Hepatocytes Produced from Human Tooth Pulp into Swine with Cirrhosis: Two Transplantations with Time Interval

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Abstract

We treated the liver cirrhosis by two step transplantations of hepatocyte produced from human exfoliated primary tooth (SHED) into the swine with cirrhosis. SHED at three of the passages was separated by magnetic sorting with CD117 antibody. The CD117+SHED hepatic separation was developed in DMEM which is enhanced with insulin-transferrin-selenium-x (ITS-x), incipient organism tropic-factors (ETF) and hepatocyte-development factor (HGF) for five days: IMDM enhanced with ITS-x, ETF, HGF, dexamethasone and endostatin for 11 days. F344-Nude rats were employed for this study. Carbon tetrachloride (CCl4) was administrated by intraperitoneal injection for 15 weeks to induce cirrhosis. Hepatocyte-like-cells (2x106 cells/ animal) suspended in Hank`s Balanced Salt Solution were transplanted into the spleen. The vehicle was injected to the positive control group. Non-cirrhosis-models were used as negative control group. Animals were sacrificed for four weeks after the transplantation. Then five weeks later, the second transplantation was carried out, and then the swine were euthanized. Immunocytochemistry perception of the hepatically separated cells unequivocally showed positive recoloring for egg whites, IGF-1,?- feto-protein, HNF4? what's more, CPS-1. The histopathological investigation, HE and Masson???s trichrome recoloring, demonstrated a major abatement of creature tissue inside the transplantation bunch with contrasting with the positive benchmark group. Healthy liver tissues were recovered by the transplantation. Moreover, serological test results revealed significance differences between the groups. Serum ALT levels of the test group dramatically decreased to at least one third compared to the positive control group. Activities of albumin, bilirubin, BUN, HA levels were also recovered. By just one transplantation albumin value was improved, but two steps showed far better improvement. The two stages transplantations of hepatocyte-like cells from human tooth relocated into the liver with serious disappointment showed their ability to perform emphatically on account of radical diminishing fibrous tissues. Together, these findings suggested that two steps transplantation may be a future potential protocol for treating chronic liver injuries like cirrhosis. Stem cells are clonogenic cells capable of self-renewal and multi-lineage differentiation. Post- natal stem cells/adult stem cells were first isolated from bone marrow. They were later isolated from the neural tissue, retina, and even the skin. The bone marrow-derived stem cells are most widely researched and utilized in clinical settings. Dental pulp stem cells (DPSC) were first discovered in the year 2000, from an extracted impacted third molar by Gronthos et al. DPSCs are considered to be cranial neural crest cells (CNCCs). A group of NCCs migrate from the neural crest and is temporally formed between ectoderm and neural plate during neural tube formation. They play an important role in embryo development. During the migration, the NCCs translate into mesenchymal cells. The CNCCs concentrate in facial and pharyngeal arches they form sensory VII, IX, X cranial nerves, thymus, thyroid follicular cells, parathyroid, and cornea. They also form the orofacial mesenchymal organs including facial skeleton such as maxilla, mandible, dentin/pulp complex, cementum, periodontal ligament (PDL), and alveolar bone. The natural function of DPSCs in the production of odontoblasts to create reparative dentin aids in the regeneration of tooth structures. However, they are also effective in the repair of tissues outside the tooth. The ease of isolation of DPSCs from discarded or removed teeth offers a promising source of autologous cells, and their similarities with bone marrow stromal cells (BMSCs) suggest applications in musculoskeletal regenerative medicine. DPSCs are effective for various diseases, such as spinal cord injuries (SCIs), Parkinson's disease (PDs), Alzheimer's disease, cerebral ischemia, myocardial infarction, muscular dystrophy, diabetes, liver diseases, eye diseases, immune diseases, and oral diseases. Other types of human dental pulp-derived stem cells (HDPSCs) include dental pulp of human exfoliated deciduous teeth, root apical papilla of human teeth, and dental pulp of human supernumerary teeth, namely, stem cells from human exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), and human supernumerary tooth-derived stem cells (SNTSCs) were identified in the year 2003, 2006, and 2013 retrospectively. In addition to this, stem cells can be isolated from various tissues, including oral parts such as alveolar bone, PDL, dental follicle, oral mucosa, and gingival. The regeneration of dentin pulp complex is based on vascularization. Vascular endothelial growth factor administration promotes vascularization but has a short half-life. This can be increased by binding to heparin. Treating stem cells under hypoxic conditions induce the cells to secrete vascularizing agents. Stem cells differentiate into various types of cells; hence, they have to be controlled using growth factors like soluble protein of the dentin matrix. DPSCs were mixed with a carrier and filled in a root canal treated extracted tooth, and the DPSC filled tooth was transplanted into dorsal surface of immune-compromised mice. Regenerated dentin deposited along to existing dentin and connective tissues beneath the de novo dentin contains blood vessels. Autologous transplantation of DPSCs is clinically tried to regenerate the dentin-pulp complex. Tubular dentin formation was observed when human pulp stem cells with scaffold (HA/tricalcium phosphate) were implanted in immunocompromised mice. Reparative dentin formation on amputated pulp was found when stem cells were combined with recombinant human bone morphogenetic protein 2 in experimental studies on animal models. Kawaguchi et al. used BMSCs for their ability to produce alveolar bone, PDL, and in vivo cementum after implantation into the

periodontal defects thereby proving to be an alternative source in the treatment of periodontal diseases. Marei et al. in their experiment on goat was able to regenerate periodontal tissues around titanium implant using autologous bone marrow stem cells with the scaffold. Transplantation of PDL derived cells into animal models was shown to regenerate periodontal tissue. Iwata et al. harvested and expanded primary canine PDL cells in vitro and also made into transplantable constructs containing PGA scaffold and PDL cell sheets. The transplantable constructs together with porous b-tricalcium phosphate induced regeneration of periodontal structures, including alveolar bone, cementum, and periodontal fibers. Liu et al. regenerated periodontal tissue in miniature swine using scaffolds seeded with PDL derived stem cells .PDLSCs can differentiate into cells which will colonize on the biocompatible scaffold, suggesting a simple and efficient autologous source of stem cells for regeneration of dental tissues. SCAP has remarkable cell migration activity; which is considered to involve root growth in tooth development.

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