matched the filtered-water procedural blank and were excluded by the pre-specified blank-exclusion rule. The polypropylene assignments in C2 (0.79) and A1 (0.71) and one polystyrene assignment in C3 run 1 (0.75) fall below the 0.80 threshold and were not counted.

Subject	Laboratory sample code	Category	Volume (mL)	Open Specy assignments (match quality)	Unassigned
C1	C3SR1+C3SR2	Control	9.5	PE ×1 (0.93)	0
C2	C36SR1+C36SR2	Control	10	PP ×1 (0.79) [< 0.80]	0

Subject	Laboratory sample code	Category	Volume (mL)	Open Specy assignments (match quality)	Unassigned
C3 (run 1)	C5SR1+C5SR2	Control	9	PS ×2 (0.75 [< 0.80], 0.98); PU ×1 (0.67) [< 0.80; graphite 0.79]; PP ×5 (0.54–0.71) [< 0.80; graphite 0.86 on one of these points]; PI ×2 (0.65, 0.71), PLA ×1 (0.69) [< 0.80]	0
C3 (run 2)	C5SR1+C5SR2	Control	9	PS ×2 (0.97, 0.98); PS ×2 (0.59, 0.60) [< 0.80; graphite 0.89–0.95]; polyamide ×1 (0.75) [< 0.80; graphite 0.86]; PP ×3 (0.46, 0.61, 0.71) [< 0.80; graphite 0.89 on one of these points]; PU ×1 (0.75), PI ×1 (0.74), PVC ×1 (0.45) [< 0.80]; non-polymeric: niter KNO_3 (0.88, 0.92)	0
C3 (run 3)	C5SR1+C5SR2	Control	10	none above threshold; PI ×13 (0.62–0.72), PP ×2 (0.54, 0.61), PVC ×1 (0.66), polyisoprene ×1 (0.40) [< 0.80]; non-polymeric: graphite (0.69), niter KNO_3 (0.89)	0
C4	C4SR1+C4SR2	Control	9	none above threshold; PS ×3 (0.52, 0.57, 0.58), PP ×3 (0.44, 0.63, 0.66), PI ×3 (0.68, 0.68, 0.71), polyamide ×2 (0.71 [graphite 0.85], 0.75), LDPE ×1 (0.64), PLA ×1 (0.47), polysulfone ×1 (0.48) [< 0.80]; non-polymeric: okenite (0.64), hingganite (0.65)	1
C5	C2SR1+C2SR2	Control	9.5	none above threshold; PP ×1 (0.65) [< 0.80]; non-polymeric: niter KNO_3 (0.93)	1
C6	C1SR1+C1SR2	Control	8	none above threshold; PP ×5 (0.58–0.72) [< 0.80; graphite 0.80 on one of these points]; PI ×3 (0.54, 0.68, 0.70), PS ×1 (0.67) [< 0.80]; non-polymeric: graphite (0.85)	0
C7	C33SR1+C33SR2	Control	10	none; no particle located on the filter and no point-by-point output recorded	0
C8	C7SR1+C7SR2	Control	9	none; no particle located on the filter and no point-by-point output recorded	0
A1	A28SR1+A28SR2	AMI patient	9.5	PE ×2 (0.91, 0.95); PP ×1 (0.71) [< 0.80]; PBT ×2 (0.96, 0.95) [blank match — excluded]	0
A2	A20SR1+A20SR2	AMI patient	9.5	PI ×1 (0.64) [< 0.80]; non-polymeric: niter KNO_3 (0.93, 0.94)	0
A3	A14SR1+A14SR2	AMI patient	9.5	PE ×1 (0.97); LDPE ×3 (0.62, 0.67, 0.75) [< 0.80; graphite 0.77–0.79 on the same points]; PP ×8 (0.42–0.70) [< 0.80; graphite 0.85–0.91 on 4 of these points]; polyacrylonitrile ×1 (0.66), PLA ×1 (0.49), polyphenylsulfone ×1 (0.50) [< 0.80]; non-polymeric: cotton (0.71), diamond (0.76), vaesite (0.63), beryl (0.53)	0
A4	A9SR1+A9SR2	AMI patient	9	PU ×1 (0.80) [graphite 0.87 on the same point]; PU ×1 (0.76) [< 0.80; graphite 0.88]; LDPE ×1 (0.67) [< 0.80; graphite 0.88]; PP ×6 (0.49, 0.58, 0.62, 0.64, 0.66, 0.66) [< 0.80]; PI ×1 (0.63) [< 0.80]; non-polymeric: niter KNO_3 (0.92, 0.89), valentinite (0.88, 0.94), azurite (0.62)	0
A5	A21SR1+A21SR2	AMI patient	10	EVA ×1 (0.93) [Open Specy top match returned as polyethylene, 0.94; assigned EVA on the acetate carbonyl band near 1740 cm^{-1}, see ref. 10]; PU ×1 (0.73) [< 0.80; graphite 0.87]; PP ×14 (0.54–0.74) [< 0.80; graphite 0.68–0.90 on 8 of these points]; PVC ×1 (0.51), PS ×1 (0.42), PI ×2 (0.62, 0.71) [< 0.80]; non-polymeric: niter KNO_3 (0.91), lausenite (0.94), sakahite (0.93)	0
A6	A37SR1+A37SR2	AMI patient	6.5	PP ×4 (0.47, 0.50, 0.53, 0.56) [< 0.80]; non-polymeric: graphite (0.71)	0
A2 (post-PCI)	D20SR1+D20SR2	AMI patient	9	PE ×1 (0.96); LDPE ×1 (0.67) [< 0.80]; PP ×1 (0.51) [< 0.80]; PI ×1 (0.73) [< 0.80]	0
A3 (post-PCI)	D14SR1+D14SR2	AMI patient	9	PE ×1 (0.93); PU ×1 (0.71) [< 0.80]; LDPE ×1 (0.67) [< 0.80; graphite 0.85]; PS ×1 (0.62) [< 0.80; graphite 0.73]; PP ×9 (0.55–0.66) [< 0.80]; PVC ×2 (0.41, 0.45), PI ×2 (0.64, 0.64) [< 0.80]; non-polymeric: niter KNO_3 (0.16–0.87), hydrocerussite (0.82–0.87), plumbotsumite (0.83), likasite (0.88), carphatite (0.84), orlandite (0.90), rouaite (0.68)	6
Procedural blank	Filtered water	Procedural blank	-	PBT ×2 (0.95, 0.73)	0
Procedural blank	Digestion solution	Procedural blank	-	none	0
Positive control	Mix of known microplastics	Positive control	0	PE ×5 (0.96–0.98); EVA ×3 (0.92–0.95); EVOH ×1 (0.88)	0
Positive control	Goat blood with lab coat fibres	Positive control	10	PET ×1 (0.34) [< 0.80]; PP ×1 (0.97) on a separate coverall-fibre mount; non-polymeric: cotton and niter KNO_3 (recorded without a correlation value)	0

