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A novel protein sampling method using styrene maleic acid is non-destructive to cardiovascular cells and tissues

**Background** Protein biomarker detection is a key tool for medical diagnostics, typically using concentration of markers or their release from tissue. We sought to establish if proteins normally retained by living cells can be extracted non-destructively, with a view to extending this to biomarker harvest. Styrene maleic acid (SMA) is a polymer that extracts nanodiscs of biological membranes (containing proteins) from cells.

**Hypothesis** SMA samples proteins directly from human cardiovascular cells without significantly impact on viability, acting as a novel 'biopsy' method.

**Methods** We applied SMA at 1.25 to 25 parts per million (ppm) in saline for 10 minutes at 37°C to human primary cardiovascular cells: cardiac fibroblasts (CFs) and vascular smooth muscle cells (VSMCs) in vitro, and rat vascular tissue ex vivo. Assays of cell membrane integrity (calcein AM) and cell/tissue viability (MTT, propidium iodide) were performed. Protein isolation in SMA 'biopsies' was confirmed by SDS-PAGE, Western blotting and mass spectrometry, with proteomic analyses to identify proteins' identities and sub-cellular locations. Statistics: ANOVA plus Tukey's test, significance:  $p < 0.05$ ; data are mean  $\pm$  SEM.

**Results** SMA at 6.25 ppm did not significantly reduce cell integrity (with cells treated by 0% SMA as 100% viability controls) in CFs (83.4  $\pm$  6.6% of control, n=8) or VSMCs (78.6  $\pm$  9.9% of control, n=8). Cell viability at 72 hours post-SMA was not reduced in CFs (104.4  $\pm$  7.8% of control, n=6) or VSMCs (102.5  $\pm$  3.4% of control, n=6). No increased cell death was seen in SMA-treated vascular tissue. An array of proteins was recovered from both cell types (CFs: 73  $\pm$  17; VSMCs: 79  $\pm$  3) and from vascular tissue, ranging in size from 20-200 kDa. Proteins were obtained from peripheral (extracellular vesicles; exosomes) and central (cytoskeleton; cytosol; organelles) cellular locations. These included cell-specific proteins (vinculin; alpha actinin 4) and heat shock proteins (A8; B1; 90AA; 90AB).

**Conclusion** We demonstrate the ability of SMA 'biopsy' to non-lethally sample an extensive range of proteins from cells and tissue, thus devising a new potential tool for protein sampling to identify new physiological and/or pathological markers, especially in advancing vascular pathology.