**Background:** For radial extracorporeal shock waves (rESWs) conflicting reports exist in the literature between studies demonstrating the successful treatment of human nonunions and successful induction of new bone formation *in vivo* and its proposed inefficacy in osteoblast cell cultures *in vitro*.

**Hypothesis:** Matrix-embedded osteocytes but not only superficial bone-lining osteoblasts orchestrate bone turnover. Expression of bone remodeling signals in 3D osteocyte cell cultures *in vitro* can be mechanically induced by rESWs.

**Methods:** Murine long bone osteocyte-like Y4 cells (MLO-Y4) were embedded within 3D collagen matrix and exposed to different rESWs. MLO-Y4 cells were sampled after rESW exposure, and analyzed for survival, apoptosis, proliferation and gene expression patterns of positive (*BMP7*, *OPG*) and negative regulators of bone mass (*RANKL*, *Sclerostin*).

**Results:** Cell functions and gene expression changed at all rESW exposure protocols and time points. The highest ratios of *RANKL/OPG* indicative for cellular signaling towards induction of osteoclastogenesis were found seven days after exposure to rESWs at 1 bar and 2 bar.

**Conclusion:** Radial ESWs induce bone remodeling signals in MLO-Y4 osteocyte-like cells. This preliminary study provides the first molecular *in vitro* evidence for the clinically observed success of rESW therapy in treating bone related diseases such as pseudoarthrosis.