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### **Original article**

# Stem cell factor in gingival crevicular fluid in periodontal health and disease

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**Background:** Stem cell factor is a pleiotropic cytokine produced by several cell types including fibroblasts, bone marrow stromal cells, mast cells, and endothelial cells. In addition, stem cell factor is an important hematopoietic growth factor, which binds to and activates the ligand for the typosine kinase-type receptor *c-kit*.

*Materials and methods:* Forty-five subjects (aged 24 to 75 years) were classified into the following three groups according to their periodontal tissue status as group I (clinically healthy gingiva with no loss of attachment), group II (gingivitis with no attachment loss), and group III (periodontitis). GCF samples collected from each patient were examined for stem cell factor level using enzyme-linked immunosorbant assay.

**Results:** The maximum level of stem cell factor in GCF was obtained for group III (71.8 $\pm$ 7.8 pg/g protein), and the lowest mean stem cell factor concentration in GCF was observed for group I (22.1 $\pm$ 7.3 pg/g protein). The GCF stem cell factor level of patients in group III was statistically higher than that in group II (p <0.04) and group I (p <0.001). In addition, the mean GCF levels of stem cell factor in group II (48.1 $\pm$ 7.5 pg/g protein) were significantly higher than those in group I (p <0.02). There was a positive correlation between stem cell factor in GCF and gingival inflammation index (r=0.59, p <0.001)

*Conclusion:* GCF levels of stem cell factor increased in parallel with the severity of periodontal disease. Its levels in GCF could be potentially useful as a biochemical marker of periodontal inflammation and the host response.

Keywords: Gingival crevicular fluid, gingivitis, inflammation, periodontitis, stem cell factor.

Human periodontal diseases are characterized by chronic inflammatory destruction of periodontal connective tissues and result from complex interaction between periodontal pathogens and the host defense mechanism [1]. The inflammatory host response is a fundamental feature of periodontal disorder and is regarded as the primary cause for periodontal breakdown [2]. Dental plaque is capable to initiate the local host response via recruitment and activation of inflammatory cells including monocytes/ macrophages, lymphocytes, and mast cells [3]. It has been established that under the stimulation of bacterial products, monocytes/macrophages, lymphocytes, mast cells, and local host cells produce a broad spectrum of cytokines and chemokines [4]. It was also demonstrated that stem cell factor expression was extensively upregulated and is consistently associated with an infiltrate of inflammatory cells during inflammatory disease [5].

Stem cell factor, also known as mast cell growth factor, steel factor, and *c-kit* ligand, is a multifunctional growth factor for hematopoietic progenitors and mast cells [6]. Stem cell factor receptor has been characterized as the *c-kit* proto-oncogene product, a member of the type III receptor tyrosine kinase family [7]. Several studies demonstrate that stem cell factor is synthesized by fibroblasts, endothelial cells, and mast cells [8]. Mast cell proliferation, survival,

**Objectives:** Analyze concentration of stem cell factor within gingival crevicular fluid (GCF) in both periodontal health and disease and to determine the correlation of stem cell factor in GCF and inflammatory status of periodontal tissues.

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and differentiation are under the control of the surrounding stroma. There is mounting evidence that overexpression of stem cell factor may be implicated in proliferation, inflammation, and wound healing [9].

Gingival crevicular fluid (GCF) is a serum transudate or inflammatory exudate in combination with white blood cells, periodontal cells, and oral bacteria. The composition of the GCF is the consequence of the interaction between the bacterial biofilm adherent to the tooth surfaces and the cells of the periodontal tissues. GCF levels of various tissue degradation products, inflammatory mediators like cytokines and growth factors increase with time in periodontal disease. GCF accumulation is a noninvasive, relatively simple and site specific procedure. Numerous GCF constituents have been characterized to identify biomarkers, which exhibit a great potential for serving as diagnostic or prognostic markers of the periodontal health and disease [10]. It is believed that the measurement of stem cell factor in GCF could be of use for prediction of the prognosis of periodontal disease. To our knowledge, there are no reports on the association of gingival crevicular fluid levels of stem cell factor with disease severity in patients with periodontal disease. Therefore, we hypothesized that stem cell factor in GCF could be associated with the severity of periodontal disease. Accordingly, the present study was conducted to investigate stem cell factor levels in the gingival crevicular fluid of patients with periodontal disease and compare with clinically healthy subjects in order to determine the relationships between gingival crevicular fluid levels of stem cell factor with the severity of periodontal disease.

