Q2 INSIGHT

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# Cord Blood Transplant Treats Very Early-Onset Inflammatory Bowel Disease

**Very Early-Onset Inflammatory Bowel Disease (VEOIBD) is a subgroup of the larger group of Inflammatory Bowel Diseases which include disorders such as Crohn's disease and ulcerative colitis. VEOIBD is distinct in that it affects children younger than 6 years of age and does not respond well to conventional therapies. Patients with VEOIBD often show severe gastrointestinal symptoms but other tissues such as the blood, respiratory tract, pituitary gland, liver, skin and spleen can also be affected.**

VEOIBD is thought to be caused by a mixture of factors including genetics, gut bacteria and environmental factors. Although rare, the incidence is increasing in certain locations. For example, the incidence in Shanghai increased by 12-fold between 2000 and 2010. This study examined the use of haematopoietic stem cell transplant (HSCT) following reduced-intensity conditioning chemotherapy (RIC) on treating critically ill paediatric patients with VEOIBD with IL10RA deficiency. The results show that although the risk of treatment-related mortality is high, the treatment can be considered the only current curative therapy. The study demonstrates the ability of umbilical cord blood to treat both genetic and immune-mediated conditions.

**Purpose:**

- To determine the effect and prognosis of RIC-HSCT therapy in infants diagnosed with VEOIBD with IL10RA deficiency.
- Prognostic information collected from 4 months to 24 months following procedure.

**Methods:**

- Nine VEOIBM patients with IL10RA deficiency from 5 sites in China underwent RIC-HSCT within a 2-year period.
- All patients were diagnosed for VEOIBD according to their clinical characteristics, endoscopic and histological assessments and confirmed gene variation of IL10RA.
- 8 Patients underwent reduced-intensity conditioning (RIC) chemotherapy due to being in critical condition (low body weight and malnutrition).
- 1 Patient – Patient 4 – underwent myeloablative conditioning (MAC) chemotherapy due to a better clinical condition than the other patients.

- Following the respective chemotherapy regimens, they received umbilical cord blood transplants from unrelated health donors with HLA match rates equal or greater than 8/10.
- Post-transplant, all patients were cared for in single occupant rooms ventilated via an air particulate ventilation system and received intravenous immunoglobin and antimicrobial prophylaxis.
- The patients' physicians recorded clinical and follow-up data for 24 months following the procedure.

**Results:**

- 7 of the 9 patients achieved complete chimerism at 2-8 weeks following transplantation.
- Patients 2 and 7 were treated for CMV reactivation following transplantation.
- 3 Months after transplantation, patient 2 was treated for intestinal infection, sepsis and severe acute grade IV GVHD of the liver. Patient 2 fully recovered and achieved successful chimerism.
- Patient 4 died of idiopathic pneumonia syndrome due to HHV6 infection and chemotherapy injury at 6 months following transplantation. Chronic lung GVHD was also diagnosed at 5 months post-transplantation.
- Patients 6 and 8 died of sepsis due to intestinal infection following unsuccessful engraftment.
- All patients who achieved successful engraftment showed improvements in the stool within 1 to 3 months after transplantation.
- C-reactive protein normalised within 10 days to 4 months post-transplantation.
- For patients who achieved successful engraftment, the faecal calprotectin levels decreased to normal within 3 to 8 months post-transplantation.
- Patients who exhibited malnutrition prior to transplantation showed significant improvements and increases in body weight.
- Endoscopic evaluations carried out 6 months post-transplantation showed improvements with normal colonic mucosa with slight inflammation in the small intestine.

**Conclusion:**

- The results demonstrate that HSCT can cure VEOIBD with IL10RA deficiency – as such, it is currently the only curative therapy.
- The risk of treatment-related mortality is high.
- All of the patients involved in the study were in critical condition prior to HSCT with low body weight and malnutrition.
- Patients who received RIC had better prognoses than the patient who received MAC.

*Peng, K. et al. (2018) "Umbilical Cord Blood Transplantation Corrects Very Early-Onset Inflammatory Bowel Disease in Chinese Patients With IL10RA-Associated Immune Deficiency," Inflammatory Bowel Diseases, 24(7), pp. 1416–1427. doi: 10.1093/ibd/izy028.*

# Deciduous Autograft of Dental Pulp-Derived Stem Cells Regenerate Dental Pulp in Injured Teeth

**Dental pulp trauma accounts for 5% of injuries and is most common in young people. In 2006, a total of 403,149 visits to emergency departments in the United States had a primary diagnosis of dental pulp disease.**

Trauma to immature permanent teeth can result in loss of dental pulp, loss of blood supply, loss of nerve supply, and impaired root development. The current standard treatment is apexification whereby the root canal of the affected tooth is filled with calcium hydroxide to close the apical foramen and create an environment suitable for a root canal treatment. However, it is unable to restore lost dental pulp tissue and maintain root development. Complications following apexification include pulpal necrosis, root resorption and pulpal obliteration.