Text S2. Quality Assurance and Quality Control

Four control samples were processed alongside the clinical series under identical conditions (Table S2).

Table S2. Control samples, their purpose and outcome.

Control	Type	Purpose	Result
Filtered water	Procedural blank	Reagent and handling background	PBT ×2 (0.95, 0.73)
Digestion solution	Procedural blank	Reagent background	No particles
Polymer mixture	Positive control	Recovery through full protocol	PE ×5 (0.96–0.98), EVA ×3 (0.92–0.95), EVOH ×1 (0.88)
Coat and overall fibres	Positive control	Airborne and textile contamination	PET ×1 (0.34) below threshold; PP ×1 (0.97) on a separate coverall-fibre mount

Three consequences follow. (i) **Blank-derived exclusions.** The two PBT particles detected in patient A1 matched the filtered-water blank and were removed; two particles were excluded in total, and no other polymer found in a clinical sample was present in any blank. (ii) **Threshold evidence.** The weaker of the blank's two PBT assignments (0.73) falls inside the 0.71–0.79 band occupied by the three lowest clinical assignments, which is the empirical basis for raising the assignment threshold to 0.80. (iii) **Digestion survival.** The polymer-mixture positive control was carried through the complete digestion–filtration protocol and returned PE, EVA and EVOH in recoverable, spectrally identifiable form, indicating that these polymers are not destroyed by the procedure. This is not a quantitative recovery determination and is not presented as one; formal spike-and-recovery experiments were not performed for this cohort.

