Electronic Supplementary Material (ESI) for Analyst.

Elemental and molecular characterization of degrading blood pools

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Table S1. Band position, local coordinate, and assignment for bloodstain bulk resulting from Raman excitation at 532 nm.

Band position (cm^{-1})	Local coordinate [1,2]	Assignment [1,2]
677	$\nu(pyr\ deform)_{sym}$	ν_7
749	$\nu(pyr\ breathing)$	ν_{15}
1001	$\nu(C_\beta C_1)_{asym}$	ν_{45}
1129	$\delta(=C_\beta H_2)_4$	ν_5
1172	$\nu(pyr\ half-ring)_{asym}$	ν_{30}
1227	$\delta(C_m H)$	ν_{13} or ν_{42}
1307	$\delta(C_m H)$	ν_{21}
1334	$\nu(pyr\ half-ring)_{sym}$	ν_{41}
1434	$\nu(C_\alpha C_m)_{sym}$	ν_{20}
1588	$\nu(C_\alpha C_m)_{asym}$	ν_{37}
1640	$\nu(C_\alpha C_m)_{asym}$	ν_{10}

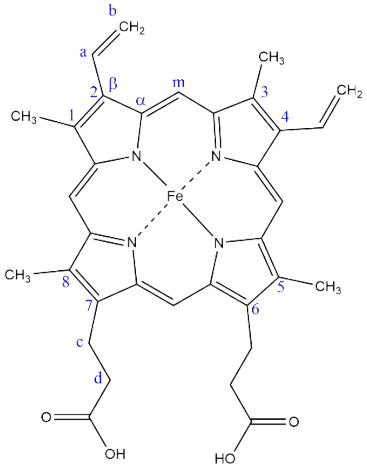

Figure S1. Molecular structure and labelling scheme of heme.[2]