Following promising preclinical results in mice, rat, and pig models for dental pulp stem cell (DPSC) implantations, this research group conducted a clinical trial to investigate DPSC transplantation for treating injured permanent incisor teeth. The clinical trial detailed below indicates the potential tissue regeneration properties of DPSCs and provides promise for future applications.

**Purpose:**

- To determine the safety and efficacy of autologous transplantation of dental pulp-derived stem cell aggregates in promoting dental p ulp regeneration in injured permanent incisor teeth.

**Methods:**

- B and T cell subsets were assessed at Wks 0, 4, 8 and 24 via flow cytometry.
- 40 male and female patients, between the ages of 7 and 12 years, with diagnosed traumatic pulp necrosis of a single permanent incisor tooth were randomly allocated in a 3:1 ratio between experimental and control groups.
- Every participant underwent treatment to remove the traumatised dental pulp.
- In the experimental group, dental pulp was isolated from a maxillary deciduous canine tooth which was digested and cultured until aggregate formation.
- Two aggregates were then implanted into the injured incisor tooth.
- In the control group, the participants received the standard apexification treatment.



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- Prior to treatment, all participants were assessed for the primary outcomes using the following tests:
  - Electric pulp vitality test, which indicates the pulpal sensory threshold.
  - Laser Doppler flowmetry, which indicates the vascular formation expressed in relative perfusion units (PU).
  - The implanted dental pulp of one participant was assessed for sensory nerve and blood vessel formation as determined by histological analysis following their exclusion post-treatment due to retraumatization.

- Prior to treatment, all participants were assessed for the secondary outcomes using cone-beam computed tomography (CBCT) to determine the root length and apical foramen.

- In order to determine safety, blood tests were performed for each participant and included counts and percentage variations for T lymphocyte subsets, B lymphocytes, and NK cells.

- Follow-up assessments took place at 1, 3, 6, 9, and 12 months after treatment.

**Results:**

- At 12 months:
  - The mean pulpal sensory threshold decreased by 43.43 ±0.86 for the experimental group and 0.17 ±0.16 for the control group.
  - The mean vascular formation increased by 7.19 ±0.77 PU for the experimental group and decreased by 0.05 ±0.48 PU for the control group.
  - The mean length of the treated root increased by 5.24 ±0.92mm for the experimental group and 0.88 ±0.22mm for the control group.
  - The mean apical foramen width decreased by 2.64 ±0.73mm for the experimental group and 0.62 ±0.22m for the control group.

- Histological analysis of the extracted treated dental pulp following retraumatization indicated the regeneration of 3D whole dental pulp tissue containing an odontoblast layer, connective tissues, and blood vessels.

- Immunoflourescence staining on the extracted pulp indicated nerve regeneration due to the overlapping NeuN and DAPI staining.

- No significant side effects were observed following implantation.

- The implantation had no effects on immune response, liver function, renal function, or myocardial function.

- Digital RVG images indicated no inflammation at the periapical area of any incisor teeth in the experimental group.

**Conclusion:**

- The results demonstrate that DPSC aggregate implantation promoted 3D dental pulp regeneration and partial tooth recovery in injured incisors.

- Incisor teeth that received DPSC implantation showed significant improvements in sensation, nerve formation, vascular formation, root growth, and apical foramen width.

- DPSC implantation should be considered safe due to the absence of any observed significant negative effects on the participants.

- DPSC implantation should be considered effective for treating dental pulp trauma in young adult incisor teeth.

*Xuan, Kun, et al. "Deciduous Autologous Tooth Stem Cells Regenerate Dental Pulp after Implantation into Injured Teeth." Science Translational Medicine, vol. 10, no. 455, Aug. 2018, p. eaaf3227, doi:10.1126/scitranslmed.aaf3227.*

## Mesenchymal Stem Cells (MSCs) to assist the development of therapies for the regeneration of mineralized tissues (tooth dentin and bone).

