Healing of periodontal tissues destroyed by periodontal disease does not usually result in the regeneration of the periodontium. One of the often repeated reasons is the lack of progenitor cell populations capable of restoring the different tissues of the periodontium. The fibroblast is the predominant cell type in the soft connective tissue of the periodontium. These cells were described as the architect, builder and caretaker of the periodontum. It is not clear whether a single progenitor cell for fibroblasts, osteoblasts and cementoblasts exists in the adult periodontal tissue. Several early studies have focused on the identification of progenitor cell populations in the periodontal ligament of experimental animals. The lack of specific markers for the fibroblast progenitors has hampered the precise identification of the origin and location of these cells. It has been suggested that the cells of fibroblastic series may resemble, other well

Immunolocalization of CD 34 positive progenitor cells in healthy human gingiva - a pilot study

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Received November 21, 2007

Background & objectives: The gingiva is a tissue with a high turnover rate of both epithelial and connective tissue cells. In an attempt to identify the possible source of cells which maintain the tissue turnover, we used CD 34, a well established marker of peripheral blood stem cell in healthy human gingiva to determine the origin of progenitor cells in healthy gingiva.

Methods: Healthy human gingival samples (n=15) were collected from patients undergoing orthodontic extraction. Immunohistochemistry was done on 5 micron paraffin fixed section using the primary antibody CD34 and a universal secondary immunoperoxidase kit. The sections were examined for a golden brown stain indicative of a positive staining.

Results: Of the 15 samples 12 demonstrated a positive staining for the endothelial cells. Of these 12 samples, 11 demonstrated positive staining for stromal and paravascular cells and 10 a positive staining for the basal epithelium layers.

Interpretation & conclusions: The presence of CD 34 positive cells in gingiva in stromal, paravascular location, and basal layer of the gingival epithelium was demonstrated. We speculate that these could be fibroblastic progenitors originating from the peripheral blood stem cells and the positivity stained epithelial cells could be gingival epithelial stem cells.

Key words CD 34 expression - gingival - immunohistochemistry - progenitor cells
described self renewal systems in mammals, such as
the skin, liver, peripheral blood, etc. CD 34 is a surface
glycophosphoprotein expressed on haemopoietic
stem and progenitor cells, small vessel endothelial
cells and embryonic fibroblasts. CD 34 positivity has
been demonstrated for fibroblasts and stromal cells in
tissues such as skin, thyroid gland, testicular stroma
and also in the oral tissues like sub mandibular gland,
and oral mucosa. The gingiva is a highly vascular
tissue. The progenitors in gingiva may be arising from
the peripheral blood haemopoietic stem cells which are
CD 34 positive. To test the above hypothesis, CD 34
was used as a marker in the present study to identify
putative progenitor cells in the healthy human gingiva
and their location. The objective was to determine the
possible origin of progenitor cells in healthy human
gingiva and its implications in regeneration of the
periodontium.

Material & Methods

The subjects who gave informed consent were
selected, the others were not included. A total of
twenty patients were chosen based on inclusion and
exclusion criteria. The small sample size is because
the study is a pilot study. No special procedure was
followed for randomization. All the subjects had
visited the Out Patient Department of Oral Surgery,
Sri Ramachandra Dental College, Porur, Chennai,
during April to August 2005 for the purpose of
extraction of teeth for orthodontic purposes. Healthy,
non smoker individuals, who had not undergone any
periodontal treatment in the past six months were
selected. Among these patients, those who had healthy
gingiva as determined by clinical findings such as
colour, consistency, absence of bleeding on probing,
absence of probing depth/loss of attachment were
included in the study. Informed written consent was
obtained from each patient prior to sample collection.
The study has been approved by Institutional Ethics
Committee of the Sri Ramachandra University. The
healthy gingival samples were collected by surgical
excision of a part of the papilla either distal or mesial
to the tooth to be removed. The gingival samples were
washed in sterile saline to remove the blood and fixed
with 4 per cent buffered formalin for 24 h following
which these were embedded in paraffin wax. The
embedding in paraffin wax and subsequent steps of
tissue sectioning and staining were performed in the
Pathology Division of Bharat Scans, Chennai. Two
5 µm thick sections were taken on 3-aminopropyltri-
ethoxysilane (APES) (Sigma, USA) coated slides,
one of which was used for hematoxylin and eosin
(H&E) (Merck, Germany) staining, the other used
for immunohistochemistry. The sections were de-
paraffinized and re-hydrated and antigen retrieval (by
microwave processing) was performed. The primary
antibody used was the antibody to CD 34 class III
epitope (Chemicon International, USA Catalog
No- CBL 555). The primary antibody was diluted
with phosphate buffered saline, pH 7.4 to obtain
an end dilution of 1: 20. A Universal Secondary
Immu-no-peroxidase Kit was used (B-SAP Universal
Staining Kit- Span Diagnostics). It contains the
secondary antibody tagged with peroxidase and also
the substrate for the enzyme. Following staining, the
sections were examined under the microscope (Zeiss
Microscope-Axiostar Plus with attached Samsung
Photomicrography equipment, Romania) for a golden
brown stain which is indicative of positive staining.
A section of a pyogenic granuloma obtained from lip
lesion was used as a positive control specimen for the
antibody to CD 34. A drop of phosphate buffered saline
(pH 7.4) was used instead of the primary antibody in
the negative control specimens. Counting of cells in the
positively stained sections was done under 100 X oil immersion. The results were expressed as the
number of positively stained cells per 10 high power
fields (modification of the method described by Mesa
et al). Basically, in the modified method, the counting
was done for 10 consecutive high power fields.

