# Recent Progress in Translation from Bench to a Pilot Clinical Study on Total Pulp Regeneration

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## Abstract

Based on a preclinical bench study in dogs, a pilot clinical study was completed. Dental pulp stem cell (DPSC) subsets were isolated by mobilization by granulocyte colony-stimulating factor and expanded in good manufacturing practice conditions. The safety and efficacy of their autologous transplantation for total pulp regeneration was assessed in 5 patients with irreversible pulpitis. The quality control of the DPSC subsets was ensured by the absence of contamination and karyotype aberrations, and positive expression of stem cell markers. The clinical safety assessment was based on laboratory and radiographic evaluations, demonstrating no evidence of toxicity and adverse events. The efficacy was determined by the recovery of a sound positive response to the electric pulp test within 4 weeks and by the robust signal intensity of magnetic resonance imaging in the root canal at 24 weeks. The functional recovery of pulp tissue was determined by lateral mineralized tissue formation detected by cone beam computed tomography. This review presents a summary of the accumulating data in translation from bench to a pilot clinical study, demonstrating potential clinical utility of DPSC subsets for total pulp regeneration in endodontics. *(J Endod 2017;■:1–5)*

## Key Words

Clinical study, dental pulp stem cells (DPSCs), efficacy, granulocyte colony-stimulating factor (G-CSF), pulpectomy, pulp regeneration, safety

**Significance**

The safety and efficacy of human dental pulp stem cells for complete pulp regeneration was assessed in a pilot clinical study. This review presents a summary of the recent progress in translation from bench to a pilot clinical study. The possibility of regeneration of the dentin-pulp complex in the endodontic clinic is becoming a reality for patients to maintain tooth function in the aged.

The regenerative therapy for dentin/pulp is a promising approach for endodontic treatment to preserve tooth function, and to prevent secondary caries and tooth fracture. Furthermore, it may contribute to the functional survival and endurance of the tooth, leading to good health and longevity. We have demonstrated that mesenchymal stem/stromal cells (MSCs) have therapeutic potential beyond their differentiation potential into committed cell types, through secretome, which promotes endogenous cell migration, antiapoptosis, neovascularization, anti-inflammation, and immunomodulation (1–4). MSC secretome includes soluble factors and factors released in extracellular vesicles (exosomes and microvesicles) (5, 6). The better understanding of regenerative mechanism by the MSC secretome and paracrine activity will make regenerative therapies more effective for millions of patients suffering from devastating diseases (7). We have developed a protocol for autologous transplantation of dental pulp stem cell (DPSC) subsets into pulpectomized teeth for pulp regeneration. We present here the manufacturing process of the clinical-grade human DPSC subsets in accordance with good manufacturing practices (GMPs) and demonstrate evaluation of the safety and quality control evaluation of the cell products for clinical study (8). Furthermore, a clinical study protocol was prepared in accordance with the principles of the Declaration of Helsinki and the Japanese guidelines of human stem cell clinical research. Then, after approval by the institutional review boards for the human stem cell clinical research both at the National Center for Geriatrics and Gerontology and Aichi Gakuin University School of Dentistry, we conducted a pilot clinical study of pulp regeneration (8) based on the preclinical experimental results (9). Thus, a summary of the pilot clinical study results is reported in this article.

## Manufacturing Protocol of Human DPSC Subsets

Clinical-grade human mesenchymal stem cells should be manufactured in accordance with the standard of the manufacturing management and the quality control of pharmaceutical products (GMPs). The safety, efficacy, and reproducibility of cell products and compliance with GMPs must be ensured by reagents and particularly media and serum used, donor eligibility and screening, facilities, environmental controls, and storage (10). An open system for cell processing is usually necessary for use of a class A (Food and Drug Administration class 100 environment) cabinet set up in a class B (Food and Drug Administration class 10,000) room. The environment must

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be quantified and regulated to achieve and maintain the level (10). Patients between ages 20 and 55 years having a tooth with irreversible pulpitis were enrolled in the clinical study and having simultaneously another tooth indicated for extraction. Patients were excluded if there was evidence of infection with virus, bacteria, fungi, or mycoplasma, or severe cardiovascular disease, diabetes, osteoporosis, pregnancy, mental disease, and/or allergy to atelocollagen. In the cell processing, the freshly extracted autologous teeth were transported to a GMP-compliant facility under strict control of temperature at 0 to 10°C. DPSC subsets were isolated and expanded according to a standard operating procedure under strict GMP conditions in a totally enclosed system of the Isolator with $H_2O_2$ vapor decontamination system (Panasonic Healthcare Co. Ltd., Tokyo, Japan) for aseptic condition. The Isolator has an important advantage over a cabinet in maintenance of the class A level located in a class C (class 100,000) room and in taking less time, less than 2 hours for change over. The Isolator also can prevent crossover pollution and save running cost compared with a cabinet. Every manufacturing process is recorded for quality control by computerized process management system of the Isolator, preventing and minimizing human error. The Isolator guarantees the quality control with validation. After enzymatic dissociation of pulp tissue, primary pulp cells were plated at low cell density to make colonies. Primary MSCs can be seeded after MSC enrichment with immunomagnetic devices or fluorescence-activated cell sorting with known MSC-expressed antigens. These enrichment methods, however, are not suitable for obtaining clinical-grade DPSC subsets due to lack of known DPSC-expressed antigens, including STRO-1, C-kit/CD117, CD34, CD51, CD140$\alpha$, CD146, CD271, and CD105 (11–16) for clinical use. Thus, we have further enriched DPSC subsets with high regenerative potential from the colony-derived DPSCs, using granulocyte colony-stimulating factor (G-CSF)–induced stem cell mobilization method (2), and they are termed mobilized DPSCs.