The textile-contamination control, prepared from laboratory coat and overall fibres carried through the protocol, returned a single assignment at 0.34, which the threshold correctly rejects; a separate mount of coverall fibres returned polypropylene at 0.97, confirming that the laboratory garments are a potential polypropylene source and that our workflow would detect such a fibre if one reached a filter. No polypropylene assignment in any clinical sample reached the threshold. Together with the carbonaceous background described below, this constitutes the airborne-contamination characterisation for this workflow.

Text S2b. The Carbonaceous Background

The most consistent analytical observation in this dataset concerns the material that falls below the assignment threshold. Weak polymer assignments in the 0.40–0.76 range were returned in nearly every clinical sample with point-by-point output, including every such sample in which no particle met the threshold. They are not randomly distributed. Across the thirteen clinical samples for which the complete point-by-point Open Specy output was retained, 126 points returned a polymer at 0.40–0.76 and nothing at or above the threshold (Table S2b). On 28 of those 126 points the untargeted library search simultaneously returned a graphite reference, and on every one of the 28 the graphite match was higher than the polymer — typically 0.68–0.95 for graphite against 0.40–0.76 for the polymer. There is not one point in this dataset at which a sub-threshold polymer outranked a graphite match returned alongside it. Counting every non-polymeric reference rather than graphite alone, a non-polymeric match outranked the polymer on 36 of the 126 points. The measured spectra in the graphite-ranked cases consist of two broad bands near 1590 and 1330 cm^{-1} with little structure elsewhere, the characteristic D and G bands of disordered carbon.

Table S2b. Sub-threshold polymer assignments and their non-polymeric competing matches, in the thirteen clinical samples for which the complete point-by-point Open Specy output was retained. Samples C1, C2 and A1 are omitted because only the assigned particle was retained for them, and C7 and C8 because no particle was located on the filter. Counts are points, not particles: a point is one measured spectrum, and the counts include only points at which the best polymer match fell below 0.80. "Of which a graphite match ranked higher" counts the points at which the search returned a graphite reference at all; on every one of those points graphite outranked the polymer, with no exception in this dataset. Samples A6, C3 run 3 and C6 each returned a graphite match on a point carrying no polymer assignment (0.71, 0.69 and 0.85 respectively); those points are not counted here. The full itemisation is in Table S1.

Sample	Points with a polymer match below 0.80	Of which a graphite match ranked higher	Graphite range	Polymer range
C3 run 1 (C5SR1)	10	2	0.79–0.86	0.54–0.75
C3 run 2 (C5SR1)	9	4	0.86–0.95	0.45–0.75
C3 run 3 (C5SR1)	17	0	—	0.40–0.72
C4 (C4SR1)	14	1	0.85	0.44–0.75

Sample	Points with a polymer match below 0.80	Of which a graphite match ranked higher	Graphite range	Polymer range
C5 (C2SR1)	1	0	—	0.65
C6 (C1SR1)	9	1	0.80	0.54–0.72
Controls, subtotal	60	8 (13%)	0.79–0.95	0.40–0.75
A2 (A20SR1)	1	0	—	0.64
A3 (A14SR1)	14	7	0.77–0.91	0.42–0.75
A4 (A9SR1)	9	2	0.88	0.49–0.76
A5 (A21SR1)	19	9	0.68–0.90	0.42–0.74
A6 (A37SR1)	4	0	—	0.47–0.56
AMI patients, subtotal	47	18 (38%)	0.68–0.91	0.42–0.76
A2 post-PCI (D20SR1)	3	0	—	0.51–0.73
A3 post-PCI (D14SR1)	16	2	0.73–0.85	0.41–0.71
Total	126	28 (22%)	0.68–0.95	0.40–0.76