Results

Of the 15 healthy gingival samples, 12 exhibited a
positive staining for the CD 34 antigen. Eleven of these
12 samples demonstrated the presence of positively
stained stromal/paravascular cells in the connective
tissue (Fig. 1), and ten demonstrated positive staining
for the basal and parabasal layers of the epithelium (Fig.
2). All the 12 samples demonstrated a positive staining
for the endothelial cells. Cell membrane positivity
for the CD 34 antigen was detected in the basal and
parabasal layers of the epithelium. Of the ten positive
samples, four exhibited a focal positive staining,
whereas six exhibited a uniform staining of the entire
basal layer. Of the eleven samples that stained positive
for the connective tissue cells, six demonstrated more
number of stromal cells than paravascular cells, and
four demonstrated more number of para-vascular cells,
with one section showing equal distribution of cells in
the paravascular and stromal locations. Some cells had
a fibroblastic morphology, whereas the others had a
round or oval shape.
The positive control sample (pyogenic granuloma) demonstrated a positive staining of the endothelial cells (Fig. 3) and the negative control sample was characterized by complete absence of the golden brown stain (Fig. 4).

**Discussion**

Of the two prominent fibroblast populations in the periodontium- the gingival and periodontal ligament fibroblasts, the origin of gingival fibroblast is less clear. Tencate and co-workers\(^1\) suggested that gingival fibroblasts may be derived from the dental follicle cells or from the non-odontogenic gingival connective tissue. However, the origin of these cells in the adult periodontium is not known. The gingival epithelium as well as connective tissue have a very high turnover rate and new cells are constantly produced to replace cells, which are lost through cell death or migration. Thus, existence of a lineage of cells, which constantly replenish the fibroblast population, cannot be doubted.

Krause and co-workers\(^1\) have shown that a single donor haemopoietic stem cell (HSC) could do more than just repopulate the marrow and haemopoietic system in the recipient irradiated mice. They found epithelial cells derived from the donor stem cells in the lungs, gut, and skin of the recipient mice. The most well characterized marker for the peripheral blood stem cells in humans as well as other mammals has been the sialomucin CD 34. Berardi et al\(^1\), found

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*Fig. 1.* Photomicrograph of a section of healthy human gingiva with positive staining of a para-vascular and stromal cell.

*Fig 2.* Photomicrograph of a section of healthy human gingiva with a cell membrane positive staining of the basal and parabasal layer of gingival epithelium.

*Fig. 3.* Photomicrograph of a section of pyogenic granuloma (Positive control) with a positive staining of the endothelial cells.

*Fig. 4.* Photomicrograph of a section of healthy human gingiva in which no primary antibody was used. (Negative control) with no golden brown staining.

Figs 1-4 - 40 X magnification.
that CD 34 is expressed on a population of pluripotent progenitors that can be enriched from the human bone marrow. In another study Schmidt et al.3 determined that CD 34 positive fibrocytes in the peripheral blood were precursors of bronchial fibroblasts.

A paravascular location for the fibroblast progenitors has been described by Nemeth et al.8 in the gingiva of the cynomolgus monkey. An earlier work by Pender et al.76 did not describe any paravascular location but identified two distinct progenitor connective tissue cell populations in the rat gingiva; one population was located in the mid papilla region and the other close to the epithelial attachment and cementum. Our study showed that CD 34 positive cells were present in the healthy human gingiva; similar to lower species, some of these cells had a parascular location whereas some demonstrated a stromal location. Three of the 15 gingival samples were not stained. This could possibly be due to technical errors in processing or these sections might not have contained the target cells.

CD 34 positive cells have been identified to be markers for stem cells in the epidermis of mouse skin30,31. Song et al.32 studied the pathogenesis of Pterygium; a proliferative disease affecting the conjunctiva of humans. They hypothesized that adult stem cells from human bone marrow contributed to the development of Pterygium. They found that the basal layer epithelial cells were CD 34 positive in addition to being positive to a few other markers. In our study, we found cell membrane positivity for the CD 34 antigen in the basal and parabasal layers of the gingival epithelium.

The present study had certain limitations, such as the lack of use of negative markers (Thy-1, c-kit etc.) which would have more definitively established the presence of stem cells of haemopoietic origin.

In conclusion, in this pilot study, we demonstrated the presence of CD 34 positive cells in the healthy human gingiva. These cells in the paravascular location could be fibroblast progenitors and the possible precursors of these progenitors could be the peripheral blood stem cells. The results of this preliminary study warrant further investigation.

References


6. Brown J, Greaves MF, Molgaard HV . The gene encoding the human progenitor cell antigen (CD34) and CD 34 positive cells have been identified to be markers for stem cells in the epidermis of mouse skin30,31. Song et al.32 studied the pathogenesis of Pterygium; a proliferative disease affecting the conjunctiva of humans. They hypothesized that adult stem cells from human bone marrow contributed to the development of Pterygium. They found that the basal layer epithelial cells were CD 34 positive in addition to being positive to a few other markers. In our study, we found cell membrane positivity for the CD 34 antigen in the basal and parabasal layers of the gingival epithelium.

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References


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