J Periodont Res 2006; 41; 547–553

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00904.x

Location of putative stem cells in human periodontal ligament

S. C. Chen[1], V. Marino[1], S. Gronthos[2], P. M. Bartold[1]

[1]Colgate Australian Clinical Dental Research Centre, Dental School, University of Adelaide, SA, Australia and [2]Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science/Hanson Institute, Adelaide, SA, Australia

Chen SC, Marino V, Gronthos S, Bartold PM. Location of putative stem cells in human periodontal ligament. J Periodont Res 2006; 547–553. © 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Background and Objective: The origin of cells in the mature periodontium, and the location of their progenitors, are still unknown. It is also unknown whether inflammation influences the number and distribution of these cells within the periodontium. Molecules such as STRO-1, CD146 and CD44 have been identified on a variety of mesenchymal stem cells. The aim of this study was to identify and localize putative stem cells in diseased and healthy human periodontal ligament using cell-surface markers for mesenchymal stem cells.

Material and Methods: Healthy and periodontitis-affected teeth were collected, fixed in 10% neutral-buffered formalin, decalcified and embedded in paraffin in preparation for immunohistochemistry. Antibodies against STRO-1, CD146 and CD44 were used to identify putative stem cells in the periodontal ligament.

Results: Putative stem cells were identified in both healthy and diseased periodontal ligament. They were mainly located in the paravascular region and small clusters of cells were also found in the extravascular region. Wider distributions of these cells were detected in sections of diseased ligament.

Conclusion: Within the periodontal ligament of both healthy and diseased teeth, cells have been identified consistent with their identification as putative stem cells. The presence of an inflammatory reaction associated with periodontitis may enhance the number of these cells.

P. M. Bartold, Colgate Australian Clinical Dental Research Centre, Dental School, University of Adelaide, South Australia, 5005, Australia
Tel: +61 8 8303 3435
Fax: +61 8 8303 6429
e-mail: mark.bartold@adelaide.edu.au

Key words: cementum; periodontal ligament; stem cells

Accepted for publication April 5, 2006

Heterogeneous populations of cells, such as osteoblasts, fibroblasts and cementoblasts, contribute to each of the unique compartments of the periodontium. The presence of multiple cell types within the periodontal ligament has led to speculation that cells in this tissue may share common ancestral cells (1). During the development of teeth, periodontal ligament cells are believed to originate from ancestral cells in the dental follicle (2–5). However, the origin of renewed periodontal ligament cells is less certain after development of the teeth has been completed.

A group of progenitor cells has been identified in mouse periodontal ligament within the paravascular areas. These cells originate from the endosteal spaces of alveolar bone, they subsequently differentiate and proliferate in the paravascular areas of the periodontal ligament and finally migrate to areas near the cementum and alveolar bone (1,6–9). Characteristics of these progenitor cells, such as the sparse distribution within the proliferative compartment, cell morphology, self-renewal capacity and slow cycling rate, suggest that they could be adult stem cells with multilineage potential (10,11). However, to date, no cell-surface markers have been available for using to confirm the existence and location of stem cells in the periodontal ligament of human teeth.

Various cell-surface antigens have been used as markers for stem cells and their isolation from other tissues (12). In particular, STRO-1 surface antigen has been shown to be non-reactive to hematopoietic stem cells and it can isolate a homogeneous group of mesenchymal stem cells that are capable of forming clonogenic fibroblast colonies *in vitro* (13). However, owing to its cross-reaction with glycophorin-A-positive nucleated red cells and a small subset of B lymphocytes, the use of STRO-1

alone is not sufficient to obtain pure populations of stem cells. More recently, the isolation of highly enriched populations of stem cells with clonogenic and multilineage potentials have been based on the use of flow cytometry with STRO-1 immunoglobulin in combination with antibodies directed to vascular cell adhesion molecule-1 (VCAM-1) and cellular adhesion molecule (CD146/MUC-18) (14–17). Stem cell populations isolated from dental pulp and bone marrow using this technique expressed markers associated with endothelium, smooth muscle, bone and fibroblasts. In addition, the cell-surface antigen, CD44, was also expressed in these cell populations (16). Recent studies have shown that human periodontal ligament stem cells (PDLSC) can also be isolated and culture expanded from digested periodontal tissue by immunoselection, based on their cell-surface expression of STRO-1 (18). The applications of STRO-1 and other mesenchymal stem cell-associated antibodies may assist in the identification of putative stem cells in the periodontal ligament.

