Electronic Supplementary Material (ESI) for Analyst.


**Supplementary Information**

# Detection of early osteogenic commitment in primary cells using Raman spectroscopy

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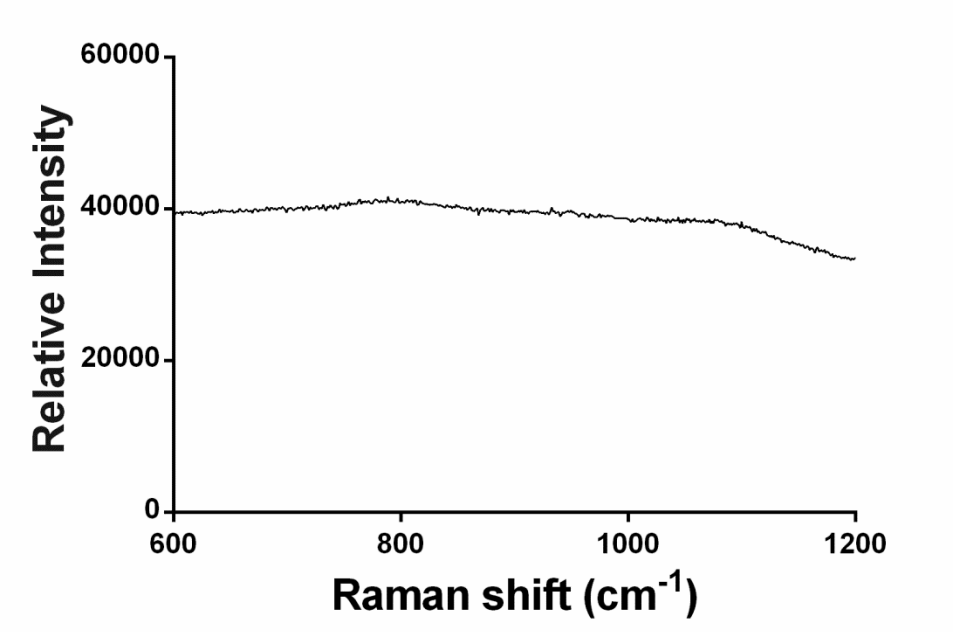


**Figure S1. Raw spectrum taken of a quartz cover slip acquired with a 532 nm laser. Only broad peaks are observed in this spectral region of interest.**