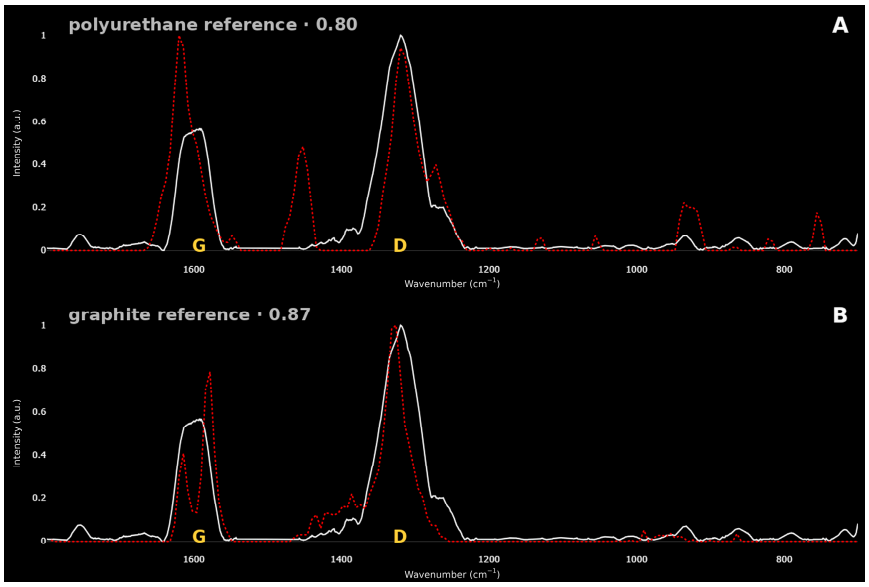


Figure S1. Competing polyurethane and graphite reference matches for the particle of patient A4. The same measured spectrum (white) from patient A4 compared with two reference spectra (red, dotted). (A) Polyurethane reference, Pearson correlation 0.80. (B) Graphite reference, 0.87. The two broad bands labelled G (near 1590 cm^{-1}) and D (near 1330 cm^{-1}) are the first-order Raman bands of disordered carbon (ref. 12 of the main text); the graphite reference reproduces them, whereas the polyurethane reference carries additional bands near 1450 and 930 cm^{-1} that the measurement does not show. This particle is retained because a polymer reached the assignment threshold, and is flagged in Table 1 of the main text; the retention rule and its rationale are given in Section 2.3 of the main text.

We therefore interpret the sub-threshold band as carbonaceous procedural and environmental background rather than as weakly matched circulating polymer, and this is the principal empirical basis for the 0.80 threshold. **The practical implication is general: a microplastic count obtained from a complex biological matrix at a permissive correlation threshold may be counting carbon.**

The same phenomenon has a converse that we have been careful not to ignore. Carbon black is a ubiquitous polymer additive and is resonance-enhanced at 785 nm excitation, so a genuinely plastic particle that happens to be pigmented will return a graphite match above its own polymer. The polyurethane particle in patient A4 is exactly that case: a polymer assignment at 0.80 with graphite at 0.87 on the same point (Figure S1). We retain such particles when the polymer itself reaches the threshold, and declare the competing match, rather than adopting a top-ranked-match rule that would systematically discard dark and pigmented microplastics.

Text S3. Associations with Biological Sex and Age

Neither biological sex nor age was associated with circulating microplastics in this cohort (Figure S2). Subject-level prevalence was 44.4% (4/9) in men and 40.0% (2/5) in women (OR 1.20, Fisher's exact p = 1.000), and

particle density did not differ (mean per-subject density 0.047 vs 0.063 particles/mL; Mann–Whitney p = 1.000). The median age of MP-positive subjects was 59.0 years (IQR 50.8–63.5) versus 62.5 years (IQR 42.8–72.8) in MP-negative subjects (Mann–Whitney p = 0.698), and age did not correlate with particle count (Spearman ρ = −0.122, p = 0.677) or particle density (Spearman ρ = −0.109, p = 0.712).