Periodontitis is an inflammatory disease that results in the destruction of periodontal connective tissue matrix and cells, loss of fibrous attachment and the resorption of alveolar bone (19). In animal models, the healing of periodontal ligament involves an increase in the number of progenitor cells in the paravascular area and then migration of these cells to the areas of alveolar bone and cementum adjacent to the wound defect (1). It is uncertain whether inflammatory changes in the periodontium can influence stem cell distribution in the tissues.

In this study, we hypothesize that the progenitor cells, previously identified in the periodontal ligament, are putative stem cells, the location and distribution of which are affected by the inflammatory changes. The aim of this study was to identify periodontal ligament stem cells and to investigate their distribution in human periodontal ligament from healthy and periodontitis-affected teeth using immunohistochemistry.

Materials and methods

Human tooth collection

A total of 50 teeth were collected for this study. Twenty-five periodontitis-affected teeth, which had a hopeless prognosis, were extracted from 14 individuals at the Periodontal Department of the University of Adelaide. Twenty-five healthy teeth, which comprised mainly premolars and wisdom teeth, were also collected from 20 individuals at the Oral Maxillofacial Department of the Adelaide Dental Hospital. This study was approved by the Human Ethics Committee of the University of Adelaide Ethics Number H-57-2003). The research objectives were explained to the patients and informed consent was obtained before sample collection.

Tooth preparation

The teeth were fixed in 0.1 M phosphate buffer [phosphate-buffered saline (PBS)], containing 4% paraformaldehyde, for 1 d. They were then decalcified at room temperature in neutral PBS containing 4% EDTA. Radiographs of the teeth were taken to ensure complete decalcification before sectioning. The decalcified teeth were washed in distilled water for 2 h and stored in 70% alcohol prior to embedding. Each tooth was cut into two or three sections along the longitudinal plane of the teeth. These sections were then coded, dehydrated through graded alcohol solutions and histolene, and embedded in paraffin wax blocks. Each paraffin-embedded sample was cut into 5-μm sections and placed onto 3-amino propyltriethoxysaline (APT) (Sigma, St Louis, MO, USA)-coated glass slides. Haematoxylin and eosin staining was carried out on selected sections to check for the presence of periodontal ligament before staining for immunohistochemistry. The number of teeth with intact periodontal ligament present was recorded.

Immunohistochemistry staining

All stages of the immunostaining process were carried out at room temperature (23°C) unless otherwise stated. To identify mesenchymal stem cells in the periodontal ligament, mouse monoclonal antibodies (mAbs) were used, including anti-STRO-1, CC9 (anti-CD146) and H9H11 (anti-CD44) (15,17).

The demonstration of cell-surface antigens was significantly improved by pretreatment with antigen-retrieval reagents that broke the protein cross-links formed during formalin fixation. For antigen retrieval of STRO-1 surface antigen, the sections were placed in preheated citrate buffer at pH 6 and heated at 95°C in a water bath for 20 min. For antigen retrieval of CD146 surface antigens, the sections were placed in preheated citrate buffer at pH 6 and heated at 80°C in a water bath for 20 min. The slides were allowed to cool to room temperature for 30 min before washing in PBS. No antigen-retrieval procedures were necessary for the CD44 surface antigens.

Endogenous peroxidase activity was blocked by incubation, for 20 min, in PBS containing 1% hydrogen peroxide and 0.1% sodium azide. The sections were then rinsed in PBS. The primary antibodies, at concentrations previously determined to give optimal staining (STRO-1, 4 μg/ml; CD146, 4 μg/ml; and CD44, 2 μg/ml), were applied to the sections and allowed to incubate at room temperature overnight.

For the negative-control sections, the primary antibodies were omitted. As stem cells have been previously identified in the dental pulp, this tissue was used as a positive control (15,17).