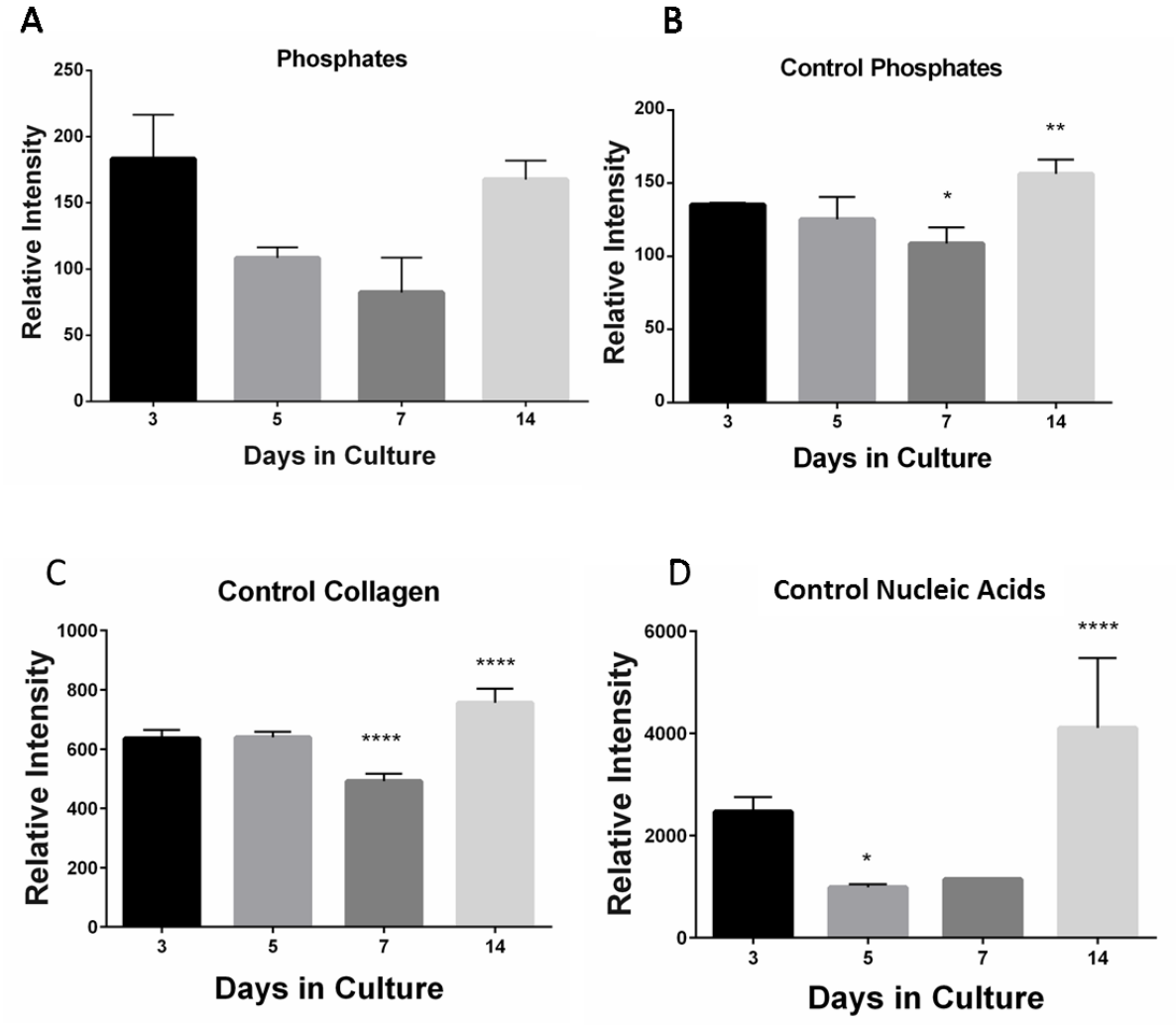


**Figure S2. Deconvolution analysis of phosphates, collagen and DNA** . (A) Deconvolution of Raman peaks from the spectral region $\nu_1 PO_4^{3-}$ between 948 and 970 cm-1 of COBs cultured in osteogenic media. Data presented as ± SEM deconvolutions ($p< 0.05$). (B) Deconvolution of the $\nu_1 PO_4^{3-}$ region of non- osteogenic COBs. Data presented as mean of deconvolutions ± SEM ($p< 0.05$ *). (C) Deconvolution of collagen matrix component 1450 $cm^{-1}$ of non- osteogenic COBs. Data presented as mean of deconvolution ± SEM ($p<0.0001$ ****). (D) Deconvolution of nucleic acid peak 782 $cm^{-1}$ of non- osteogenic COBs. Data presented as mean of deconvolution ± SEM ($p< 0.05$ *$p<0.0001$ ****)

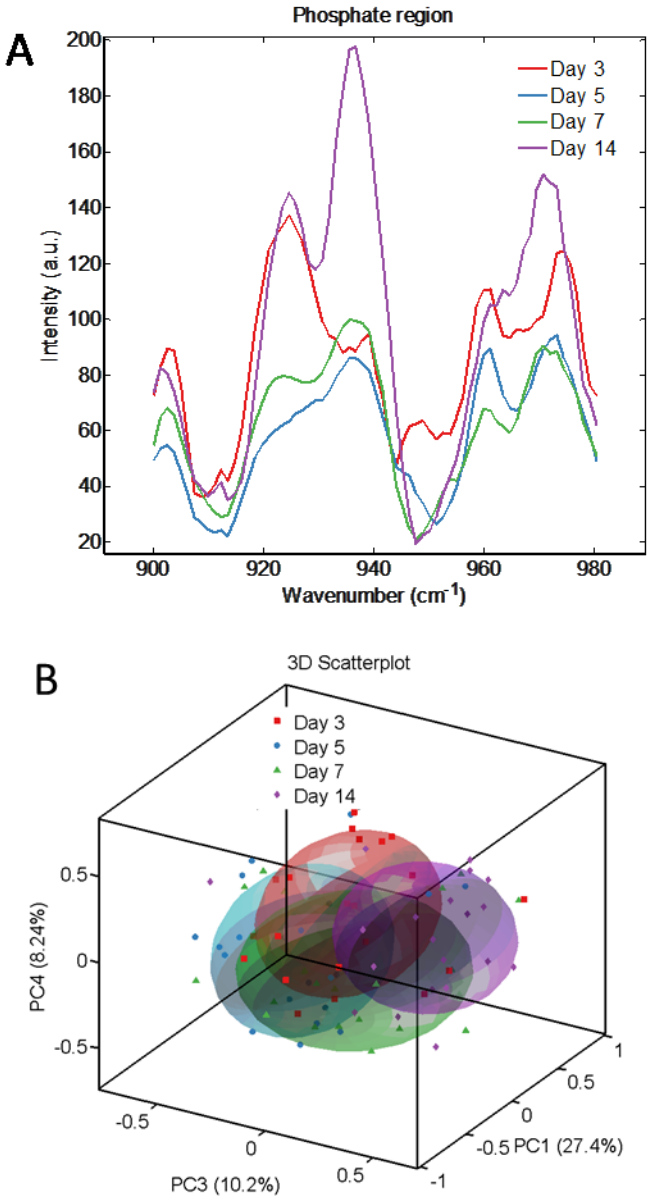


**Figure S3. (A)** Spectra of the $\nu_1$ $PO_4^{3-}$ region of COBs cultured for 3, 5, 7 and 14 days. Class mean spectra after background subtraction and wavelet denoising are presented. These are zoomed in versions of spectra presented in Figure 1 in the main text. (B) Shows PCA on the spectra for the above phosphate region alone. While there is overlap, clustering within each class and some segregation of the different time-points is seen in PC1, PC3 and PC4. PC2 showed less distinction ability than PC3 and PC4.