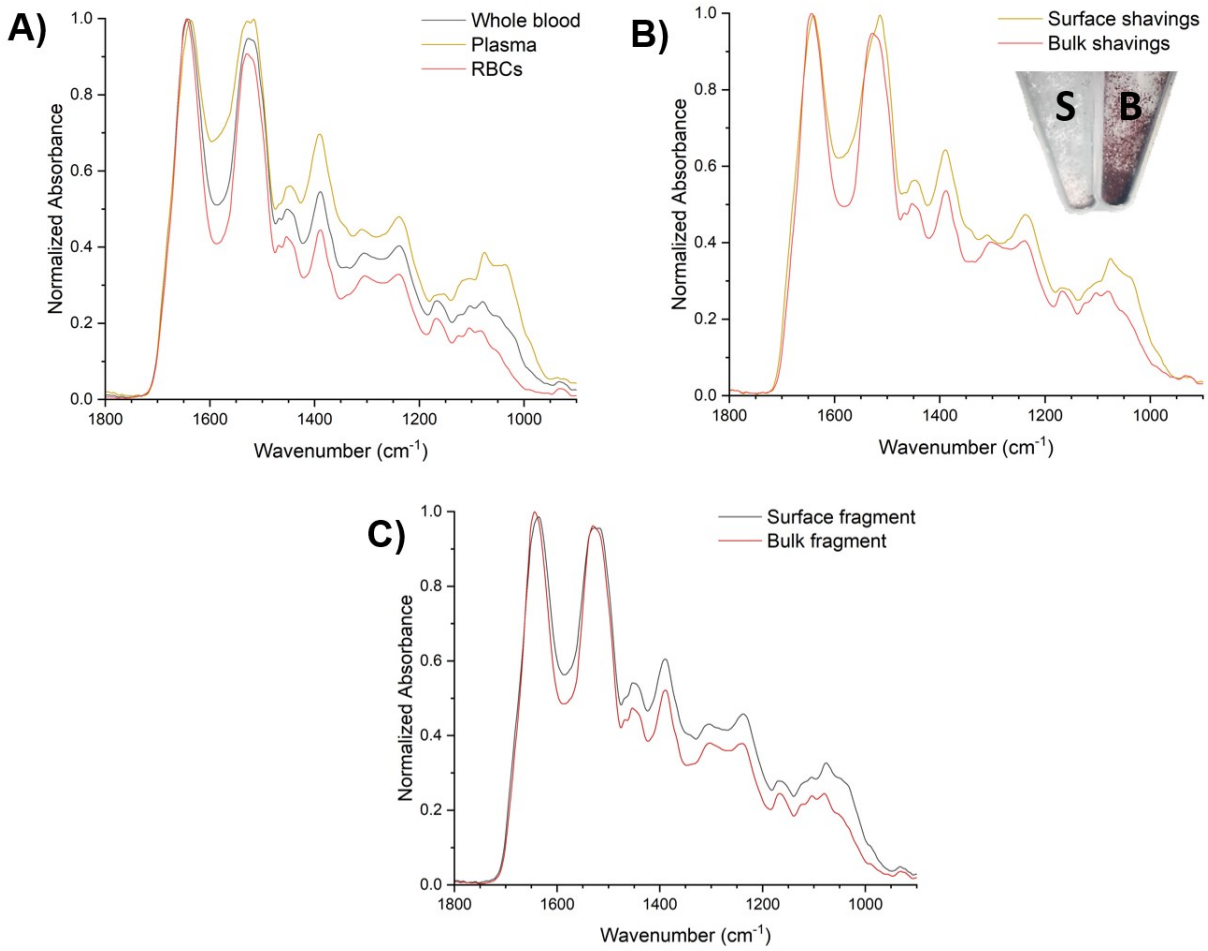

Figure S2. ATR-FTIR spectra of (A) dried and ground whole blood and blood components, (B) blood pool shavings collected from different layers of a blood pool, either the surface or bulk. Insert photo is shaved and collected layer samples of the surface (marked 'S') and the bulk ('B'), and (C) unmodified blood pool fragment ('Surface fragment') and blood pool fragment following removal of top 500 μm ('Bulk fragment').