We note, as a point of statistical caution, that absence of a detectable association in a cohort of 14 subjects does not establish absence of confounding. No claim that these variables do not act as confounders would be supportable at this sample size.

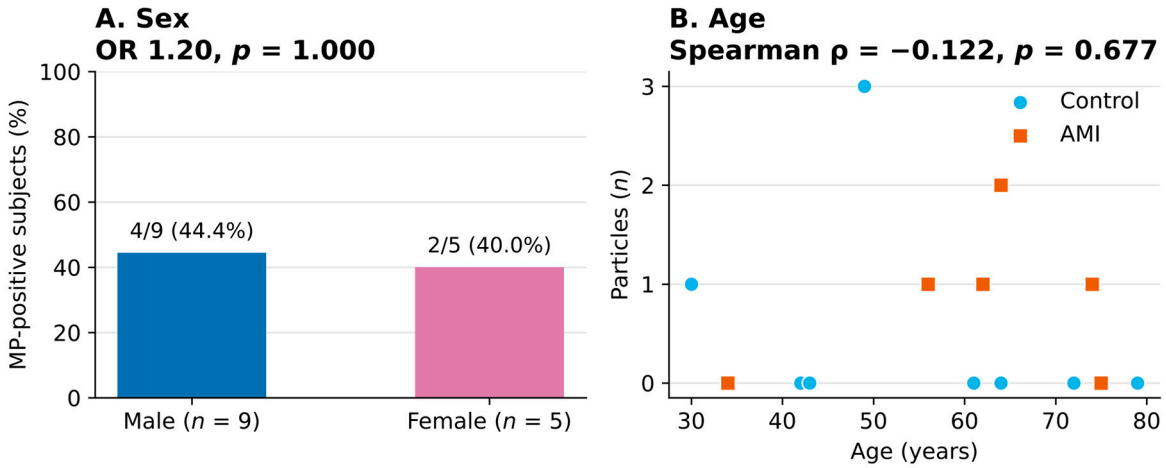


Figure S2. Associations of circulating microplastics with biological sex and age (N = 14). (A) Subject-level prevalence by sex. (B) Particle count by age, with controls and AMI patients distinguished. Neither association approaches significance.

Text S4. Exploratory Multivariate Analysis

Including clinical group and sex among the input features of a principal component analysis would make any recovery of those labels by the clustering circular, and it could not then be treated as an unsupervised finding. They are therefore excluded from the feature matrix.

The analysis uses only age, smoking status and individual polymer counts. The first two components explain 50.4% of the variance (PC1 30.6%, PC2 19.8%); PC1 loads principally on smoking status and polystyrene count. Agglomerative clustering (Ward linkage) on this feature set does not recover clinical group in either a two-cluster solution (p = 1.000) or a three-cluster solution (p = 0.352), and does not recover sex. The structure that does emerge isolates the two subjects carrying polystyrene or EVA, which restates the polymer data rather than discovering anything independently of it (Figure S3).

We report this as a negative result. With 14 subjects, six features and eight subjects carrying no particles at all, we do not consider the analysis capable of supporting phenotypic claims, and make none on its basis.

