J Clin Periodontol 2011; 38: 148–156 doi: 10.1111/j.1600-051X.2010.01651.x

Journal of Clinical Periodontology

Effect of periodontal treatment on circulating CD34$^+$ cells and peripheral vascular endothelial function: a randomized controlled trial

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Li X, Tse HF, Yiu KH, Li LSW, Jin L. Effect of periodontal treatment on circulating CD34$^+$ cells and peripheral vascular endothelial function: a randomized controlled trial. J Clin Periodontol 2011; 38: 148–156. doi: 10.1111/j.1600-051X.2010.01651.x.

Abstract

Aim: Periodontal disease is associated with endothelial dysfunction and increased circulating progenitor cell (CPC) count. This study sought to investigate the effect of periodontal treatment on CPC count and vascular endothelial function.

Materials and Methods: A single-blind, randomized controlled trial was conducted in 50 otherwise healthy subjects with moderate-to-severe chronic periodontitis. They were randomly assigned into Treatment group ($n = 25$), in whom periodontal treatment was conducted immediately, and Control group ($n = 25$), in whom periodontal treatment was postponed until the completion of this 3-month study. CPCs and peripheral endothelial function were evaluated at baseline and 3-month follow-up using flow cytometry and peripheral arterial tonometry, respectively.

Results: Based on the intention-to-treat analysis, periodontal treatment exhibited neutral effects on endothelial function [treatment effect: 0.03, 95% confidence interval (CI): -0.29 to 0.35, $p = 0.85$]. However, circulating CD34$^+$ cells count significantly decreased in the Treatment group compared with the controls (treatment effect: -29.85 cells/μl, 95% CI: -52.62 to -7.08, $p = 0.011$). The reduction of circulating CD34$^+$ count was positively correlated with the decrease in sites% with bleeding on probing or periodontal pockets $\geqslant 4$ mm.

Conclusions: This study suggests that treatment of periodontitis has neutral effects on peripheral endothelial function but significantly decreases circulating CD34$^+$ cell count.

Key words: atherosclerosis; clinical trial; endothelial function; inflammation; periodontal disease; progenitor cells

Accepted for publication 20 October 2010

Conflict of interest and source of funding

The authors declare that they have no conflict of interests.

This study was supported by the Hong Kong Research Grants Council (HKU 7518/05M and HKU766909M), The University of Hong Kong (CRCG Funds 200507176137, 200607176038, 200707176095 and 200907176052) and the Sun Chieh Yeh Heart Foundation. This clinical trial was registered at http://www.hkclinicaltrials.com, No. HKCTR-458.

Periodontal disease is initiated by pathogenic plaque biofilms and characterized by bacteria-induced inflammatory destruction of tooth-supporting structures and alveolar bone. Emerging evidence shows that periodontal disease is associated with an increased level of systemic inflammation and subclinical atherosclerosis (Cairo et al. 2008, Persson & Persson 2008, Behle et al. 2009, Buhlin et al. 2009a, b). Of interest, periodontal treatment could significantly improve vascular endothelial function (Blum et al. 2007, Tonetti et al. 2007, Piconi et al. 2009, Tonetti 2009), which plays a crucial role in almost every stage of atherosclerosis and has been used as an endpoint in clinical research of cardiovascular disease (CVD) (Deanfield et al. 2007, Fadini et al. 2008). Nevertheless, the underlying mechanisms of such beneficial effects remain largely unknown. A recent study reports that periodontal conditions are related in a dose-dependent manner with asymmetric

dimethylarginine, a potent serological marker of endothelial function (Tsioufis et al. 2010). This finding suggests that the cross-talk between periodontal infection and endothelium may be beyond systemic inflammation.

A surrogate marker for endothelial function and cumulative cardiovascular risk is the count of circulating endothelial progenitor cells (EPCs) (Hill et al. 2003, Fadini et al. 2008, Fadini et al. 2010). Circulating EPCs are characterized by their expression of surface markers including CD34, CD133 and kinase insert domain-containing receptor (KDR). Currently, both $CD34^+$ cells and $CD133^+$ cells are considered as circulating progenitor cells (CPCs) (Fadini et al. 2006). Interestingly, clinical studies suggest that $CD34^+$ cells are more closely related to the risk of CVD than other factors including high-sensitivity C-reactive protein (hs-CRP) (Fadini et al. 2006). On the other hand, circulating $CD34^+$ cells serve not only as a pool of EPCs but also as haematopoietic stem cells, which can differentiate into monocytes and macrophages, and contribute to inflammatory response (Komor et al. 2005). Our recent study indeed shows that an increased level of circulating EPCs is significantly associated with moderate-to-severe periodontitis (Li et al. 2009). However, it is unknown whether the increased level of EPCs is related to uncontrolled periodontal inflammation or other relevant factors that remain unclear. It is hypothesized that periodontal treatment decreases the number of circulating EPCs. This study sought to investigate the effect of periodontal treatment on CPC count measured by flow cytometry and peripheral vascular endothelial function by means of pulse amplitude tonometry (PAT).