#### Materials and methods

Ethical clearance of the present study was acquired from the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University. This study was conducted in compliance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from the patients who agreed to participate voluntarily in the study.

#### **Population**

The study population consisted of 45 subjects (39 females and 6 males) attending the outpatient clinic of the Department of Dentistry, King Chulalongkorn Memorial Hospital. The mean age of the patients was

42.1±1.5 years (range: 24-75 years). All subjects were informed regarding personal data, oral hygiene care, and related medical history. Patients with a history of diabetes mellitus, ischemic heart disease, or any other conditions contributing to atherosclerosis, pregnancy condition, and seizure were excluded from the study. The inclusion criteria were dentated patients with six teeth as followed by classification (or six mentionedteeth). If any patients loss some of them, they should be compensated by other adjacent teeth. Six teeth were examined such as right upper first molar and lateral incisor, left upper first premolar, left lower first molar and lateral incisor and right lower first premolar, etc. The patients were categorized into three groups according to their periodontal tissue status. Group I (healthy) consisted of 15 subjects who had clinically healthy gingiva without loss of clinical attachment, and they were aged between 24 and 50 years. Group II (gingivitis) comprised 15 patients who showed clinical signs of gingival inflammation without attachment loss. Patients in this group were between 24 and 75 years of age. Fifteen patients aged between 31 and 60 years who had signs of clinical inflammation with loss of clinical attachment constituted group III (periodontitis).

#### Gingival crevicular fluid collection

The GCF was accumulated from the four preselected sites of six mentioned-teeth, the four deepest sulcuses were selected in each patient. The identified test sites were gently air-dried, isolated with cotton rolls, and supragingival plaque was lightly removed with sterile periodontal curette.

The GCF was collected from four sites per patient using Durapore filter membranes (pore size=0.22 µm; Millipore, Bedford, USA). The Durapore strip was placed gently at the entrance of the gingival sulcus/ crevice until the light resistance was felt, care being taken to avoid mechanical injury, and left in place for 60 seconds. Samples that were suspected to be contaminated with blood and saliva were excluded from the study. After collection of the gingival fluid, the strips were immediately transferred in microcentrifuge tubes and stored frozen at -80°C for subsequent analysis. On the day of the analysis, 250 µL of phosphate buffer saline (pH 7.4) was added to the tubes containing the paper strips. The tubes were vigorously shaken for 15 minutes and then centrifuged at 2000 g for five minutes, with the strips kept at the collar of the tube to elute GCF components completely.

## Enzyme-linked immunosorbent assay for stem cell factor

Double-blind quantitative detection of stem cell factor in GCF was performed using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, USA) according to manufacturer's protocols. Briefly, standards of recombinant human stem cell factor, GCF samples were added to 96-well microtiter plates precoated with mouse monoclonal antibody against human stem cell factor and incubated for two hours at room temperature. Then, the wells were washed four times with washing buffer and incubated for two hours at room temperature with a horseradish peroxidase-conjugated monoclonal antibody against stem cell factor. After four washes, substrate solution was added to each well, and the plate was incubated for thirty minutes at room temperature in the dark. Finally, the reaction was stopped with the stop solution, and absorbance was measured at 450 nm using an automated microplate reader. Recombinant human stem cell factor was used to generate a linear standard calibration curve (range: 0-1000 pg/mL), allowing the concentration of stem cell factor in each sample to be determined. Protein concentrations in all the GCF samples were evaluated using the BCA protein assay kit (Pierce Biotechnology, Rockford, USA). Then, the stem cell factor concentration in the samples was determined as pg/g of protein.

