**Introduction:** Maturation and pathological adaptation of cardiac myocytes are processes associated with remodeling of mitochondrial structure and function, which can directly impact myocyte contractility due to their high energetic demands. However, the factors that underlie mitochondrial remodeling, including contributions from the chemical and/or mechanical properties of the extracellular matrix (ECM), are not fully understood. We hypothesize that the composition and elasticity of the ECM regulate mitochondrial function in cardiac myocytes.

**Methods:** Cell culture microplates were coated with gelatin hydrogels or polydimethylsiloxane (PDMS) blends of varying elastic moduli. Four formulations of gelatin hydrogels (17 kPa, 27 kPa, 58 kPa, and 72 kPa) and three of PDMS (1.6 kPa, 27 kPa, and 2.7 MPa) were used. Wells were sterilized and oxidized using a UVO cleaner. PDMS-coated microplate wells were uniformly coated with fibronectin (referred to as PDMS-fibronectin) or gelatin (referred to as PDMS-gelatin) solution. Plates were then seeded with neonatal rat ventricular myocytes. After five days, a standard mitochondrial stress test was performed using a Seahorse XFe24 Extracellular Flux Analyzer. Measured oxygen consumption rates (OCR) were normalized to total protein content. Additional plates were fixed and immunostained to quantify cell density.

**Results & Discussion:** Total protein content was equivalent for all tissues, indicating similar tissue confluence. For tissues on hydrogels, mitochondrial functional metrics were independent of elasticity (Fig. 1 A–C). For samples on PDMS, there were some variations due to elasticity and protein coating (Fig. 1 A–C). Tissues on hydrogels presented consistently higher basal respiration and ATP production OCRs than tissues on all PDMS substrates (Fig. 1A–B). Lastly, spare respiratory capacity was mostly preserved irrespective of biomaterial or elasticity (Fig. 1C).

**Conclusions:** Our data suggest that gelatin hydrogels enhance baseline mitochondrial function compared to matrix-coated PDMS, independent of protein ligand or elasticity. These data indicate that the physical and chemical properties of the ECM might have a critical impact on metabolism in cardiac myocytes. These data also emphasize that the ECM must be carefully designed when engineering physiologically-relevant microphysiological systems due to its many effects on myocyte phenotype, including metabolism.

**Figure 1:** Regulation of Mitochondrial Function by ECM in Cardiac Myocytes. Average oxygen consumption rates (OCRs) associated with basal respiration (A), ATP production (B), and spare respiratory capacity (C). Whiskers extend to the most extreme points not considered outliers, and outliers are represented by crosses. n=16 for all conditions. Letters above each box indicate a statistical difference (p<0.05) with the condition represented by the same letter on the x-axis. For example, “a” indicates p<0.05 compared to gelatin hydrogel, 17 kPa.