Materials and Methods

Eligibility criteria for participants

A single-blind, randomized controlled trial based on intention-to-treat strategy was conducted in healthy subjects without any established CVD (Fig. 1). Eligibility criteria for inclusion in the trial consisted of two steps for assessment. For Step 1, a health screening programme involving 647 subjects was conducted at the Department of Medicine, Queen Mary Hospital between 2006 and 2007 to investigate the predictive role of coronary calcium score on CVD. Subjects with any of the following conditions were excluded from the study: (i) documented CVD including stroke, coronary artery diseases, valvular heart diseases, cardiomyopathy, heart failure, cardiac arrhythmias and neoplastic disorders; (ii) a coronary calcium score of $\geqslant 10$ as detected by multi-slice cardiac computed tomography within the last 12 months; (iii) systemic illnesses and recent infection, febrile illness or inflammatory disease; (iv) use of antibiotics, anti-inflammatory or immunosuppressant agents in the last 3 months; (v) former or current smokers; (vi) symptoms of angina; (vii) diabetes mellitus; and (viii) major depression. As a result, a total of 187 healthy subjects were identified. Among them, 100 subjects (53.5%) were randomly selected and then contacted by phone; and subsequently 86 subjects (36 males and 50 females, aged 35–80 years) agreed to participate in the study. For Step 2, 86 subjects were invited to the Prince Philip Dental Hospital to receive periodontal examination between November 2007 and April 2008; and of them, 61 subjects with moderate-to-severe chronic periodontitis as described in our recent report (Li et al. 2009) were invited to participate in the clinical trial, whereas the edentulous subjects and those with no or mild chronic periodontitis were excluded. Of these 61 subjects, 11 declined to participate and two were edentulous (Fig. 1). As a result, 50 subjects were enrolled in this study. This clinical trial was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster, and registered at http://www.hkclinical