After overnight incubation with the primary antibodies, the sections were washed in PBS and a secondary horseradish peroxidase-conjugated antibody [1% goat antimouse immunoglobulin; Dako, Glostrup, Demark, diluted with 10% normal human serum in PBS/1% bovine serum albumin (BSA)] was applied to each section. After incubation for 30 min, the sections were washed in PBS and a tertiary horseradish peroxidase-conjugated antibody (1 : 60 swine anti-goat immunoglobulin; Biosource, Camarillo, CA, USA; diluted with

10% normal human serum in PBS/1% BSA) was then applied and incubated for 30 min at room temperature. The sections were then washed in PBS for 15 min. Horseradish peroxidase substrate, 3-amino 9-ethylcarbazole (AEC) (Sigma, St Louis, MO, USA), was used for antibody detection. The sections were counterstained with haematoxylin and then mounted in aqueous media before light microscopic analysis.

Analysis of results

The slides were examined and photographed (D-1 camera, Nikon, Kanagawa, Japan) under light microscopy (Microphot – FXT; Nikon). The pattern and distribution of immunohistochemistry staining were recorded for each of the primary antibodies used. The location of positive staining was categorized into the coronal half, apical half, or furcation area of the root. The distribution of positive staining was divided into paravascular area of the periodontal ligament, extravascular area of the periodontal ligament, or region near the cementum. As this was a descriptive immunohistochemical study, statistical analysis of the data was not undertaken.

Results

Among the 25 healthy teeth studied, 19 had periodontal ligament present, three were positive for the STRO-1 antigen, eight were positive for the CD146 antigen and six were positive for the CD44 antigen. Among the 25 periodontitis-affected teeth, 17 had periodontal ligament present, two were positive for the STRO-1 antigen, 10 were positive for the CD146 antigen and nine were positive for the CD44 antigen (Table 1). In the healthy sections, periodontal ligament was found in the coronal, apical and furcation areas of the root. In the diseased sections, periodontal ligament was found only in the apical and furcation areas of the root.

STRO-1 antigen

Healthy tooth — In the periodontal ligament of healthy teeth, positive STRO-1 staining of cells was detected in the coronal and apical as well furcation areas of the root. The distribution of the staining was sparse and mainly observed on the cells around the paravascular area in the periodontal ligament. In two of the healthy sections, positive staining for STRO-1 was also detected in cells near the cementum. The cells which stained positive for STRO-1 comprised several different morphologies. One group of cells had elongated nuclei and elongated cytoplasm that resembled endothelial cells (Fig. 1A). The other group had enlarged round or oval-shaped, intense haematoxylin-stained nuclei with a smaller amount of cytoplasm (Fig. 1B).

Table 1. Summary of antibody detection and periodontal ligament presence in all specimens (healthy and diseased) processed for immunohistochemistry

	Number of teeth	PDL present	STRO-1	CD146	CD44
Healthy tooth	25	19	3	8	6
Diseased tooth	25	17	2	10	9

PDL, periodontal ligament.