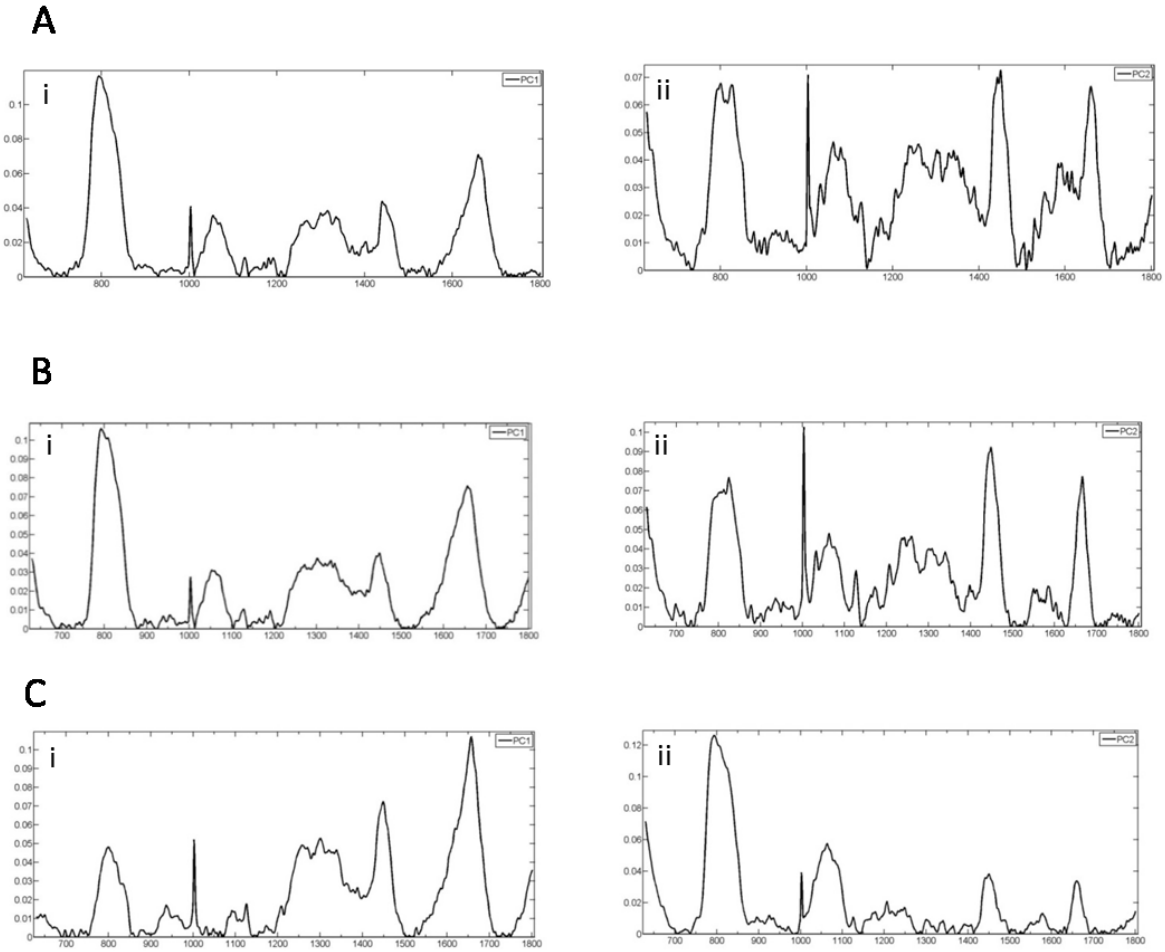


**Figure S4. PC loadings from pairwise PCA analysis.** (A) PCA output day 5 and day 3. i) The loading of PC1 indicates that the entire spectrum contributes to the group separation between these time points. ii) PC2 loadings also show variance over the whole spectrum. (B) PCA output day 7 and day 5. i) The loading of PC1 indicates that the entire spectrum contributes to the group separation between these time points. Peaks in the phosphate region (~950 $cm^{-1}$) though a small percentage, also contribute to the variance between the groups at day 7 and day 5. ii) PC2 contributes to 21.3% of the variance, here the 950 $cm^{-1}$ phosphate region contributes to a larger percentage of the overall variance. (C) PCA output day 14 and day 7. i) The loading of PC1 indicates that whilst the entire spectrum contributes to the group separation between these time points, Amide I, $CH_2$ wag and Amide III contribute more to the overall variance. ii) PC2 loadings show variance to a lesser extent from nucleic acids and carbonate region.