ELSEVIER

Cell Biology International 31 (2007) 1191–1197

Cell Biology International

www.elsevier.com/locate/cellbi

Mesenchymal progenitor cells in adult human dental pulp and their ability to form bone when transplanted into immunocompromised mice

Sayuri Otaki [a], Shigeru Ueshima [b,c,*], Kohei Shiraishi [d], Kazuo Sugiyama [d], Suguru Hamada [e], Masatomo Yorimoto [a,f], Osamu Matsuo [b]

[a] *Department of Plastic Surgery, Kinki University School of Medicine, Ohnohigashi 377-2, Osakasayama-shi, Osaka 589-8511, Japan*
[b] *Department of Physiology, Kinki University School of Medicine, Ohnohigashi 377-2, Osakasayama-shi, Osaka 589-8511, Japan*
[c] *Department of Food Science and Nutrition, Kinki University School of Agriculture, Nakamachi 3327-204, Nara-shi, Nara 631-8505, Japan*
[d] *Department of Chemistry and Environmental Technology, Faculty of Engineering, Kinki University, Takayaumenobe 1, Higashihiroshima-shi, Hiroshima 739-2116, Japan*
[e] *Department of Stomatology, Kinki University School of Medicine, Ohnohigashi 377-2, Osakasayama-shi, Osaka 589-8511, Japan*
[f] *Yorimoto Dental Clinic, Nakano 3-11-15, Higashisumiyoshi-ku, Osaka-shi, Osaka 546-0012, Japan*

Received 27 December 2006; revised 19 March 2007; accepted 3 April 2007

Abstract

The technique of tissue engineering is developing for the restoration of lost tissues. This new technique requires cells that fabricate tissue. Mesenchymal stem cells in bone marrow have been used as the cell source for this technique; however, dental pulp cells have recently been shown to possess stem-cell-like properties.

We earlier demonstrated that dental pulp cells proliferate and produce an extracellular matrix that subsequently becomes mineralized *in vitro*. We now report that such dental pulp cells (first to eighth passage) produced bone instead of dentin when those cells were implanted into subcutaneous sites in immunocompromised mice with HA/TCP powder as their carrier. This evidence shows that dental pulp cells are the common progenitors of odontoblasts and osteoblasts, or dental pulp cells are mesenchymal stem cells themselves.

It is expected that dental pulp cells can be a useful candidate cell source for tissue engineering, and contain the potential of new therapeutic approaches for the restoration of damaged or diseased tissue.

Keywords: Dental pulp; Mesenchymal progenitor cell; Bone formation

1. Introduction

Autologous tissue grafting for the restoration of lost tissues is limited by several factors, including the availability of sufficient donor tissue. One solution to this problem may be to develop substituted tissue grafts using the technique of tissue engineering. This new technique requires cells that give rise to the tissue to be fabricated. The cells used for such purposes can be either stem cells or progenitor cells, but they cannot be terminally differentiated cells, such as osteoblasts and odontoblasts, since those cells will not proliferate after transplantation.

Abbreviations: EPMA, electron probe microanalysis; RT-PCR, reverse transcription-polymerase chain reaction; DSPP, dentin sialophosphoprotein; HA/TCP, hydroxyapatite/tricalcium phosphate; PBS, phosphate-buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

* Corresponding author. Department of Physiology, Kinki University School of Medicine, Ohnohigashi 377-2, Osakasayama-shi, Osaka 589-8511, Japan. Tel.: +81 72 366 0221x3165; fax: +81 72 366 2853.

E-mail address: sueshima@nara.kindai.ac.jp (S. Ueshima).

1065-6995/$ - see front matter © 2007 International Federation for Cell Biology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.cellbi.2007.04.001

Stem cells are defined as cells that have the capacity for self-renewal and broad potential to give rise to diverse differentiated progenies. Several stem cells have been isolated from human tissue: Embryonic stem cells have been cultured from human fetal tissue and have shown the ability to differentiate into a variety of cell types found in embryonic germ layers (Thomson et al., 1998). Many adult tissues also contain populations of stem cells that have the capacity for renewal after trauma, disease, or aging. Bone marrow contains hematopoietic stem cells and mesenchymal stem cells, the brain contains neural stem cells, and the gut and epidermis contain epithelial stem cells. These cells may be found within the tissue or in other tissues that serve as stem cell reservoirs. For example, although adult hematopoietic stem cells that renew circulating blood elements reside mostly in bone marrow, these cells can be found in other tissues, too (Till and McCulloch, 1961).

