Electronic Supplementary Material (ESI) for Lab on a Chip.
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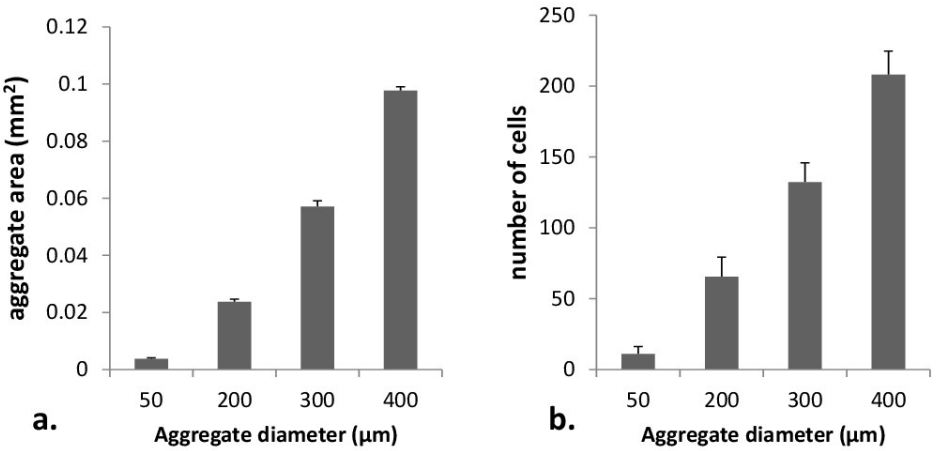
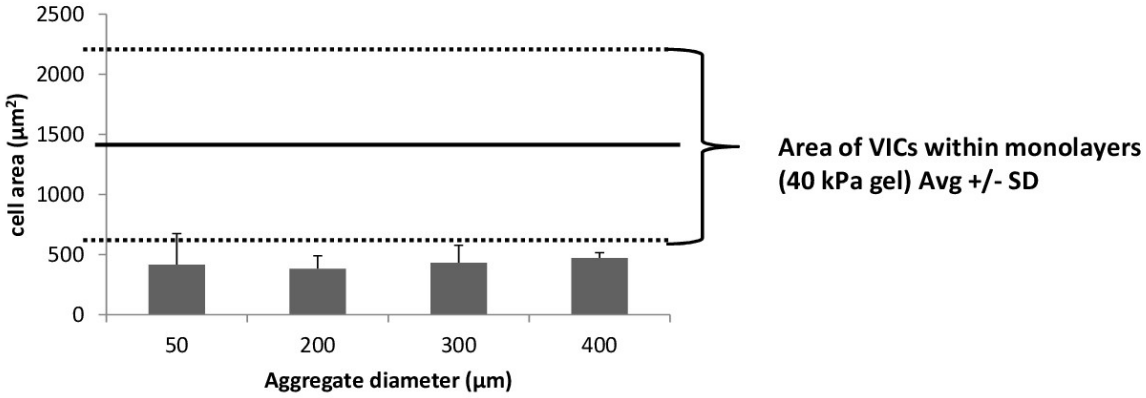
# Supporting Information for Reproducible *in vitro* model for dystrophic calcification of cardiac valvular interstitial cells: Insights into the mechanisms Calcific Aortic Valvular Disease

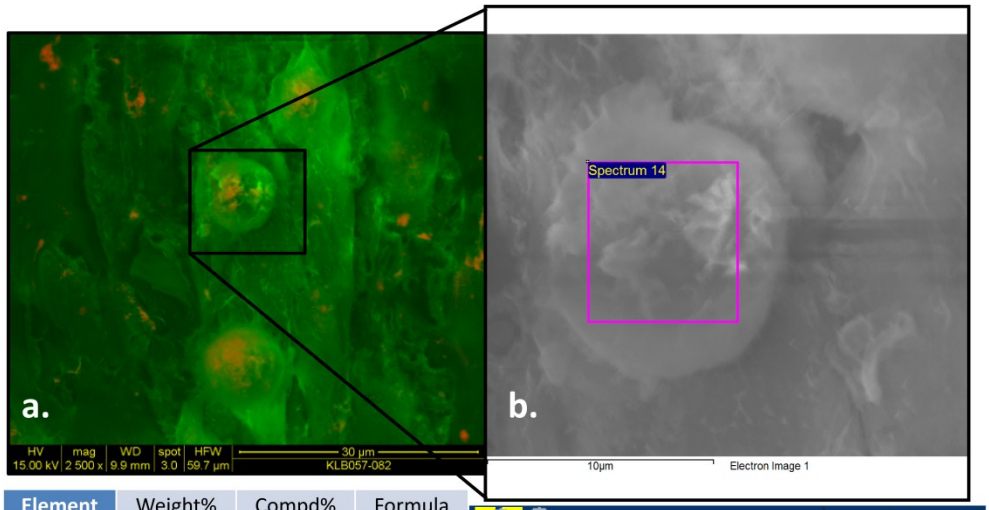



**Supplementary Figure1: Micro-contact printing method allows for tight control of aggregate size.** a) Aggregates created with micro-contact printing have < 4% variability in size. This control over sizes allows for repeatable calcification time-courses b) Number of cells contained within the aggregate increases with aggregate size.