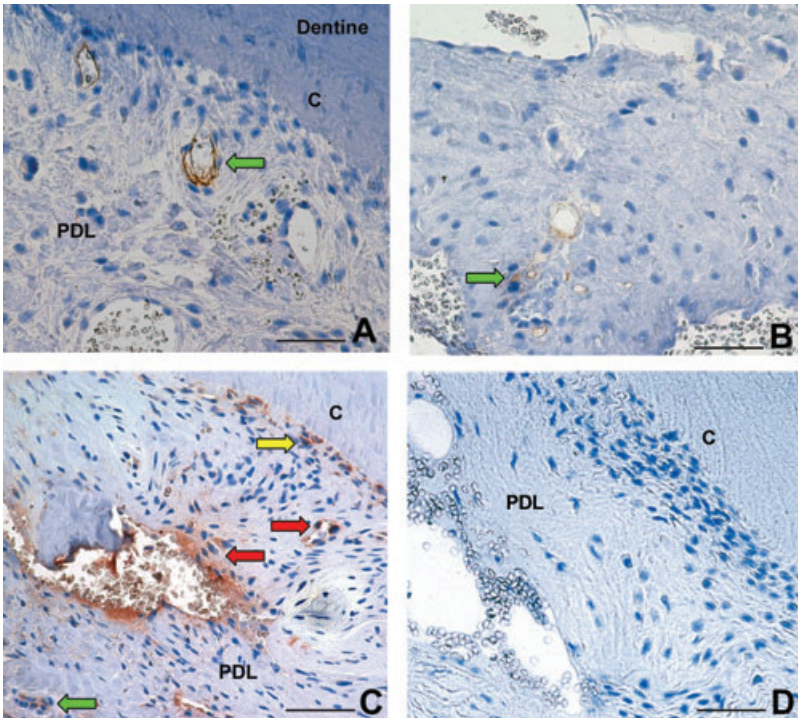

Fig. 1. STRO-1 surface antigen in the periodontal ligament of healthy and periodontitis-affected teeth. (A) Staining occurred around the paravascular area near the apex of the root in healthy periodontal ligament. These cells have elongated nuclei and cytoplasm that resembles endothelial cells. C, cementum; PDL, periodontal ligament. Bar = 50 μm (magnification ×90). (B) Cells in the extravascular area that stain positive for STRO-1 antigen in healthy periodontal ligament. These cells have round nuclei with a small amount of cytoplasm. Bar = 50 μm (magnification ×90). (C) STRO-1 surface antigen in the apical periodontal ligament of a periodontitis-affected tooth. STRO-1 surface antigen was widely distributed in the diseased sections compared with the healthy section. Apart from the positive staining in the paravascular area (red arrow), a small cluster of cells was also observed in the extravascular area (green arrow) and cementum (yellow arrow). Cells with different morphologies can be observed. C, cementum; PDL, periodontal ligament. Bar = 50 μm (magnification ×90). (D) Control section of STRO-1. C, cementum; PDL, periodontal ligament. Bar = 50 μm (magnification ×90).

Periodontitis-affected tooth — In the periodontal ligament of periodontitis-affected teeth, cells staining positive for STRO-1 were detected in the middle and apical portions of the root. More areas of cells with positive staining were observed in the diseased teeth than in the healthy teeth. Positive staining was mainly detected in cells around the paravascular areas of the periodontal ligament; additionally, small clusters of stained cells were also detected in the extravascular spaces and along the cementum. The cells in the paravascular area also comprised two different morphologies. One group had elongated cytoplasm and elongated nuclei which resembled endothelial cells. The second group had large round, or oval-shaped nuclei with more intense haematoxylin staining and a smaller amount of cytoplasm. The cells in the extravascular areas and near the cementum had the appearance of the second group of cells described above (Fig. 1C). Control staining is shown in Fig. 1D.

CD146-CC9 surface antigen

Healthy tooth — Cells expressing the CD146 antigen were observed in the coronal, apical and furcation areas of the healthy teeth. The distribution of these cells was mainly found in the paravascular area within the periodontal ligament (Fig. 2A). A few sparsely distributed solitary stained cells were observed in the extravascular spaces of the periodontal ligament. In addition, some cells in small clusters were also observed in the extravascular spaces. Cells expressing CD146 could be divided into two different morphologies. The cells in the paravascular area had elongated nuclei and elongated cytoplasm, which resembled endothelial cells. The second type of cells observed in this area had round or oval-shaped nuclei which were intensely stained by haematoxylin and had a small amount of cytoplasm. Cells in the extravascular area shared the morphologies of the second group described above (Fig. 2A).

Periodontitis-affected tooth — Cells expressing the CD146 antigen were observed in the apical and furcation area of the periodontal ligament in periodontitis-affected teeth. The distribution of cells which were positive for CD146 in the periodontal ligament of diseased teeth was similar to that for healthy teeth. However, a larger abundance of cells with positive staining was observed in these areas of the periodontal ligament. Small groups of cells were sometimes observed in the extravascular area and near the cementum. The cells in the paravascular area resembled endothelial cells. Other cells with round or oval-shaped nuclei, intensely stained by haematoxylin and with small amounts of cytoplasm, were observed in the paravascular area, the extravascular area as well as near the cementum (Fig. 2B,D).

