Tissue grafts are the gold standard for replacing large volume tissue defects. Yet, they present several risks, including infection, low functional outcomes, and reduced graft integrity. Tissue engineering (TE) combines cells and biomaterial scaffolds to foster tissue growth and remodeling. Bone marrow stromal cells (BMSCs) have been shown to respond to the stiffness of their microenvironment, resulting in differentiation into different lineages. 3D porous chitosan–alginate (CA) scaffolds have been previously demonstrated for bone TE with osteoblasts and BMSCs; however, only a single scaffold composition (4 wt%) was studied. Three CA scaffold compositions (2, 4, 6 wt% CA) were produced. Scanning electron microscopy images were obtained to determine average pore sizes for 2, 4, and 6 wt% CA scaffolds, which were 250, 210, and 220 µm. Compression testing was performed on CA scaffolds in dry and wet conditions, where higher concentrations yielded higher stiffnesses ranging from 0.22 to 5.34 kPa and 20 to 40 Pa, respectively. Fourier transform infrared spectroscopy performed on the CA scaffolds confirmed polyelectrolyte complex formation for all compositions. Human BMSCs from three donors were seeded on CA scaffolds, cultured in growth media for 14 days, then cultured in adipogenic or osteogenic differentiation media for 28 days to promote differentiation. Our hypothesis was that scaffold stiffness would influence BMSC differentiation, with softer scaffolds promoting adipogenesis and stiffer scaffolds promoting osteogenesis. BMSCs formed multicellular spheroids in all CA scaffold concentrations, while the 2 wt% CA scaffolds had smaller spheroids compared to the 4 wt% and 6 wt% CA scaffolds. Osteogenic and adipogenic differentiation were evaluated with Alizarin Red and Oil Red O staining, respectively. While positive staining was observed in all scaffold compositions, more robust differentiation was expected, thereby disproving our hypothesis. The polysaccharide composition of the CA scaffolds likely contributed to the spheroid formation and limited differentiation.