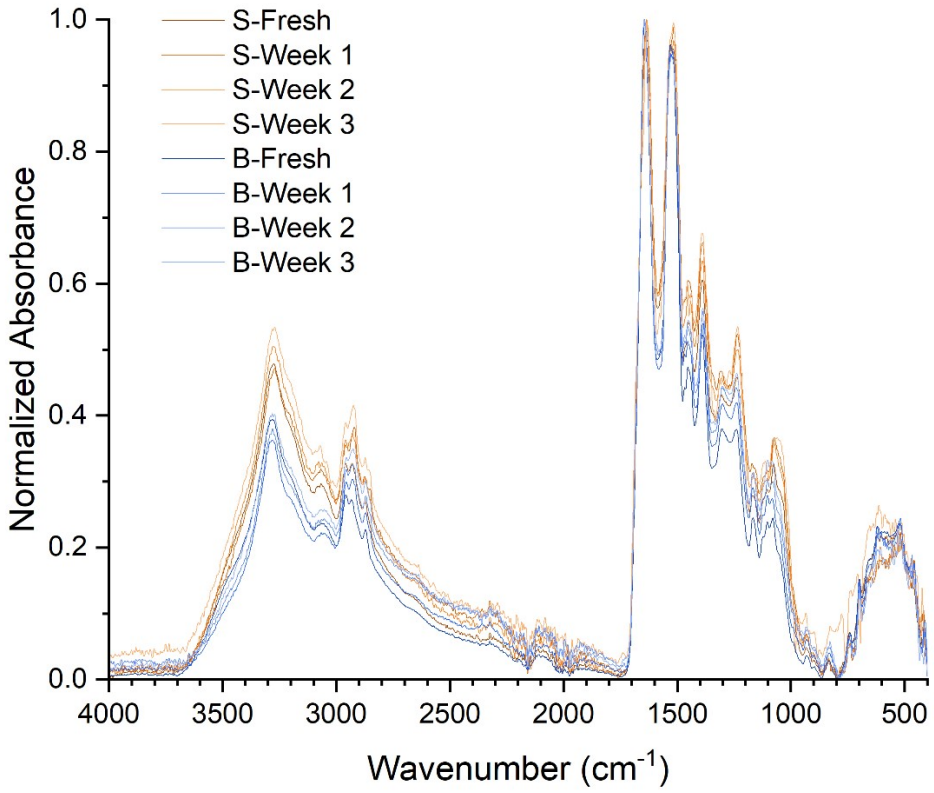

Figure S3. Overlayed averaged ATR-FTIR spectra (n=3) for blood pool surface (S) and bulk (B) fragments after being aged in an ambient environment for 18 days, then aged for 1, 2, and 3 weeks in the same environment.

Table S2. ATR-FTIR band assignments.

Frequency (cm^{-1})	**Assignment** [3–5]	**Component** [3–5]
~1080	PO_2^- symmetric stretch	nucleic acids, phospholipids
	C-O stretch	glycogen
~1103	C-O symmetric stretch	carbohydrates
~1166	C-O vibration	carbohydrates
~1239	PO_2^- asymmetric stretch	nucleic acids
~1302	Amide III: N-H bend, C-N stretch,	proteins
	O=C-N bend	proteins
~1388	C=O stretch of COO^-	fatty acids, nucleic acids
~1453	C-H bending	lipids
~1531	Amide II: N-H bend, C-N stretch	α-helices of proteins
~1640	Amide I: C=O stretch	α-helices of proteins
~2934	C-H asymmetric stretch from CH_2	lipids
~2957	C-H asymmetric stretch from CH_3	lipids
~3281	Amide A: N-H stretch	proteins
	O-H stretch	polysaccharides