Mesenchymal stem cells that give rise to progenitors for several mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, adipose, and stroma have been referred to by different names. The original term ''colony-forming unit-fibroblast (CFU-F)'' or ''marrow stromal fibroblasts (MSF)'' (Castro-Malaspina et al., 1980; Piersma et al., 1985; Kuznetsov et al., 1997b) has been gradually replaced by ''marrow stromal cells (MSC)'' (Prockop, 1997), ''mesenchymal stem cells (MSC)'' (Caplan, 1994), or ''mesenchymal progenitor cells'' (Conget and Minguell, 1999). *In vitro* and animal implant studies have indicated that there are multipotent ''mesenchymal stem cells'' or mixtures of committed ''mesenchymal progenitor cells'', each with restricted potential (Haynesworth et al., 1992; Bergman et al., 1996; Cassiede et al., 1996; Young et al., 1998; Wakitani et al., 1994; Kadiyala et al., 1997; Pittenger et al., 1999). Mesenchymal stem cells, even if they have a fibroblastic appearance, have proved to be different from normal skin fibroblasts or other mature fibroblasts that do not have self-renewal capability and multi-lineage differentiation capacity (Pittenger et al., 1999).

Mesenchymal stem cells in bone marrow have mainly been used as cell source for tissue engineering; however, dental pulp cells were recently shown to differentiate into odontoblasts, adipocytes and neural-like cells, indicating there are ''mesenchymal progenitor cells'' or ''mesenchymal stem cells'' in dental pulp (Gronthos et al., 2002). We earlier demonstrated that dental pulp cells expressed mRNA for osteocalcin (common marker of osteoblasts and odontoblasts), and not dentin sialophosphoprotein (DSPP) (odontoblast-specific marker) after differentiation. Further, they produced extracellular matrix *in vitro* which is proved to contain large amount of calcium and phosphate by EPMA (Ca:P:Na:Cl = 72:68:7:7) (Otaki, 2006).

For the purpose of further investigating the potential of dental pulp cells as ''mesenchymal progenitor cells'' or ''mesenchymal stem cells'', we transplanted dental pulp cells into immunocompromised mice and observed the differentiation of those cells *in vivo*.

2. Materials and methods

2.1. Cell culture

Human teeth (18–31 years old) extracted during caries treatment were collected with the patients' informed consent under guidelines set by the Kinki University School of Medicine. The teeth were grooved around the cementum-enamel junction using dental fissure burs without revealing the pulp chamber. The teeth were kept in 4 °C phosphate-buffered saline (PBS), cleaned with 70% alcohol, washed with sterile PBS, and cracked open using a sterilized chisel on the clean bench within 6 hours after extraction. Pulp tissues were gently separated from the dental cavity, apical parts of the pulp were dissected to strictly avoid contamination of periodontal tissue, minced with scalpels, and rinsed with PBS. The explants were cultured in 75 cm^2 tissue culture flasks (Iwaki Glass, Tokyo, Japan) in MEM (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% FCS (Gibco Laboratories, Grand Island, NY, USA), 2 mM L-glutamine (Wako Pure Chemical Industries Ltd., Osaka, Japan), and 10 mM β-glycerophosphate (Lot 71K5421, Sigma–Aldrich Co., St. Louis, MO, USA). The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2, 95% air. The culture medium was changed once a week. Confluent cultures were collected by trypsinization [0.2% trypsin (DIFCO Laboratories, Detroit, MN, USA) and 0.02% EDTA (Wako Pure Chemical Industries Ltd., Osaka, Japan)], and subcultured in the same conditions. Cell morphology was examined routinely by phase contrast microscopy under an inverted microscope.

2.2. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was prepared from cultured cells using an RNeasy Mini Kit (Lot 4096698, Qiagen, Tokyo, Japan). Specific primers to published sequences of human type I collagen, fibronectin, osteocalcin, dentin sialophosphoprotein (DSPP), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed and prepared (Table 1). RT-PCR was performed using Ready-to-Go RT-PCR Beads (Lot 20659, Amersham Biosciences, Piscataway, NJ, USA) following a two-step protocol with oligo (dT) priming. After amplification, 5 μl of each reaction was analyzed by 1.7% agarose gel electrophoresis, and visualized by ethidium bromide staining.

2.3. Transplantation into immunocompromised mice

Approximately 6×10^6 cells (first to eighth passage) were mixed with 60 mg of hydroxyapatite/tricalcium phosphate (HA/TCP) powder (item 97-1109-231-00, Lot 56566200, Zimmer, Warsaw, IN, USA) and then

Table 1
The sequences of human-specific primers

Specific marker	Sequence of primer	The length of amplified fragments (bp)
Type I collagen	Sense 5′-GGCGGCCAGGGCTCCGACCC-3′; antisense, 5′-AATTCCTGGTCTGGGGCACC-3′	347
Fibronectin	Sense 5′ -CTCCAAGTACCCCCTGAGGAA-3′; antisense, 5′-CCAGGAGACTGTGAGCACTCC-3′	442
Osteocalcin	Sense 5′-CATGAGAGCCCTCACA-3′; antisense, 5′-AGAGCGACACCCTAGAC-3′	314
DSPP	Sense 5′-GGCAGTGACTCAAAAGGAGC-3′; antisense, 5′-TGCTGTCACTGTCACTGCTG-3′	253
GAPDH	Sense 5′ -GCAGGGGGGAGCCAAAAGGG-3′; antisense, 5′-TGCCAGCCCCAGCGTCAAAG-3′	566