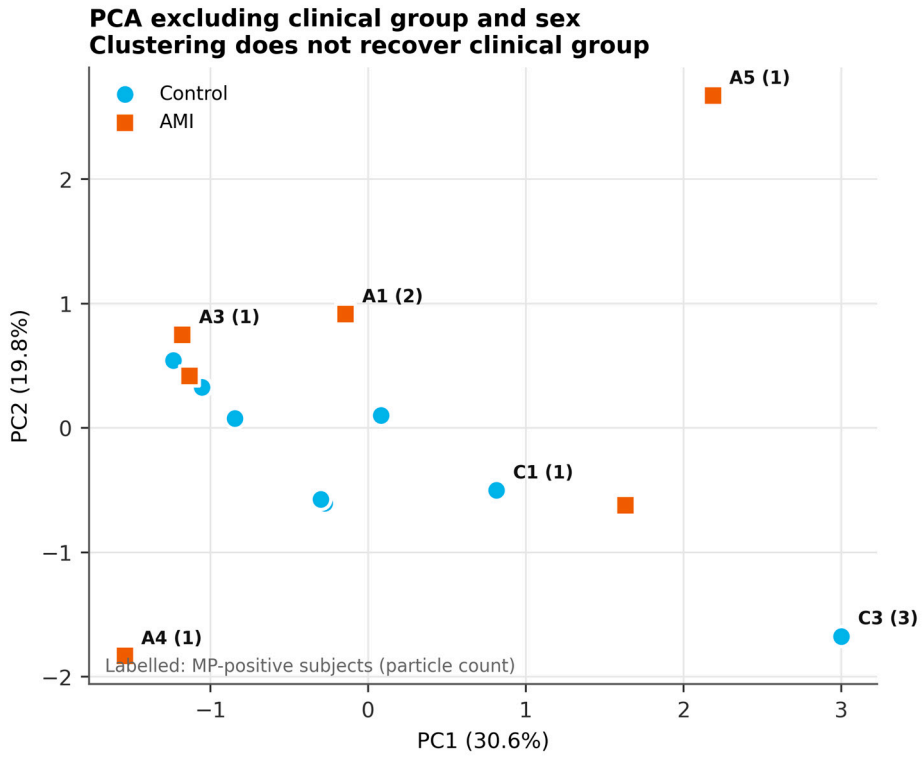


Figure S3. Principal component analysis of the cohort excluding clinical group and sex from the feature matrix. Circles, controls; squares, AMI patients. Labels indicate MP-positive subjects and their particle counts. Clinical groups are intermixed across the projection.

Text S5. Paired Pre- and Post-Procedural Samples

Two patients provided a second peripheral blood sample after percutaneous coronary intervention (Table S3). These samples are excluded from every analysis in the main manuscript, because they represent a different exposure state from the pre-procedural samples that constitute the cohort. They are reported here because they bear on how blood is sampled in this field.

Table S3. Paired pre- and post-procedural samples. In A2 the post-procedural sample yielded a particle that the pre-procedural sample did not; in A3 both samples yielded one PE particle, and one post-procedural sample contained six particles whose spectra returned no library match above threshold — the highest count of unassigned particles in the entire series.

Patient	Pre-PCI sample	Result	Post-PCI sample	Result	Unassigned particles (post)
A2 (M, 34, active smoker)	A20, 9.5 mL	No particles	D20, 9.0 mL	PE ×1 (0.96)	0
A3 (F, 74, never-smoker)	A14, 9.5 mL	PE ×1 (0.97)	D14, 9.0 mL	PE ×1 (0.93)	6

With two patients this is an observation and not a finding, and we draw no inference from it. We report it because percutaneous coronary intervention introduces catheters, guidewires, balloons, stents and contrast media directly into the arterial circulation, and because the principal clinical series in this field obtain blood through precisely those devices. Whether instrumentation contributes measurable particles to periprocedural blood is a question that a properly powered paired study could answer straightforwardly, and we consider it a priority for the field.

Text S6. Sub-Analysis Restricted to AMI Patients

For completeness we report the smoking sub-analysis restricted to the six AMI patients. Under Classification 1, prevalence was 50.0% (1/2) in active smokers and 75.0% (3/4) in never- and former smokers (OR 0.33, $p = 1.000$). Under Classification 2, prevalence was 66.7% (2/3) in ever-smokers and 66.7% (2/3) in never-smokers (OR 1.00, $p = 1.000$), with mean per-subject particle densities of 0.104 and 0.072 particles/mL respectively (Mann–Whitney $p = 1.000$).

With two to four subjects per stratum we do not consider these comparisons interpretable, and they are not carried into the Conclusions of the main manuscript.