#### Statistical analysis

Baseline demographic and clinical characteristics were summarized by a mean and standard error of the mean ( $\pm$  SEM). Comparisons between the groups were performed using one-way analysis of variance (ANOVA). Differences among the three groups were analyzed with a post hoc Tukey test. Pearson's correlation coefficient (r) was performed to determine the correlation between the stem cell factor levels in GCF and the severity of periodontal disease. A p-value <0.05 was considered to indicate statistical significance. The statistical analysis was carried out with the statistical package for social sciences (SPSS) software, version 16.0 for Windows (SPSS, Chicago, USA).

#### Results

The demographic data of the study population were illustrated in **Table 1**.

Forty-five participants were recruited for the measurement of stem cell factor in gingival crevicular fluid and clinical periodontal assessments. In group I, stem cell factor was between 0 and 75.0 pg/g protein, in group II, it ranged between 5.1 and 108.0 pg/g protein, and in group III, values fell between 27.5 and 152.3 pg/g protein. The mean, median and the range values of the GCF levels of stem cell factor in group I, II and III, as well as the standard error of the mean are displayed in **Table 2**.

Variables	Parameters	GCF Stem cell factor Number (%)	
All		45 (100)	
Age at visit in years (mean±SEM)		42.1±1.5	
Body mass index	$> 30  \text{kg/m}^2$	4 (8.9)	
	$25-30 \text{ kg/m}^2$	12 (26.7)	
	$<25 \text{ kg/m}^2$	29 (64.4)	
Gender	Male	6(13.3)	
	Female	39 (86.7)	
When visits dentist	Not regular	14(31.1)	
	Regular	31 (68.9)	
Alcohol intake	Yes	10(22.2)	
	No	35 (77.8)	
Smoking history	Yes	3 (6.7)	
	No	42 (93.3)	
Hypertension	Yes	2 (4.4)	
	No	43 (95.6)	

Table 1. Demographic data and subject characteristics of study population.

Group	Minimum level of stem cell factor (pg/g protein)	Maximum level of stem cell factor (pg/g protein)	Difference between maximum and minimum levels of stem cell factor (pg/g protein)	Mean (pg/g protein)	Median (pg/g protein)	SEM
Ι	0	75.0	75.0	22.1	8.1	7.3
II	5.1	108.0	102.9	48.1	32.8	7.5
III	27.5	152.3	124.8	71.8	67.6	7.8

Table 2. Descriptive statistical values of stem cell factor levels in the gingival crevicular fluid of the three groups.

The GCF stem cell factor levels from group I were 22.1 $\pm$ 7.3 pg/g protein, those from group II were 48.1 $\pm$ 7.5 pg/g protein, and those from group III were 71.8 $\pm$ 7.8 pg/g protein (**Fig. 1**). The results demonstrated that GCF stem cell factor levels in group II (p <0.04) and group I (p <0.001). Moreover, the mean GCF levels of stem cell factor in group II were significantly higher than those in group I (p <0.02). The analysis of variance revealed that the difference in GCF levels of stem cell factor between these groups is statistically significant at p <0.001.

In addition, we investigated the relationship between stem cell factor in GCF and the severity of periodontal disease. The present study showed that there was a positive correlation between stem cell factor in GCF and gingival inflammation index (r=0.59, p < 0.001) (**Fig. 2**).

#### Discussion

Periodontal diseases result from the inflammatory processes that occur in the tissues around the teeth in response to microbial dental plaque on the teeth. They are characterized by the classic hallmarks of the inflammatory response, including erythema and edema [2]. The progression of periodontal diseases relies upon complex interaction between periodontal pathogens and cells of the host immune system. These interactions are mediated by cytokines produced by numerous inflammatory cells in periodontal tissues, and these cytokines represent a major component of the immune response to bacterial antigens. These responses either result in the successful removal of the pathogens or lead to host-mediated destruction of the periodontal tissues [11].

Gingival crevicular fluid is a serum ultrafiltrate of blood deriving from the vasculature subjacent to the



Fig. 1 Graph showing statistically significant differences in mean stem cell factor concentration among the three groups.



