Motor neurons control muscle movement for speech, walking, breathing and swallowing. Approximately 1 in 400 people suffer debilitating motor neuron disease during their lifetime, with reduced life expectancy to 2.5 years. Understanding how motor neurons develop into circuits is critical to design cell therapies to treat motor neuron disease. Our objective was to establish a culture system that allows study of mechanical and material cues on motor neuron development, including an option for real-time imaging of circuit formation. We developed a system that uses surface acoustic waves (SAW) to dynamically control pressure applied to embryonic stem cells (ESC) during differentiation into motor neuron-like (MN) cells and formation of multicellular networks resembling neural circuits. SAW affect cell migration in other cell types, but their impact on stem cells is unknown. We first overcame technical hurdles of combining SAW piezoelectric crystals with biological assays, including adhesion of fragile ESC to SAW chips and birefringence interference with microscopy measurements. Cells can now be patterned with a variety of SAW profiles (producing lines, grids, or traveling waves), substrate stiffness, and topologies. ESC can be grown on SAW chips throughout differentiation into MN cells, or after they have been pre-differentiated. Applied SAW enabled greater physical separation between cells, which resulted in easier visualization of single cell behavior during circuit formation. Ongoing studies are measuring the effect of SAW-induced pressure waves on cell signaling and gene expression. In future, this system can be used to study how mechanical forces and material cues affect motor neuron development.