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Andrew J. Smith[1,2], Kathleen E. Wright[1], Stephen P. Muench[1,3], Sophie Schumann[4], Adrian Whitehouse[3,4], Karen E. Porter[5], John Colyer[1]

[1]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, United Kingdom

[2]Centre for Human & Applied Physiological Sciences and Centre for Stem Cells & Regenerative Medicine, Faculty of Life Sciences & Medicine, King's College, London, Guy's Campus, London SE1 1UL, United Kingdom

[3]The Astbury Centre for Structural Molecular Biology, Faculty of Biological Sciences, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, United Kingdom

[4]School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, United Kingdom

[5]Division of Cardiovascular & Diabetes Research, Leeds Institute of Cardiovascular & Metabolic Medicine (LICAMM), Faculty of Medicine and Health, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, United Kingdom

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℃ 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±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±6.6% of control, n=8) or VSMCs (78.6±9.9% of control, n=8). Cell viability at 72 hours post-SMA was not reduced in CFs (104.4±7.8% of control, n=6) or VSMCs (102.5±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±17; VSMCs: 79±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.