Examination of Mineralized Nodules Formed in Dental Pulp Cell Cultures Isolated from Rat Incisor

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Abstract: In order to develop vital pulp therapy, it is important to establish an in vitro experimental system reflecting the odontoblast differentiation process containing terminal differentiation, i.e., mineralized dentin formation. Previously, we had established an in vitro culture system using dental pulp cells enzymatically isolated from rat lower incisors. This culture system can demonstrate characters of differentiated odontoblast-like cells forming mineralized dentin-like matrix. However, much about the mechanism of dentinogenesis remains to be researched. The purpose of this study was to analyze the characters of mineralized nodules formed in the cell layer of dental pulp cells, and to determine whether or not this culture system can be a useful tool to examine dentinogenesis. During the 20-day culture period, the cells began to form mineralized nodules from day 10, the nodules became larger by day 20, and the nodules surrounded the cells expressing alkaline phosphatase (ALP). ALP activity in the cell layers was maximum on day 15, and gradually decreased on day 20. The calcium content in the layers was low by day 10, and significantly increased from day 15. Real-time PCR demonstrated that the expression of bone gla protein (BGP) was first determined on day 15 and drastically increased on day 20, and that dentin sialo protein (DSPP) gradually increased to day 15, and remarkably decreased on day 20. Immnohistochemistry was performed in the sections prepared from the cell layers embedded in paraffin, and antibodies used in the stainings were anti insulin-like growth factor (IGF)-1, anti bone morphogenetic protein (BMP)-4, anti DSPP, anti BGP, and anti osteopontin (OPN). The matrices of mineralized nodules were strongly stained with all antibodies. These results demonstrate that this culture system is useful for studying the odontoblast differentiation process.

Key words: Dental pulp cells, In vitro, Mineralized nodules, Rat