Integrated photodynamic Raman theranostic system for cancer diagnosis, treatment, and post-treatment molecular monitoring

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Supplementary Information

Figure S1 | Photosensitisers for Raman-PDT theranostics. (A-C) Chemical structures of photosensitisers investigated for Raman-PDT theranostic system; (A) Protoporphyrin IX (PPIX), (B) Verteporfin, (C) Temoporfin. (D-F) Normalised fluorescence emission spectra (ex 405 nm) of (D) PPIX, (E) Verteporfin, (F) Temoporfin. (G-I) Normalised fluorescence emission spectra (ex 785 nm) of (G) PPIX, (H) Verteporfin, (I) Temoporfin.
Figure S2 | Raw Raman spectra of photosensitizer solutions. (A-C) Raw Raman spectra of (A) PPIX, (B) Temoporfin, and (C) Verteporfin serial dilutions as compared to PBS ($n = 5$). Major peaks seen in (B) and (C) correspond to background traces of solvents used in preparation of Temoporfin and Verteporfin solutions. (D) Peak fluorescence backgrounds for photosensitizer serial dilutions (mean ± S.D., $n = 5$).
**Figure S3** | Photosensitiser cell viability assays. (A-C) Cell viability assays of MDA-MB-231 cells incubated with (A) 5-ALA, (B) Temoporfin, (C) Verteporfin. (D-F) Cell viability assays of MDA-MB-436 cells incubated with (D) 5-ALA, (E) Temoporfin, (F) Verteporfin. (mean ± S.D., N = 3, n = 6) (Error bars: mean ± STD) (Multiple comparisons t-test, Bonferroni post hoc correction, *P < 0.05, **P < 0.01, ***P < 0.001).

**Figure S4** | Raman difference spectra of photosensitiser cells. (A-C) Raman difference spectra (10 s integration time) of cells in the presence of different photosensitisers (phenol red-free DMEM (Control), 5-ALA (10000 µM), Verteporfin (100 ng/mL), or Temoporfin (10 ng/mL)), calculated as ‘PS Cell – Control Cell’ for (A) A549 cells, (B) MDA-MB-231 cells, and (C) MDA-MB-436 cells (N = 10, n = 5).

**Figure S5** | Raw Raman spectra of photosensitiser cells. (A-C) Raw Raman spectral acquisitions (10 s integration time) of (A) A549 cells, (B) MDA-MB-231 cells, and (C) MDA-MB-436 cells in the presence of different photosensitisers (phenol red-free DMEM (Control), 5-ALA (10000 µM), Verteporfin (100 ng/mL), or Temoporfin (10 ng/mL)) (N = 10, n = 5).
Figure S6 | Mean spectral coefficient of variation and signal-to-noise ratio of photosensitiser cells. (A-B) Mean spectral coefficient of variation of (A) raw and (B) processed Raman photosensitiser cell spectra. (C-D) Mean SNR of (C) raw and (D) processed Raman photosensitiser cell spectra (N = 10, n = 5) (Error bars: mean ± STD) (Two-way analysis of variance (ANOVA), Tukey’s honest significant differences (HSD) post hoc correction, * P < 0.05, ** P < 0.01, *** P < 0.001).
Figure S7 | Photosensitiser cell Raman spectra PLS-DA. (A-I) Matrix plot of (A, E, I) latent variables 1-3 for PLS-DA of processed Raman spectra performed across the three cell lines, A549, MDA-MB-231, and MDA-MB-436 (blind to the presence or absence of different photosensitisers) (N = 40, n = 5). (J-L) PLS-DA latent variables 4-6. Percentages indicate percentage variance explained by each latent variable.
Figure S8 | Confirmation of PPIX uptake in SW1222 tumours in vivo. (A) Mean raw Raman spectra of control flanks and tumours in mice pre-5-ALA induced PPIX and 4 hours post-5-ALA injection (50 mg/kg) (n = 18-20, N = 5). (B) Emission spectra of control tumours and PPIX positive tumours following re-administration of 5-ALA (50 mg/kg) with a 4-hour incubation time 6 days post PDT treatment immediately prior to tumour excision. (C) Quantification of PPIX tumour concentration for control and PPIX positive tumours.

Figure S9 | PPIX+ SW1222 tumours Raman spectra PLS-DA. (A-I) Matrix plot of (A, E, I) latent variables 1-3 for PLS-DA of processed Raman spectra for control tissue and tumour tissue pre-5-ALA induced PPIX and 4h post 5-ALA injection (50 mg/kg) (n = 18-20, N = 5). Percentages indicate percentage variance explained by each latent variable.