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論文内容の要旨

１．Purpose：

The purpose of this study was to investigate the growth and differentiation potential of dental pulp cells (DPCs) in three-dimensional (3-D) collagen type-1 scaffold *in vitro* and *in vivo*.

２．Materials and Methods：

In this experiment GFP and Sprague-Dawley rats were used. Dental pulp cells (DPCs) were obtained and sub cultured up to three passages. 3rd passage dental pulp cells growth behavior and differentiation in 2-D culture and 3-D collagen scaffold were evaluated. DPCs expression of bone related mRNA (ALP, BSP and OPN) were evaluated in control and in 3-D collagen scaffold. Mineralized dentin tubes were prepared from Sprague-Dawley male rat central incisors. When DPCs were cultured up to 3 passages, two types of graft were prepared, cultured dental pulp cells suspended in a-MEM, cultured DPCs mixed with collagen type-1 gel scaffold. These grafts were injected into mineralized dentin tubes, which were then transplanted into rectus abdominus muscles. Cultured dental pulp cells suspended in a-MEM, without mixing with collagen scaffold in mineralized dentin tubes were used as control. Rats were sacrified at 7 days after the transplantation. H E, immunohistochemical and immunofluorescence staining methods were used for evaluation. Primary anti-bodies alkaline phosphatase (ALP), bone sialophosphoprotein (BSP) osteopontin (OPN), were used for immunohistochemical staining evaluation. PHALLOIDIN and DAPI were used for immunofluorescence staining evaluation of the cell behavior in 2-D and 3-D scaffold, under confocal microscope. While rabbit anti FAK anti body, which is primarily cell attachment protein used to evaluate the cell proliferation and differentiation in 2-D culture medium and 3-D collagen type-1 scaffold.

３．Results：

Histological and immunohistochemical results showed that DPCs in collagen gel were spindle shaped and showed significantly greater expression of ALP, BSP and OPN *in vitro* than the controls. Transplanted DPCs in collagen type-1 gel showed greater positive immunoreactivity for ALP, BSP and OPN than the controls.

４．Conclusion：

These results suggest that expanded DPCs maintain their multilineage potential in collagen gel. DPCs were differentiated into osteoblastic cells earlier than when cultured without collagen gel, as evidenced by the mRNA expression and immunohistochemical staining of osteogenic antibodies ALP, BSP and OPN *in vitro* and *in vivo*, respectively.

論　文　審　査　の　要　旨

本論文は，Ⅰ型コラーゲンゲルに混和した培養歯髄細胞の動態を *in vitro*及び *in vivo*で検索したものである。本審査委員会は，平成23年２月24日に副査を山田教授，中川教授，下野教授のもと行われた。まずSultan Zeb Khan大学院生から論文内容の説明がなされた。その後，各審査委員より次のような質問がなされた。１）腹直筋への抜去歯の移植方法について，２）抜去歯内部へのコラーゲンゲルおよび培養歯髄細胞の埋入方法について，３）コラーゲンゲル内での培養歯髄細胞の分化能についてなど多くの質疑が行われ，それぞれ１）腹直筋の筋層にそってポケットを作成し，同部位へ移植した，２）培養歯髄細胞とコラーゲンゲルの混和試料はシリンジを用いて挿入した，３）コラーゲンゲルに入れて移植した培養歯髄細胞はオステオポンチンおよび骨関連タンパクの発現が高まったとし，概ね妥当な回答が得られた。その他，目的の明確化，実験方法の説明文章の整理，用語の統一，付図およびその説明の補足などの修正すべき点が指摘され，訂正が行われた。

その結果，本研究で得られた結果は，今後の歯学の進歩，発展に寄与するところ大であり，学位授与に値するものと判定した。