Fig. 2 Stem cell factor levels in gingival crevicular fluid positively correlated with severity of periodontal disease (r=0.59, p < 0.001).

sulcus. GCF flow rates and the elevated concentration of substances that stimulate innate and adaptive immune responses are associated with the severity of periodontal inflammation. The detection of some biomarkers can provide useful information concerning tissue destruction. Since most patterns of periodontal disease are site specific, one approach is to analyze the molecules in the GCF that correlate with the degree of disease severity and tissue deterioration [12]. Numerous GCF composites have been characterized to identify biochemical parameters that might be used to monitor the progression of gingival inflammation and possess a potential for prognostic indicators of the periodontal health and disease [13].

Stem cell factor or *c-kit* ligand is a cytokine produced by several cell types including fibroblasts, bone marrow stromal cells, mast cells, and endothelial cells, and it is critical in hematopoiesis [14]. Stem cell factor is encoded by the Steel (*Sl*) locus in mice and is the ligand for the tyrosine kinase-type receptor *c-kit* [15]. However, the role of stem cell factor in the pathogenesis of periodontal disease has not been fully elucidated. In the present study, we examined the GCF levels of stem cell factor and determined the relationship between stem cell factor levels and the periodontal tissue status.

The present report has established the presence of stem cell factor in GCF of healthy or gingivitis individual, and provided evidence of a possible association between stem cell factor production and clinical outcome. The current study illustrated that the mean concentration of stem cell factor in GCF was elevated progressively from health to periodontal disease, with the average concentration in gingivitis falling in between the former values. There are two possible mechanisms to explain the reason of high stem cell factor levels in GCF. Elevated stem cell factor levels in GCF are plausibly caused by either the release of stem cell factor residing in gingival and periodontal tissues, or by increased production, or both. Another possible explanation is that mast cells have been identified in both healthy and inflamed gingival, in different numbers at various sites [16-21]. The number of mast cells significantly increased in gingivitis/ periodontitis lesions compared with clinically healthy gingival tissues [22]. Therefore, mast cells and endothelial cells in the local tissues could express increase endogenous stem cell factor in GCF. Moreover, stem cell factor could be originated from blood or salivary gland. In addition, the spillover of these increased GCF stem cell factor levels from diseased periodontal tissues could lead to a concomitant increase in serum stem cell factor.

This study inevitably has some limitations. Firstly, the cross-sectional study design precludes us from evaluating the exact nature of the elevated GCF levels of stem cell factor. Secondly, the method used to determine the severity of periodontal disease might not be the best assessment of local inflammation. Furthermore, the diversity of the stem cell factor concentration in each group can be ascribed to the various stages of the disease process at the time of sample collection. Few samples from group II showed values nearing those of group III, which could be attributable to near conversion of a gingivitis lesion without attachment loss to a gingivitis lesion with attachment loss that is not clinically detectable or it could be due to a limited sample size. A few of the samples from group I also showed values nearing those of group II, which can be attributed to the subclinical levels of inflammation in clinically healthy tissues. Despite these limitations, we observed a significant relationship between GCF levels of stem cell factor and periodontal disease.

In conclusion, the present study showed that GCF levels of stem cell factor tended to be higher in patients with gingivitis compared to healthy subjects. Patients with periodontitis had significantly increased GCF levels of stem cell factor compared to patients with gingivitis and periodontally healthy controls. Stem cell factor concentration in GCF was positively correlated with the clinical severity of periodontal disease. These findings indicate that stem cell factor may play a crucial role in the host immune response during periodontal inflammation. To our knowledge, this was the first study to evaluate the GCF levels of stem cell factor in periodontal health and disease. Further studies are needed to investigate protein and mRNA expression levels of stem cell factor in gingival tissues in different periodontal diseases to provide information into the expression patterns of this molecule. In addition, studies determining the GCF levels of stem cell factor after periodontal treatment might further elucidate the role of stem cell factor in the development of periodontal disease.

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