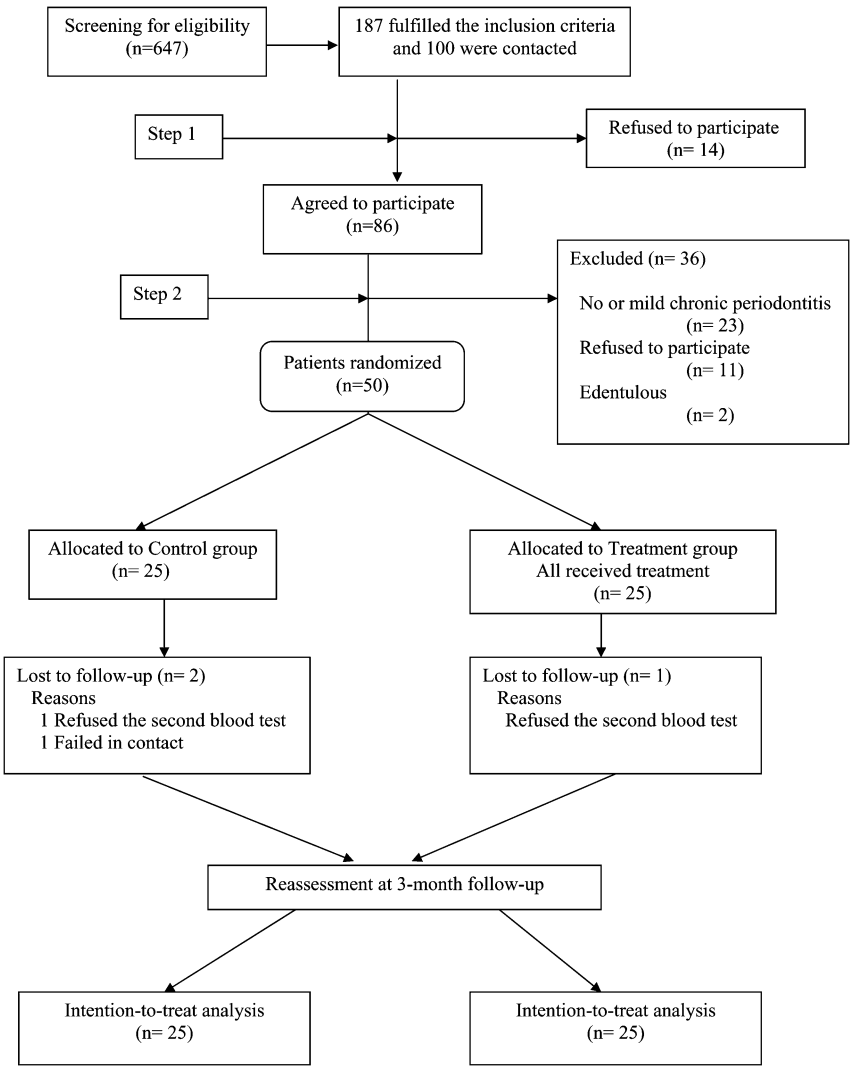

Fig. 1. Flow chart of the participants throughout the study.

trials.com (No. HKCTR-458). Oral and written informed consent were obtained from all participants before the study.

Study design

A total of 50 subjects fulfilling with the eligibility criteria were randomly assigned in a 1:1 ratio into two groups: Treatment group ($n = 25$) in whom periodontal treatment was conducted immediately; and Control group ($n = 25$) in whom periodontal treatment was postponed until the completion of this 3-month study. This trial started in February 2009 and ended in November 2009. Among those 50 subjects who underwent randomization, 47 subjects (24 in the Treatment group and 23 in the Control group) completed the trial (Fig. 1). Two subjects (one in each group) refused the second blood test and one subject in the Control group was lost to follow-up during the trial. All subjects underwent blood sampling, test of peripheral vascular endothelial function and periodontal examination at baseline and 3-month follow-up. To prevent an imbalance of age, gender and severity of periodontitis between the two groups, a restricted randomization approach (minimization) was used (Altman & Bland 2005, Hewitt & Torgerson 2006). Hong Kong Identity Card number of the participants was used to implement the random allocation. The primary investigator generated the allocation sequence and kept the detail of group assignment. Two reception staff and one research assistant were responsible to enroll participants and inform the patients back to respective hospitals. All examiners responsible for periodontal examination and medical test were blinded to the group assignments. Because of the description of the study for informed consent, participants were not blinded to group assignment.

Periodontal examination and therapy

Periodontal parameters were assessed blindly by a single investigator at baseline and 3-month follow-up. All subjects received two times of full-mouth, comprehensive periodontal examination at six sites per tooth excluding the third molars. The data included number of missing teeth, presence or absence of plaque, bleeding on probing (BOP), probing depth (PD) and gingival recession. Inter-proximal clinical attachment loss was calculated as the sum of the PD and gingival recession values (Li et al. 2009). After baseline examination, each subject was given oral hygiene instructions.

Subjects in the Treatment group immediately received one course of non-surgical periodontal therapy performed by senior dental hygienists, including supra- and sub-gingival scaling and root debridement using both hand instruments and a piezoelectric ultrasonic scaler. The periodontal therapy was completed within 1 week through two to three sessions depending on the severity of periodontitis. At the end of each session, chlorhexidine gel was applied into periodontal pockets with PD $\geqslant$ 4mm and Corsodyl 0.2% w/v Mint Mouthwash (chlorhexidine digluconate 0.2% w/v, 300 ml, GlaxoSmithKline, Maidenhead, Berkshire, UK) was prescribed to each subject for home use. In addition to periodontal therapy, periodontally involved hopeless teeth were extracted and teeth with caries or endodontic infections received restoration or root canal therapy. The subjects in the Control group received similar periodontal and other necessary dental treatments only after completion of this 3-month study.

Blood sampling

After overnight fasting, 25 ml of peripheral blood sample was obtained from each subject at baseline and 3-month follow-up assessment. Full blood counts, serum creatinine, glucose and lipid levels were measured by standard biochemical testing. hs-CRP level was measured by using a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany) with a chemistry analyzer (Hitachi 747 Analyzer, Boehringer Mannheim, Mannheim, Germany).