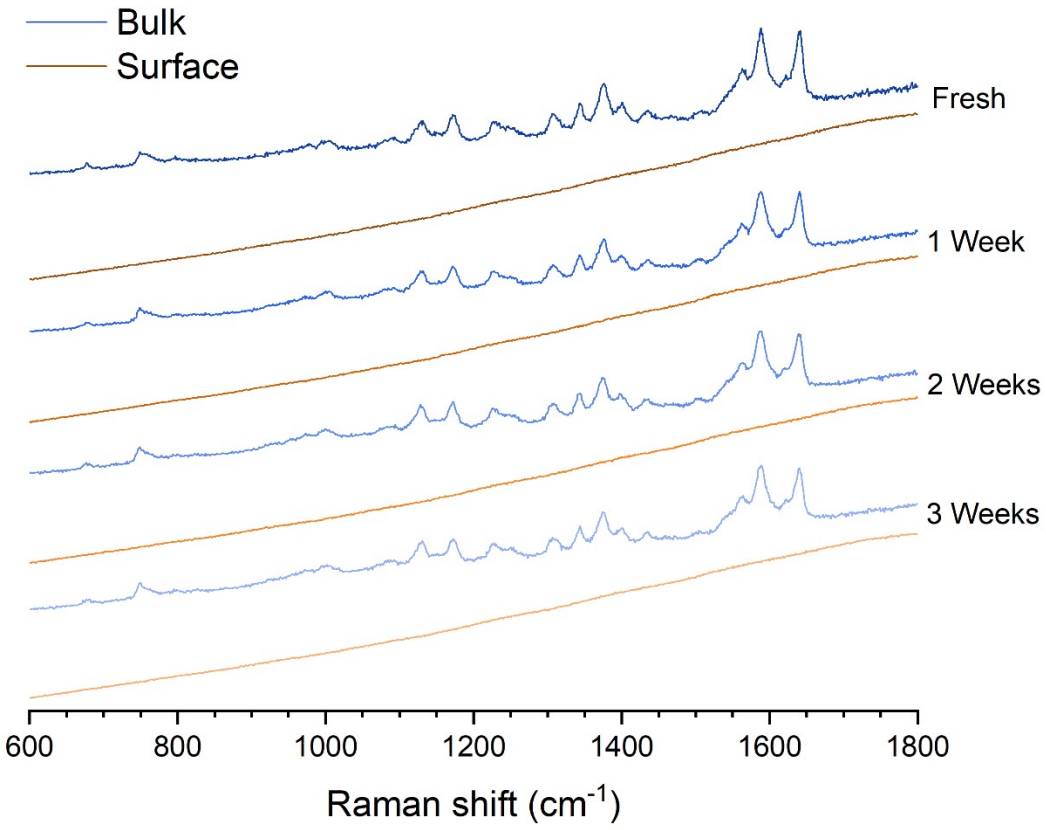

Figure S4. Averaged raw (normalized, but not baseline corrected or smoothed) Raman spectra (n=3) for blood pool surface and bulk fragments after being aged in an ambient environment for 18 days, then aged for 1, 2, and 3 weeks in the same environment.

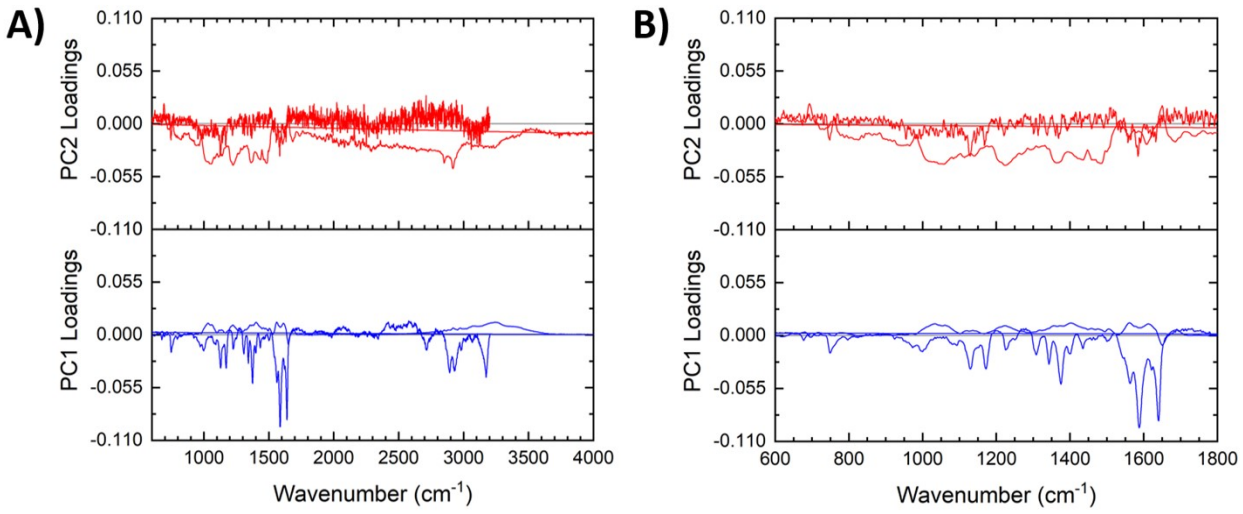

Figure S5. Loadings spectra (A) full range and (B) biofingerprint region, of PCA differentiating blood pool surface from bulk samples based on their ATR-FTIR spectra.

