Development of In Vitro 3D Culture of Human Tonsil-Derived Mesenchymal Stem Cells (T-MSC) Using Hydrogel for Parathyroid Tissue Regeneration
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Purpose
Parathyroid hormone (PTH) is the major hormone performing calcium homeostasis in the body. Loss of parathyroid gland function, hypoparathyroidism, frequently occurs during thyroid surgery, and therefore the reconstruction of a biocompatible parathyroid gland is a compelling means to restore parathyroid function. Based on our previous results showing PTH gene expression in differentiated tonsillar mesenchymal stem cells (T-MSC), here, we investigate whether biopolymer-based hydrogel further enhances a bioactive PTH protein secretion by these cells.

Methods
Using activin A (100 ng/mL) and soluble sonic hedge hoc (100 ng/mL) for 21 days, we first conducted the differentiation of T-MSC into parathyroid-like cells secreting PTH. Secreted PTH concentration was measured by an ELISA. Differentiation into parathyroid-like cells was assessed by in situ immunofluorescence microscopy, Western blot analysis, and ELISA. Osteocalcin protein expression and mineralization, as indices of osteogenic conductivity, were measured in MC3T3-E1 preosteoblast cells by Western blot analysis and Alizarin Red S staining, respectively. Second, T-MSC with or without differentiation, were incorporated into hydrogel. Survival of T-MSC loaded in hydrogel was assessed using Live/dead staining method.

Results
Differentiated T-MSC secreted PTH into conditioned medium, which was regulated by extracellular calcium levels; the lower calcium induced PTH secretion. In situ immunofluorescence microscopy revealed colocalization of PTH and chromagranin A, a marker protein for secretory vesicles. Furthermore, treatment of MC3T3-E1 preosteoblast cells with secreted PTH clearly increased the osteocalcin protein expression and induced mineralization, suggesting a role of osteogenic activity. While the morphology of loaded T-MSC in 3D hydrogel culture was different from that in normal 2D culture, similar survival was observed in cells from two culture systems.

Conclusion
To our knowledge, this is the first report showing the feasibility of development of in vitro 3D culture of parathyroid-like cells releasing a functional PTH from T-MSC. This 3D culture further enhances our understanding of in vivo cell-based therapies, especially for restoring parathyroid function.