Abstract

Bone is a highly dynamic organ that undergoes remodeling equally regulated by osteoblast-mediated bone formation and osteoclast-mediated bone resorption. To clarify the regulation of osteoblastogenesis, primary murine osteoblasts are required for an *in vitro* study. Primary osteoblasts are isolated from neonatal calvariae through digestion with collagenase. However, the number of cells collected from one pup is not sufficient for further *in vitro* experiments, leading to an increase in the use of euthanized pups. We hypothesized that the viscosity of digested calvariae and digestion solution supplemented with collagenase results in cell clumping and reduction of isolated cells from bones. We simply added Benzonase, a genetically engineered endonuclease that shears all forms of DNAs/RNAs, in order to reduce nucleic acid-mediated viscosity. We found that addition of Benzonase increased the number of collected osteoblasts by three fold compared to that without Benzonase through reduction of viscosity. Additionally, Benzonase has no effect on cellular identity and function. The new osteoblast isolation protocol with Benzonase minimizes the number of neonatal pups required for an *in vitro* study and expands the concept that isolation of other populations of cells including osteocytes that are difficult to be purified could be modified by Benzonase.