Autotransplantation of teeth, with a long history and some influential advantages such as maintaining bone volume, PDL regeneration ability and allowing dentofacial development, has always been a treatment modality for substituting missing teeth. Although in some situations that immediate transplantation could not be attained, autotransplantation with the use of tooth bank can add a new dimension to treatment planning. With the establishment of a newly-developed freezer using a magnetic field called “Cells Alive System” (CAS), cell damage caused by intracellular ice formation could be prevented. This is due to the magnetic field vibration function which prohibits water molecules to create clusters during freezing process. It is generally known that appropriate PDL regeneration after transplantation can directly affect on the enhancement of transplantation success rate and prognosis improvement. Meanwhile, revascularization capability of transplanted teeth with incomplete root formation, which is related to pulp viability, is of an outmost importance.

Thus, this study was performed with the main purposes of (1) examining the effects of long-term cryopreservation using magnetic field freezer on human periodontal ligament cells, (2) evaluating the influences of cryopreservation on pulp tissues of teeth with immature and mature roots and (3) examining clinical cases applying teeth bank.

In the first part of study, ten freshly extracted teeth were selected and divided into two groups with five in each. In the cryopreserved group, the teeth were frozen in 10% dimethyl sulfoxide for 5 years using a programmed freezer combined with a magnetic field. As for the control group, freshly extracted teeth were used. In each group, extracted PDL tissues were cultured and PDL cells phenotype and proliferation rate were recorded 15 and 25 days after initiation of cell culture in each group. Furthermore, gene expression and protein concentration of type I collagen, alkaline-phosphatase (ALP) and vascular endothelial growth factor (VEGF) were compared between the two groups. Next, freshly extracted teeth, one-year cryopreserved teeth with CAS, one-year cryopreserved with conventional freezer and 3-day dried teeth were used for observing the PDL structure under optical and transmission electron microscopes.

In the second part, pulp tissues were obtained from ten mature and immature third molars which were freshly extracted or cryopreserved for three months with 10% dimethyl sulfoxide as the cryoprotectant. Pulp tissues were cultured in α-MEM with required supplements. The first appearance of cells out of pulp tissue, phenotype of the cells, cell proliferation and the confluent date were all recorded for the four groups. For comparing the characteristics of pulp cells, expression of VEGF and nerve growth factor (NGF) mRNAs and the protein concentration in the supernatant were investigated. Finally, for confirming the revascularization capability of cryopreserved transplanted tooth with immature root formation, a relevant case is presented.
In the third part, current status of tooth bank, Hiroshima University and two patients with transplantation of cryopreserved teeth in tooth bank are reported.

The following results were obtained.

1. Phenotype of PDL cells after cryopreservation presented no alteration in all the plates. Moreover, quantitative measurement of cell proliferation in the cryopreserved group on days 15 and 25 was not significantly different from the control group. The expressions of type I collagen, ALP and VEGF mRNAs derived from PDL cells of non-cryopreserved and cryopreserved teeth exhibited no significant differences. These results were confirmed by ELISA assay in which concentration of above-mentioned proteins showed no significant difference between groups. Histological examination and the transmission electron microscopic image of cryopreserved tooth with a magnetic field did not show any destruction of cryopreserved PDL cells. In contrast, severe PDL tissue damage was seen in teeth frozen without a magnetic field and dried for three days. These results indicated that long-term cryopreservation with the use of CAS freezer cannot affect the growth rate, characteristics and structure of PDL cells.

2. Pulp cells in cryopreserved teeth with mature root formation could not survive due to poor penetration of cryoprotectant from apex. However, pulp cells of both non-cryopreserved and cryopreserved teeth with immature root formation could survive without any significant alteration in the phenotype and proliferation rate. The expression of VEGF mRNA was the highest in the immature control group among all the groups and an identical result was observed in NGF expression as well. No significant difference in the VEGF expression was found between the mature control group and the immature cryopreserved group. VEGF and NGF protein concentration presented similar results to those of gene expression without any significant differences in VEGF and NGF protein concentration between control groups with mature roots and cryopreserved groups with immature ones. Finally, proper PDL regeneration and appropriate apexogenesis after transplanting magnetically cryopreserved teeth with immature roots were clinically confirmed. Furthermore, it was discovered that teeth with incomplete root formation can exploit newly-developed magnetic freezer for eliminating the need for root canal treatment.

3. In 112 patients, who have already received their cryopreserved teeth for autotransplantation, 7 teeth have been removed and the remaining 105 teeth have been survived in the 5-year follow up. Ninety-four% survival rate via 6% failure rate for transplanted cryopreserved teeth applying CAS illustrated that cryopreserving extracted teeth can help the patient for future feasible transplantation. Clinical case reports have demonstrated that soft tissue and PDL healing, bone regeneration and functional recovering can be achieved after transplanting cryopreserved tooth.

These results have demonstrated that tooth banking with the use of magnetic field programmed freezer can be available for future autotransplantation as a treatment modality for replacing missing teeth.