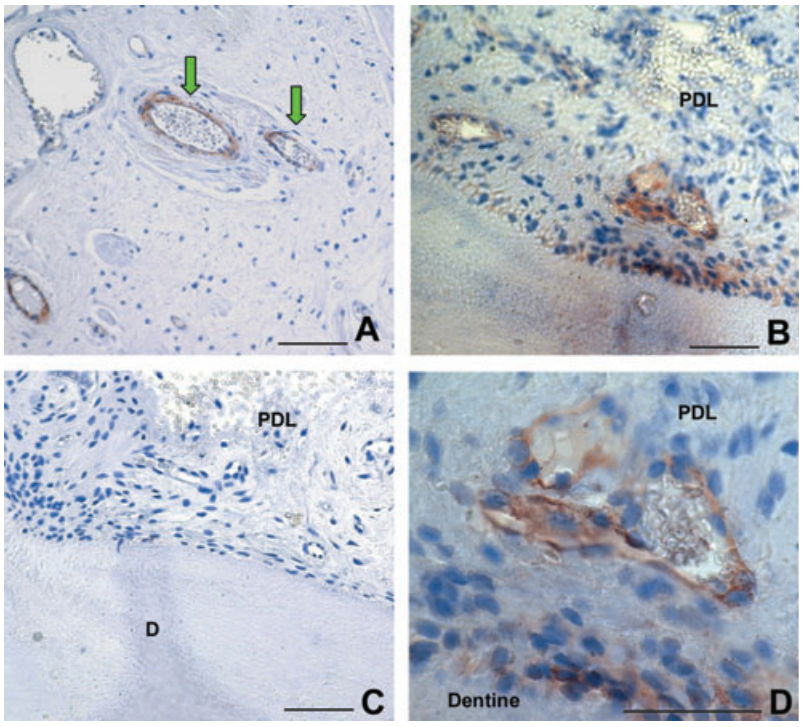

Fig. 2. CD146 antigen in the periodontal ligament of healthy and periodontitis-affected teeth. (A) Healthy tooth. Cells with CD146 antigens were mainly located in the paravascular area. These cells closely resembled endothelial cells. Bar = 50 μm (magnification ×90). (B) Diseased tooth. Cells with CD146 antigen can be observed in the paravascular space as well as in the area near the cementum. D, dentine; PDL, periodontal ligament. Bar = 50 μm (magnification ×60). (C) Control section of a diseased tooth. D, dentine; PDL, periodontal ligament. Bar = 50 μm (magnification ×50). (D) Diseased tooth. Higher magnification of (B) of the cells positive for CD146 staining highlighted their morphologies of small round nuclei with small amounts of cytoplasm. Bar = 25 μm (magnification ×150).

CD44 surface antigen

Healthy tooth — Cells expressing the CD44 surface antigen were observed in the coronal, apical and furcation areas of the periodontal ligament of healthy teeth. The distribution of the staining was less specific and general background staining was found in the periodontal ligament matrix of most sections. However, cells with oval-shaped nuclei and strong haematoxylin staining were identified. These cells were observed in the paravascular areas as well as in the extravascular areas (Fig. 3A).

Diseased tooth — In diseased teeth, cells positive for the CD44 antigen were found in the apical and furcation areas of the periodontal ligament. The pattern of distribution was similar to that of the healthy sections. However, the nonspecific background staining was more intense in the diseased sections