Analysis of circulating EPCs by fluorescence-activated cell sorting (FACS)

The circulating EPCs were defined by the expression of surface markers: $CD34^+$, $CD133^+$, $CD34^+/KDR^+$ and $CD133^+/KDR^+$; and their numbers were measured by fluorescence-activated cell analysis of peripheral blood sample as described (Lau et al. 2007, Li et al. 2009). In brief, 100 µl of peripheral blood was incubated with a phycoerythrin-conjugated monoclonal antibody against human KDR (Sigma, St. Louis, MO, USA), followed by a fluorescein isothiocyanate (FITC)-conjugated CD34 and CD133 antibodies (Beckman Coulter, Fullerton, CA, USA). FITC-labelled anti-human CD45 antibody was used for differential gating during flow analysis. FITC-labelled IgG1a (Beckman Coulter) and phycoerythrin-labelled IgG2b (Becton Dickinson, Franklin Lakes, NJ, USA) served as the isotypic control for colour compensation. Analysis was performed with an automated counter for FACS (Elite; Beckman Coulter) in which 1,000,000 events were counted (Fig. 2). The intra-observer variability testing found an intra-class correlation coefficient of 0.9 ($p < 0.001$).

Measurement of peripheral vascular endothelial function by Endo-PAT

Peripheral vascular endothelial function was evaluated using PAT index as described previously (Bonetti et al. 2004). In brief, after overnight fasting, PAT signals were measured by the Endo-PAT 2000 device (Itamar Medical, Caesarea, Israel) on the tip of middle fingers of each subject's hand. A cuff was then placed on the proximal study forearm for 5 min to interrupt arterial flow while the other arm served as a control. PAT index were then recorded for 5 min. A computerized, operator-independent algorithm automatically obtained and analysed average pulse amplitude in both fingers. The average pulse amplitude of the PAT signal was divided by the baseline value to calculate PAT index (Bonetti et al. 2004). The control arm's average pulse amplitude was used subsequently to normalize the measured PAT in the study arm. Intra-observer variability testing revealed an intra-class correlation coefficient of 0.87 [95% confidence interval (CI): 0.80–0.91, $p < 0.001$].

Statistical analysis

The primary outcome was the change in vascular endothelial function and CPCs counts. The secondary outcome measures were the change of lipid and inflammatory biomarkers as well as periodontal conditions. Based on the screening results of the 86 subjects (Li et al. 2009) and Altman's nomogram (Petrie et al. 2002), it was decided to recruit at least 20 subjects in each group to detect a difference in circulating EPC count of 90.2 cells/µl, with its standard deviation of 83.2 at the 5% level of significance and 90% detection power. Analysis and report of this trial conformed to the intention-to-treat principle and the CONSORT guidelines (Moher et al.