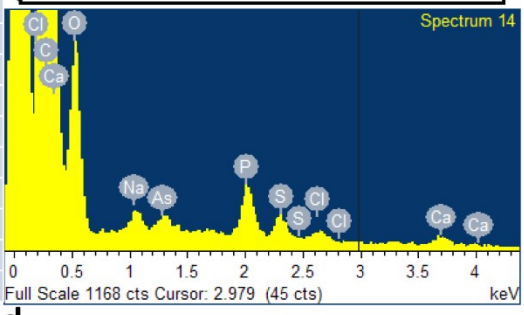

**Supplementary Figure 2: Cell area of cells within aggregates on 38.2 kPa PA gel does not change with aggregate size.** Cell area was determined by measuring aggregate area and dividing the area by the

number of nuclei within the aggregate. Cells within micro-contact printed aggregates are smaller and more dense than those in culture at confluence on TCPS.



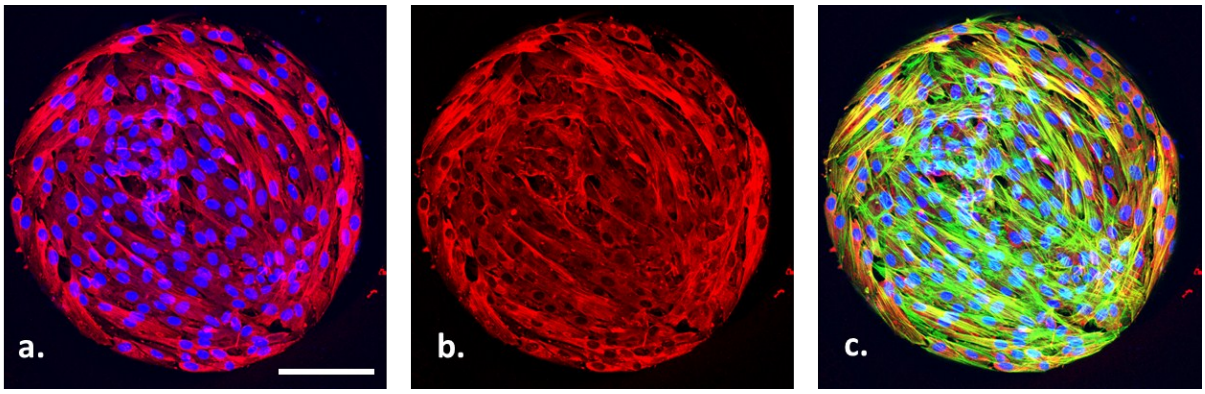

| Element | Weight% | Compd% | Formula |
|---|---|---|---|
| C | 26.62 | 97.53 | CO2 |
| Na | 0.24 | 0.32 | Na2O |
| P | 0.48 | 1.10 | P2O5 |
| S | 0.21 | 0.53 | SO3 |
| Cl | 0.10 | 0.00 | |
| Ca | 0.13 | 0.18 | CaO |
| As | 0.18 | 0.24 | As2O3 |
| O | 72.04 | | |
| Totals | 100.00 | | |

c.




d.

**Supplemental Figure 3: Rounded cells in center of aggregate contain mineralization with calcium.** a) Density dependent scanning electron image created from overlaying the electron image with the backscatter image. Regions that are denser and more likely to contain mineralization and are pseudo-colored orange. b) Electron image imported for energy dispersive spectrometry. Region of interest (ROI) selected with magenta box. c) Elemental composition of selected region. Calcium was found to be present in 0.13% in the selected ROI. d) Elemental spectrum from ROI used to calculate weight percent of the elements.




**Supplemental Figure 4: α-SMA is present within micro-contact printed valvular interstitial cell aggregates.** a) α-SMA incorporates into stress fibers, most noticeably along the periphery of micro-contact printed aggregates, indicating presence of the highly contractile myofibroblast phenotype b) nuclei (blue, hoescht)/ α-SMA (red) overlay. c) nuclei (blue, hoescht)/ α-SMA (red) /f-actin (phallodin, green) to illustrate α-SMA incorporation into stress fibers. *Scale bar = 50 μm*.