Abstract

The aim of this dissertation study was to optimize the isolation and long term cultivation protocols for human dental pulp stem cells. The protocols which showed best results were used for cultivation of dental pulp stem cell isolated from exfoliated teeth (SHED). Additional aims were to characterize DPSC and SHED and prove their ability to proliferate over Hayflick´s limit and differentiate into mature cell lines (osteoblasts, chondroblasts and adipocytes).

In order to find optimal protocols for isolation of dental pulp from tooth, we tried three different approaches. During optimization of cultivation protocol we focused on decreasing amount of fetal calf serum (FCS) from 10 % FCS in cultivation media (most often used in literature) into 2 % and thus get closer to cultivation conditions suitable for clinical usage. We compared DPSC cultivated in three different media (medium with 10 % FCS, 2 % FCS supplemented with growth factors and media with 2 % FCS supplemented with ITS and growth factors). For characterization of DPSC and SHED we used basic biological properties (proliferation activity, viability, morphology), their phenotype and karyotype.

The study demonstrated that the best protocol for isolation of dental pulp from tooth was to break the roots and extract the pulp throw this aperture. We found that enzymatic dissociation was the most successful for isolation of DPSC from dental pulp. We were able to cultivate DPSC in all three tested media, but DPSC in medium containing 2 % FCS and supplemented with ITS showed the highest proliferation rate and kept other biological properties on the same levels as DPSC cultivated in other tested media. We were able to proliferate DPSC over Hayflick´s limit and differentiate them into osteoblasts and chondroblasts-like cells. The phenotypical analysis showed that DPSC express surface markers typical for mesenchymal cells and some markers of stem cells, on the other hand they did not express any markers typical for hematopoietic cell line.

Key word

Dental pulp stem cells, exfoliated teeth, Hayflick´s limit, isolation, cultivation, phenotypical analysis