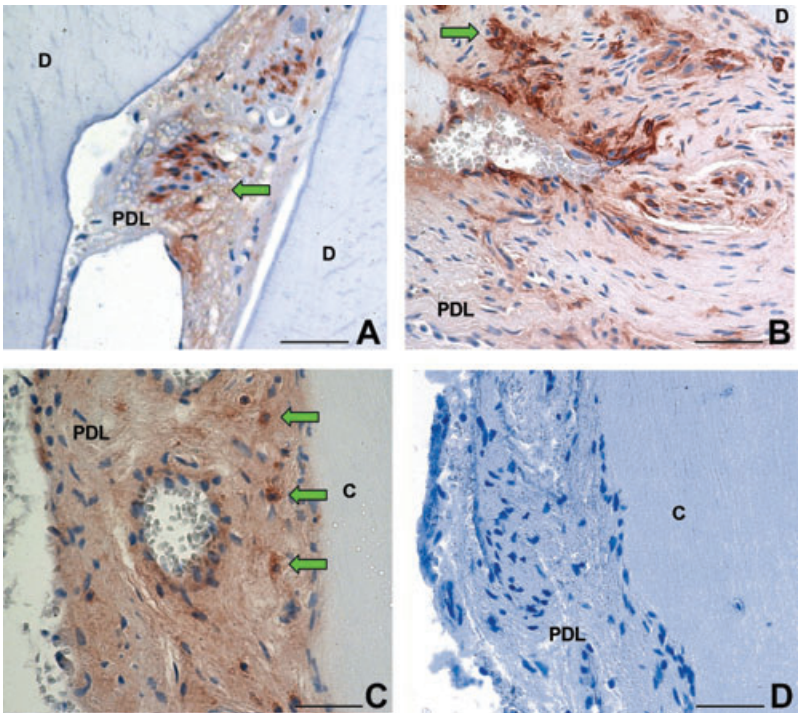

Fig. 3. CD44 antigen in the periodontal ligament of healthy and periodontitis-affected teeth. (A) Healthy tooth. D, dentine; PDL, periodontal ligament. Bar = 50 μm (magnification ×90). (B) Diseased tooth. D, dentine; PDL, periodontal ligament. The intense CD44 staining around the paravascular areas and the extravascular areas (green arrows) were consistent with STRO-1 staining. Bar = 50 μm (magnification ×90). (C) Diseased tooth. D, dentine; PDL, periodontal ligament; C, cementum. Green arrows showed cells with CD44 antigen along the region near the cementum. Bar = 25 μm (magnification ×90). (D) Control section from healthy tooth. Bar = 50 μm (magnification ×90).

(Fig. 3B). Apart from the staining in the extravascular and paravascular areas, intense staining was observed in the areas near the cementum (Fig. 3C). Cells in the extravascular area and in the region near the cementum shared similar morphologies to those observed with positive staining for CD146 and STRO-1.

Discussion

Previous immunohistochemical staining and immunofluorescence staining of frozen fragments of periodontal ligament from extracted human third molars using STRO-1 has shown that putative stem cells are present in the human periodontal ligament (20). However, because only fragments of tissue were used, it was difficult to localize the putative stem cells in relation to the surrounding tissues, and the presence of putative stem cells in periodontitis-affected tissue reamined unknown. In the present study we have shown that human periodontal ligaments from healthy and periodontitis-affected teeth contain a population of cells with immunohistochemical features consistent with putative postnatal stem cells.

Among the samples, 26% of normal teeth and 32% of periodontitis-affected teeth did not have periodontal ligament present and could not be used for analysis in this study. The mechanical trauma during extraction, the disease process and the tissue-preparation technique may all have contributed to the loss of the periodontal ligament.

In healthy teeth, the putative stem cells were found in the periodontal ligament along coronal, apical and furcation parts of the root surfaces. In periodontitis-affected teeth, the coronal part of the periodontal ligament was destroyed by the disease process. However, periodontal ligament stem cells were still found in the periodontal ligament along the apical and furcation parts of the root surfaces. As a result of the small sample size it was not possible to draw definitive conclusions as to where the putative stem cells were predominantly located along the root surfaces. Nonetheless, the overall distributions of putative stem cells in periodontal ligament were sparse. This is in concordance with previous findings (20). Although not verified by statistical analyses because of the small sample size, the distribution of such cells appeared to be more abundant in the periodontal ligaments from periodontitis-affected teeth.