DPSCs contain several trophic factors in a releasable pool that enhance the regeneration process. For successful translation of the use of DPSCs from bench side to bedside, cell production before transplantation needs optimal supplements to maintain their genetic stability and stemness and to enhance engraftment and regenerative potential. Widely used fetal bovine serum (FBS) for research provides almost all the necessary nutrients for the survival and proliferation of cells. FBS may transmit unexpected or emerging diseases, including prions and pathogenic viruses (10). In addition, human MSCs cultured with FBS express N-glycolylneuraminic acid xenoantigen, which might cause acute xenogeneic rejection of the MSCs after transplantation (17, 18). Thus, serum-free and xeno-free media are alternatives to serum containing media, and human platelet-derived growth factor–BB, basic fibroblast growth factor, and transforming growth factor–$\beta$1 are needed as supplements (19). Limited use for isolation and primary culture (20) are considered as drawbacks of serum-free and xeno-free media. However, an optimal phenotype and multidifferentiation potential have been reported (21–23). Serum-free and xeno-free media appear to promote early senescence, telomere shortening, and loss of chemokine receptors that are required for migration, engraftment, and long-term regenerative potential (20). Currently, human serum, plasma, and/or platelet lysates are alternatives for FBS in a xeno-free cell expansion protocol. Platelet lysate is obtained from platelet-rich plasma, and is enriched by growth factors, including platelet-derived growth factors, basic fibroblast growth factor, transforming growth factor-beta, and insulin-like growth factor-1. Platelet lysate can promote MSC expansion and maintain their multidifferentiation potential and retain their immunosuppressive activity (24). It is important, however, to produce clinical-grade MSC platelets free of infectious agents to prevent potential disease transmission. Autologous human serum also has stimulated the proliferation (24, 25) and maintenance of multidifferentiation potential (26–28) and immunomodulation of MSCs (29). Furthermore, MSCs cultured in autologous human serum demonstrated stable gene expression and higher migration and regenerative potential compared with MSCs cultured in FBS. Human serum may stimulate chemokine receptors mediated by microvesicles (20). Allogeneic human serum and human cord blood serum have been considered as a suitable substitute for FBS (30–32). However, allogeneic human serum caused growth arrest and cell death in MSCs during expansion (28). Therefore, autologous serum was isolated from freshly collected blood by Serum Collection Set with medical device certification (CELLAID; JMS Co. Ltd., Hiroshima, Japan), and was used for isolation and expansion of DPSC subsets in our clinical study, because safety is our primary goal. Further details about manufacturing protocol of DPSC subsets may be found in our previous article (8).

## Safety and Quality Control Tests of Human DPSC Subsets

The safety and sterility of the final cell products is critical for clinical investigations. They should be free of fungus, aerobic and anaerobic bacteria, virus, mycoplasma, and endotoxin. Genetic stability of MSCs could be determined by using karyotype analysis and comparative genomic hybridization array (33) *in situ*. The Cell Products Working Party and the Committee for Advanced Therapies with leading European experts expressed the opinion that conventional karyotype analysis can be valuable and useful to analyze genetic stability. If recurrent aberrations are identified, comparative genomic hybridization and/or fluorescence *in situ* hybridization having better sensitivity could be alternative analyses for looking for these aberrations (34).

Our previous experiments demonstrated that the expanded human DPSC subsets at the 20th passage showed no tumor formation after transplantation intratesticular site or subcutaneously in immunodeficient mice (nonobese diabetic/severe combined immunodeficiency or KSN nude mice). Also, no chromosomal alteration or karyotype abnormalities/aberrations were detected at the 20th passage (2). As quality control tests, survival rate more than 80%, total cell number more than $3.0 \times 10^6$, expression rate of stem cell markers CD29, CD44, and CD105 higher than 90%, and CD31 less than 1% were demonstrated. Significant chromosomal abnormalities/aberrations, including irregular portion of chromosomal DNA and no more than 2 chromosomes of a pair (trisomy, tetrasomy), were not detected in the karyotype. Of the 20 randomly selected cells for evaluation, 1 or 2 cells demonstrated slight aberration on chromosomal aberrations (45,X) in 2 of 5 patients. On further examination of chromosome number 45, no specific chromosome anomalies were observed. The sterility tests demonstrated no contamination in all cell products. Thus, cell products from all patients could be used for cell transplantation.

## Clinical Protocol

The primary purpose of our pilot clinical study (8) was to assess the safety, and the secondary purpose was to evaluate the potential efficacy and feasibility of autologous transplantation of clinical-grade DPSC subsets in the pulpectomized tooth. The following are treatment procedures for cell therapy for pulp regeneration:

1. Patients are registered after signing informed consents, including explanation of risks and alternative treatments or no treatment.
2. Within 4 weeks after registration, autologous serum is isolated. More than 14 days after that, autologous clinical-grade DPSC subsets are manufactured from each discarded tooth, and safety and quality control tests of the final cell products are performed as described in

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