**Congenital malformations, trauma, surgical resection and teeth diseases lead to bone defects that are challenging to repair. Currently, 2.2 million bone grafts are used annually worldwide with autografts and allografts as the major bone substitutes.**

While autografts are considered the gold standard for bone defect repair, their application is restricted by limited bone quantities from harvest and donor-site morbidity. Furthermore, the number of sub-standard repairs using autografts is as high as 30%. Allografts are readily available however; osteogenesis is inhibited by immunogenic reactions from host tissues using this method (1). There is a need for the field to find innovative ways to induce bone generation and mineralisation of tissues.

Mineralisation of tooth dentin in vivo occurs upon activation of growth factors and signalling molecules that are called dentin Extracellular Matrix Components (dEMCs). The following study is utilising purified dEMCs and investigating the affect during the mineralisation process at an in vitro model of MSC populations essentially exploring ways to generate mineralised tissues.

**Purpose:**

- To determine whether the dEMCs, that are naturally produced in vivo during tooth dentin formation, could be used as differentiation enhancers of the in vitro induced mineralization of MSCs.
- Determining the most appropriate stromal cell population and signalling cues that can be used in the process of developing effective treatments for tooth/bone mineralisation. Essentially, to contribute to the development of treatment modalities for the reconstruction of lost/damaged mineralized tissues.



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**Methods:**

- For this purpose Umbilical Cord MSCs (UCMSCs), Dental Pulp Stem Cells (DPSCs), and Adipose-derived Stem Cells (ASCs) were utilised.
- Cell viability assay was performed at the cells using the MTT assay upon incubation of cells with increasing concentrations of dEMCs. Proliferation capacity of the cells was measured with the expression of the Ki-67 proliferation marker using fluorescent microscopy.
- In vitro mineralisation assay was performed using the fluorogenic OsteoImage Mineralization Assay kit which specifically binds to the hydroxyapatite portion of the mineralized depositions.
- Microstructural analysis of mineralized deposits was performed using Scanning Electron Microscopy-SEM and Energy Dispersive X-ray Spectrometry-SEM-EDX.

**Results:**

- Osteogenic media containing 0.1 mg/ml dEMCs enhanced hydroxyapatite formation compared to osteogenic controls, both in the DPSCs and the UC-MSCs, as measured by the OsteoImage assay.
- In the DPSCs, OsteoImage revealed a nodular staining pattern of mineralization. Alizarin Red staining demonstrated an extensive stained mineralized substrate with discrete areas of strongly stained nodular structures. In the UCMSCs, mineral deposition revealed a different pattern compared to the DPSCs.
- The tissue origin of stromal cells dictates the mineralization pattern. DPSCs osteogenic cultures showed a collagen-like fibril network with randomly orientated fibrils, upon which nodular mineral accretions were deposited. UCMSCs osteogenic cultures revealed globular structures of different size and degree of coalescence aggregated upon a poorly organized fibril-like network.
- The elemental analysis of dEMCs-supplemented osteogenic cultures of DPSCs and UC-MSCs revealed the presence of the basic elements of hydroxyapatite, namely calcium (Ca) and phosphorus (P).

**Conclusion:**

- The presence of the dEMCs significantly improved the mineralization capacity of UCMSCs and DPSCs, but not ASCs, as this was demonstrated through the significantly increased detection of bone-like minerals.
- The MSCs exhibited a dose-dependent viability and proliferation capacity, indicating that fine-tuning of the concentration of the dEMCs is necessary before clinical applications.
- These findings can further aid in the development of MSC-based translational therapies for mineralized tissue repair and regeneration.

*Xenos Petridis, Bas P. Beems, Phillip L. Tomson, Ben Scheven, Ben N.G. Giepmans, Jeroen Kuipers, Luc W. M. van der Sluis, Martin C. Harmsen. Tissue Eng Part A. 2018 Dec 28. doi: 10.1089/ten.TEA.2018.0192 https://www.liebertpub.com/doi/10.1089/ten.tea.2018.0192*

*Footnote: Future Health Biobank has kindly provided research samples of Umbilical Cord MSCs, designated for research and development, for the experiments described above to the Centre for Dentistry and Oral Hygiene at the University of Groningen in the Netherlands. Future Health Biobank is committed to supporting the research and the development of cell therapies that will benefit current and future clients.*

*Ref1: An Innovative Approach for Enhancing Bone Defect Healing Using PLGA Scaffolds Seeded with Extracorporeal-shock-wave-treated Bone Marrow Mesenchymal Stem Cells (BMSCs) 2017*

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