Progenitor cells have been identified previously in the periodontal ligament of mice (10). They originate from the endosteal spaces of alveolar bone and subsequently migrate to the paravascular areas of the periodontal ligament (9,10). These progenitor cells are located in the area 0–20 μm from the perimeter of blood vessels. McCulloch hypothesized that based on cell kinetic and cell morphology observations, these progenitor cells were stem cells residing in the periodontal ligament (8). As the samples in the current study did not include fragments of alveolar bone, it was not possible to determine whether the cells identified were associated with this region. The localization of putative stem cells in the present study to the paravascular regions was consistent with the location of progenitor cells described previously.

Apart from the positive staining in the paravascular area, periodontal ligament stem cells were also identified in the extravascular areas and regions near the cementum. In the periodontal ligament of healthy teeth, putative stem cells in the extravascular area were sparse; however, in the periodontal ligament of periodontitis-affected teeth, a greater abundance of positively stained cells were identified in the extravascular area as well as near the cementum. The increase in stem cell populations in the extravascular area and the region near the cementum could be caused by the early recruitment and migration of paravascular cells, as observed in the wounded mouse periodontal ligament (1,6).

Putative stems cells may respond to changes in the extracellular matrix and inflammatory cytokines. Through subsequent differentiation and proliferation, they may contribute to the wound healing of damaged periodontal ligament.

The combination of STRO-1 and CD146 antibodies has been used to isolate homogeneous stem cell populations from periodontal ligament (18). The isolation of clonogenic and multilineage potential cells from diverse tissues has qualified these two antibodies as suitable markers for different mesenchymal stem cell populations. In the present study, these antibodies detected two distinct cell phenotypes. One group of cells resembled endothelial cells: they had elongated nuclei and elongated cytoplasm, which was typically observed near the blood vessels. This finding is probably the result of cross-reactivity of STRO-1 and CD146 antibodies with blood vessel walls, and suggests that some of the paravascular cells could be endothelial cells (17,20). Similarly, other reported mesenchymal stem cell markers, such as CD105, CD106 and CD166, are also expressed by both perivascular cells and endothelial cell populations (17,22).

Apart from the endothelial cells, the other group of cells identified in this study had round/oval-shaped prominent nuclei with intense haematoxylin staining and relatively small amounts of cytoplasm. These cells are likely to be stem cells as they share similar morphologies with the stem cells isolated from dental pulp and bone marrow, including prominent nuclei and agranular cytoplasm (1,17,23).

The isolation and identification of pure stem cells may require further investigations, based on DNA characterization and identification using more specific markers for stem cells (18).

CD44 cell-surface antigens have been reported on stem cells from bone marrow and dental pulp (20). CD44 is a very broad class of transmembrane glycoprotein that is expressed in the extracellular matrix and on the cell surface of epithelial, mesenchymal and hematopoietic cells. While CD44 is well recognized as being of importance for lymphocyte homing, its function on epithelial cells and fibroblasts is not clear, but it is presumed to be associated with cell adhesion and the binding of extracellular matrix components, such as fibronectin, laminin, collagen and hyaluronan (24,25). This surface antigen is also expressed by gingival fibroblasts, gingival epithelial tissues as well as periodontal ligament in humans; furthermore, its expression is regulated by inflammatory cytokines *in vitro* (26). This could explain the nonspecific background staining in the extracellular matrix and heterogeneous populations of cells identified by CD44 from both healthy and periodontitis-affected periodontal ligament. Thus, CD44 may not be a particularly useful histological marker for stem cells.

While staining of serial sections would have been beneficial for defining periodontal ligament stem cells, it was difficult to achieve this owing to technical difficulties. A number of sections were lost during antigen-retrieval procedures. However, the morphology and the location of cells identified by these three antigens suggests that among the heterogeneous cell population identified by these antigens, some of these cells are likely to be stem cells.

In conclusion, by using antibodies against cell-surface antigens known to be expressed on different mesenchymal stem cell populations (STRO-1, CD146 and CD44), this study has localized cells that can be considered potentially as postnatal stem cells in healthy and periodontitis-affected human periodontal ligament. This study has also highlighted the possible changes of putative stem cell populations in the periodontal ligament in periodontitis. Understanding the recruitment of these locally derived stem cells with the capacity to develop into either periodontal ligament-forming cells or mineral-forming cementoblasts, which combine to secure the connection between the cementum and the adjacent alveolar bone, will help to achieve more predictable regeneration of periodontal tissues in the future.

