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Differences of isolated dental stem cells dependent on donor age and consequences for autologous tooth replacement

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ABSTRACT

Objective: Autologous therapy *via* stem cell-based tissue regeneration is an aim to rebuild natural teeth. One option is the use of adult stem cells from the dental pulp (DPSCs), which have been shown to differentiate into several types of tissue *in vitro* and *in vivo*, especially into tooth-like structures. DPSCs are mainly isolated from the dental pulp of third molars routinely extracted for orthodontic reasons. Due to the extraction of third molars at various phases of life, DPSCs are isolated at different developmental stages of the tooth.
Design: The present study addressed the question whether DPSCs from patients of different ages were similar in their growth characteristics with respect to the stage of tooth development. Therefore DPSCs from third molars of 12–30 year-old patients were extracted, and growth characteristics, *e.g.* doubling time and maximal cell division potential were analysed. In addition, pulp and hard dental material weight were recorded.
Results: Irrespective of the age of patients almost all isolated cells reached 40–60 generations with no correlation between maximal cell division potential and patient age. Cells from patients <22 years showed a significantly faster doubling time than the cells from patients ≥22 years.
Conclusion: The age of patients at the time of stem cell isolation is not a crucial factor concerning maximal cell division potential, but does have an impact on the doubling time. However, differences in individuals regarding growth characteristics were more pronounced than age-dependent differences.

1. Introduction

The infection diseases caries and periodontitis are the main causes for the loss of teeth. Common replacements are fixed partial dentures on neighbouring teeth, implants or prostheses. Despite constant technical advances in scientific research concerning implants, artificial dental prostheses do not meet the requirements of natural teeth. The survival rates of conventional fixed partial dentures are about 95%

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after 5 years,[1] 89.1% after 10 years,[2] 85% after 15 years,[3] 66.2% after 20 years[4] and 53% after 30 years[5] in function. The major risk factors are periimplantitis, soft tissue- and technical complications, including connection- and suprastructure-related problems as well as implant fractures.[2] The aim of regenerating natural teeth instead of prosthetic reconstruction has brought stem cells into the focus of dental research.

Regenerative medicine and regenerative biology *via* personalized stem cell therapy are the buzzwords of the 21st century regarding modern medicine approaches to reach the optimal treatment for each patient.[6] There are two broad types of stem cell, embryonic and adult. Embryonic stem cells can be harvested from the early blastocyst stage, which includes the destruction of embryos in an early developmental phase. Since these cells were found to have the potential to differentiate into all kinds of tissues *in vitro*, the idea that stem cells may be used to treat loss of function at diseases is ubiquitous in life science. However, due to ethic concerns as well as other critical topics, like high malignancy potential and the use of allogenic material, the focus of tissue engineering approaches has moved from embryonic stem cells to alternatives like adult stem cells. In contrast, they can be isolated from various tissues or organs of adults without the destruction of unborn life.

Due to a relatively simple isolation and cryopreservation, adult stem cells play a crucial role in postnatal tissue development and provide an attractive progenitor cell source for tissue engineering and regenerative medicine.[6] In recent years several types of adult dental stem cell with different locations in the tooth or different isolation time points in developing teeth were discovered.[7–11] One type of adult dental stem cell is the dental pulp stem cell (DPSC). DPSCs as one source of mesenchymal stem cells (MSCs) have been shown to differentiate into several types of tissue, such as articular cartilage, bone, tendon, muscle or adipose tissues *in vitro*,[7,12,13] as well as *in vivo*.[14,15] In recent studies it was reported that DPSCs are capable of regenerating complete tooth-like structures[16,17] and rebuilding dentine structures after damage or during ageing of teeth after eruption.[18] Therefore DPSCs are interesting tools concerning hard-tissue regeneration and, in combination with storage of patients' own DPSCs, a promising approach for future therapies.

For orthodontic reasons third molars are predominantly extracted between 16 and 18 years of age, more rarely at a later time point. These extracted teeth could function as a valuable and easily available source of stem cells and therefore tissue engineering approaches. First dental stem cell banks already exist (http://www.stemsave.com 28-10-2013 http://www.biohellenika.gr/en.html 28-10-2013). However, little is known about variations of DPSCs dependent on donor age. To investigate these influences, extracts of DPSCs from a heterogeneous group of patients ranging from 12 to 30 years were compared regarding growth characteristics, especially doubling time and maximal cell division potential. In addition, pulp and hard dental material weight were recorded for potential connections between pulp weight and age.

2. Material and methods

2.1. Subjects and cell culture

Healthy human impacted third molars were collected from young adults aged between 12 and 30 years by the Department of Prosthetic Dentistry and Biomedical Materials Science at Hannover Medical School. For pulp isolation the teeth were broken in a screw clamp. The pulp tissue was digested in a solution of 4 mg/ml collagenase type I (Invitrogen, Darmstadt, Germany) and 2 mg/ml dispase (Invitrogen) for 1 h at 37 °C. The cell suspension was cultured in α-MEM (Lonza Group AG, Basel, Switzerland) plus 10% Fetal Bovine Serum (FBS, Pan Biotech GmbH, Aidenbach, Germany), 100 U/ml penicillin and 100 μg/ml streptomycin (Biochrom AG, Berlin, Germany) at 37 °C and 5% CO_2. The cells were allowed to adhere overnight and non-adherent material was removed by intense washing the next day. Over a period of approximately 7 days isolated cells formed colonies and could be used for experiments.

2.2. Weight of pulp

To calculate the ratio of pulp to hard tooth structures, the whole teeth were measured immediately after extraction and the pulp weight after removal as described above.

2.3. Immunofluorescence

Cells were grown on coverslips, fixed with 2% paraformaldehyde (PFA, Carl Roth GmbH, Karlsruhe, Germany) for 30 min at room temperature (20–25 °C) and washed with phosphate buffered saline (PBS, Biochrom AG). For the detection of intracellular antigens, cells were permeabilized with 0.1% Triton-X-100 (Sigma Aldrich Chemie GmbH, Steinheim, Germany) for 5 min at room temperature.

Cells were incubated with the following primary antibodies: anti-CD29 IgG (1:50, BD Biosciences, Heidelberg, Germany), anti-CD44 IgG (1:100, BD Biosciences) or anti-CD166 IgG (1:100, BD Biosciences) for 30 min at room temperature.

After washing with PBS, cells were incubated for 30 min at room temperature with secondary antibodies consisting of goat anti-mouse IgM-FITC (1:500, Invitrogen), goat anti-mouse IgG-CY3 (1:100, Jackson Immuno Research, Pennsylvania, USA), goat anti-rat IgG-CY3 (1:100, Jackson Immuno Research) or goat anti-mouse IgM- and IgG-Cy5 (1:100, Jackson Immuno Research). Additionally, cells were incubated for 15 min at room temperature with tetramethylrhodamine B isothiocyanate/phalloidin (1:1.000, Sigma Aldrich Chemie GmbH) or 4′,6-diamidino-2-phenylindole/DAPI (1:30.000, Invitrogen) and viewed under the fluorescence microscope.

All antibodies were diluted according to the manufacturer's description.

2.4. Differentiation assays

2.4.1. Osteogenic and adipogenic differentiation

For osteogenic and adipogenic differentiation cells were seeded at a density of 5×10^3 cells per cm^2 in α-MEM with 10% FBS,