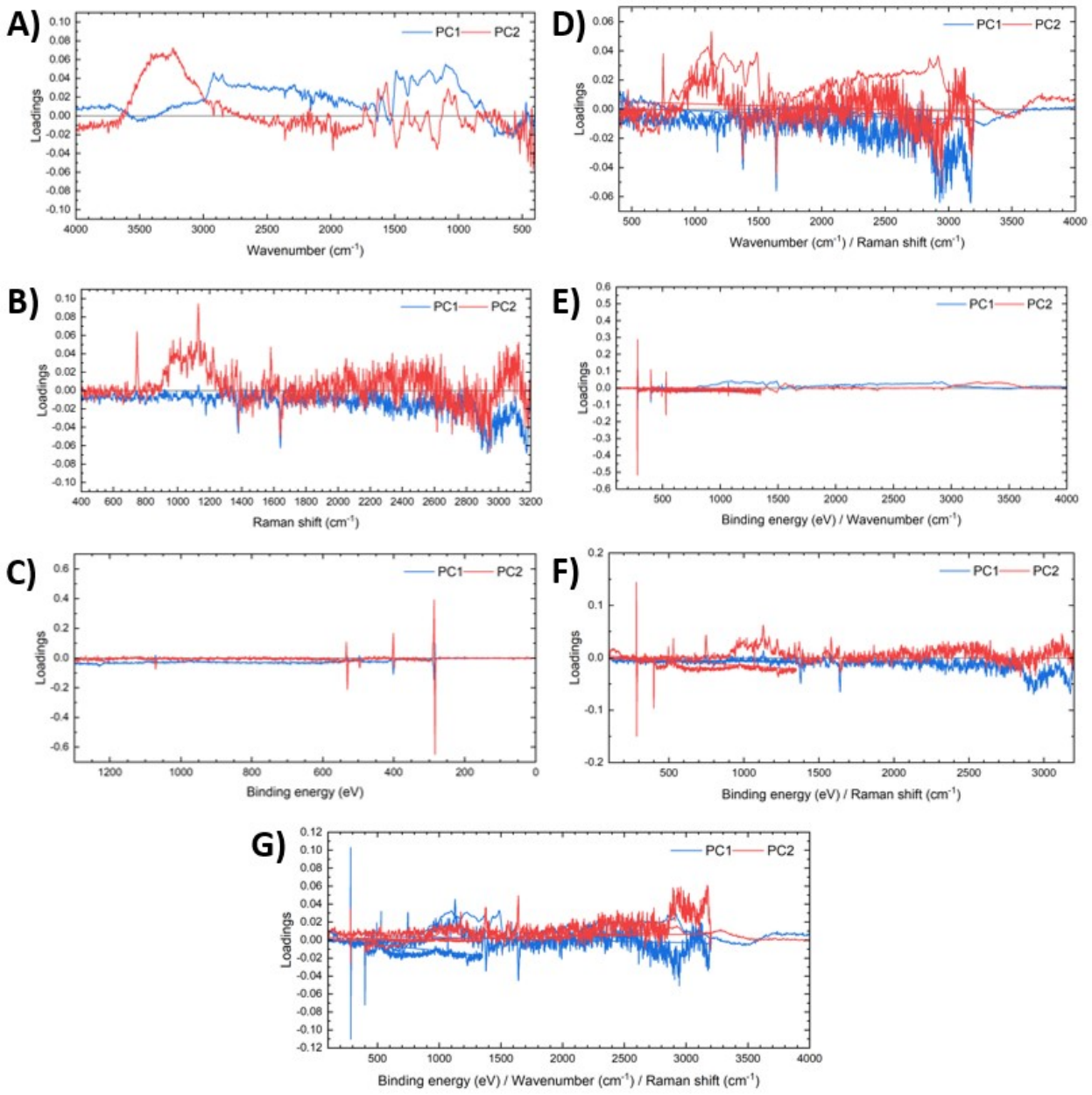

Figure S6. Loadings spectra of PCA modelling of bulk samples across measured timepoints using spectral inputs of (A) ATR-FTIR, (B) Raman, (C) XPS, (D) ATR-FTIR and Raman, (E) ATR-FTIR and XPS, (F) Raman and XPS, (G) ATR-FTIR, Raman, and XPS.

References

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