Acknowledgements

This project was supported by funds from the National Health and Medical Research Council of Australia, the Australian Dental Research Foundation and the Australian Periodontology Research Foundation

References

1. Gould TR, Melcher AH, Brunette DM. Migration and division of progenitor cell populations in periodontal ligament after wounding. *J Periodont Res* 1980;**15:**20–42.
2. Schroeder HE. The periodontium. In: Schroeder HE, ed. *Handbook of Microscopic Anatomy*. Berlin: Sprinter, 1986: 23–129.
3. Cho MI, Garant PR. Ultrastructure evidence of directed cell migration during initial cementoblast differentiation in root formation. *J Periodont Res* 1988;**23:** 268–276.
4. Cho MI, Garant PR. Radioautographic study of [^{3}H] mannose ultilization during cementoblast differentiation, formation of acellular cementum, and the development of periodontal ligament principal fibers. *Anat Rec* 1989;**223:**209–222.
5. Cho MI, Garant PR. Development and general structure of the periodontium. *Periodontol 2000* 2000;**24:**9–27.
6. McCulloch CAG, Melcher AH. Cell density and cell generation in the periodontal ligament of mice. *Am J Anat* 1983;**167:** 43–58.
7. McCulloch CAG, Melcher AH. Continuous labelling of the periodontal ligament of mice. *J Periodont Res* 1983;**18:**231–241.
8. McCulloch CAG. Progenitor cell populations in the periodontal ligament of mice. *Anat Rec* 1985;**211:**258–262.
9. McCulloch CAG, Nemeth E, Lowenberg B, Melcher A. Paravascular cells in endosteal spaces of alveolar bone contribute to periodontal ligament population. *Anat Rec* 1987;**219:**233–242.
10. McCulloch CAG, Barghava U, Melcher AH. Cell death and regulation of populations of cells in the periodontal ligament. *Cell Tiss Res* 1989;**255:**129–138.
11. Lekic P, McCulloch CAG. Periodontal ligament cell populations: the central role of fibroblasts in creating unique tissue. *Anat Rec* 1996;**245:**327–341.
12. Majumdar MK, Keane-Moore M, Buyaner D *et al.* Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci* 2003;**10:**228–241.
13. Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood* 1991;**78:**55–62.

14. Shih I. The role of CD146 (Mel-CAM) in biology and pathology. *J Pathol* 1999;**189:**4–11.
15. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* 2000; **97:**13625–13630.
16. Gronthos S, Brahim J, Li W *et al*. Stem cell properties of human dental pulp stem cells. *J Dent Res* 2002;**81:**531–535.
17. Gronthos S, Zannettino AC, Hay SJ *et al*. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. *J Cell Sci* 2003;**116:**1827–1835.
18. Seo B, Miura M, Gronthos S *et al*. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004;**364:**149–155.
19. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* 1976;**34:**235–249.
20. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and pulp. *J Bone Miner Res* 2003;**18:**696–704.
21. McCulloch CAG, Barghava U, Melcher AH. Cell death and regulation of populations of cells in the periodontal ligament. *Cell Tissue Res* 1989;**255:**129–138.
22. Pittenger MF, Mackay AM, Beck SC *et al*. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;**284:**143–147.
23. Gould TR. Ultrastructural characteristics of progenitor cell populations in periodontal ligament. *J Dent Res* 1983;**62:** 873–876.
24. Hakkinen L, Oksala O, Salo T, Rahemtulla F, Larjava H. Immunohistochemical localization of proteoglycans in human periodontium. *J Histochem Cytochem* 1993;**41:**1689–1699.
25. Bartold PM, Narayanan AS. Proteoglycans. In: Bartold PM, Narayanan AS, eds. *Biology of the Periodontal Connective Tissue*. Illinois: Quintessence Publishing Co., Inc., 1998: 121–147.
26. Hirano F, Hirano H, Hino E *et al*. CD44 isoform expression in periodontal tissues: cell-type specific regulation of alternative splicing. *J Periodont Res* 1997;**32:**634–645.