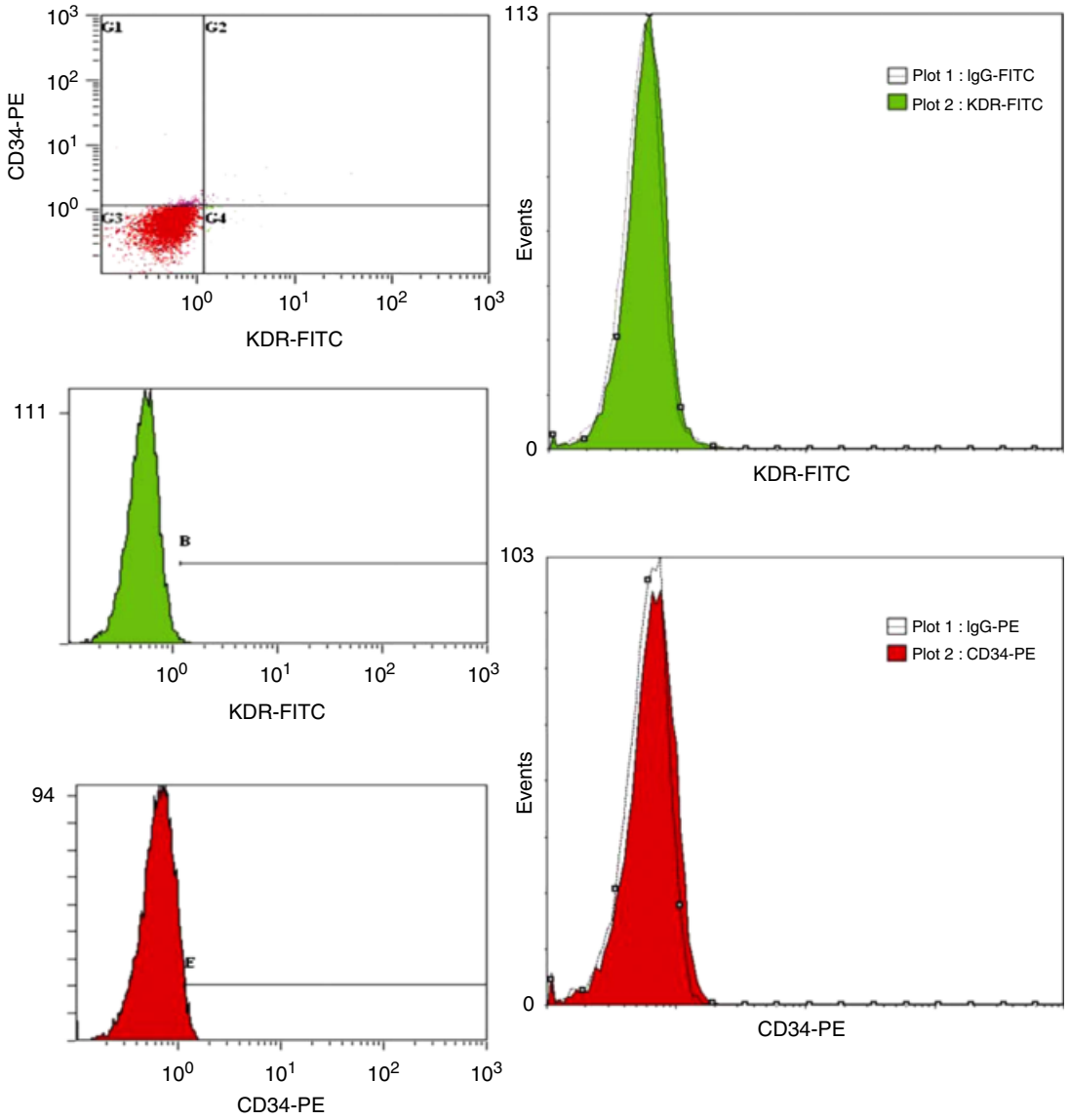

Fig. 2. A set of representative pictures of the fluorescence-activated cell sorting analyses.

2001). Missing values were imputed by use of the respective baseline values. Baseline demographic and clinical characteristics of patients were compared between the two groups using independent *t*-test or chi-square analysis as appropriate. The difference of the changes in primary or secondary outcomes between the groups was compared using the analysis of covariance for adjusting the baseline values concerned. The correlation between continuous variables was analysed by computing Spearman's correlation coefficient. Analysis of covariance was used to identify the independent variables associated with the change of circulating $CD34^+$ cells. A two-sided p-value < 0.05 was considered statistically significant using a software program (SPSS 14.0, SPSS Inc., Chicago, IL, USA).

Results

Study population

The clinical characteristics of the study subjects are shown in Table 1. The mean age of the subjects was 59 ± 11 years and 46% of them were men. There was no significant difference in demographic data and clinical characteristics of patients between the two groups ($p > 0.05$). Furthermore, at baseline endothelial function, CPC count, lipid and inflammatory biomarkers as well as periodontal conditions were all similar between the two groups (Tables 2 and 3, $p > 0.05$).

Effects of periodontal treatment

Periodontal treatment significantly improved the overall periodontal conditions ($p < 0.05$) in the Treatment group (Table 3). Nevertheless, the treatment exhibited a neutral effect on peripheral vascular endothelial function (treatment effect: 0.03, 95% CI: −0.29 to 0.35, $p = 0.85$] (Table 2). On the other hand, circulating $CD34^+$ cell count was significantly decreased after periodontal treatment (treatment effect: −29.85 cells/μl, 95% CI: −52.62 to −7.08, $p = 0.011$). However, there were no significant differences in other subsets of CPCs counts or levels of lipids and inflammatory biomarkers between the two groups ($p > 0.05$; Tables 2 and 3).

The reduction of $CD34^+$ cell count was positively correlated with the decrease in sites% with BOP ($r = 0.389$, $p = 0.005$, Fig. 3a), sites% with PD ≥ 4 mm ($r = 0.513$, $p < 0.001$, Fig. 3b),

apolipoprotein A1 ($r = 0.344$, $p = 0.015$, Fig. 3c) and lymphocytes ($r = 0.373$, $p = 0.008$, Fig. 3d). Analysis of covariance showed that Δapolipoprotein A1 (g/l), Δlymphocyte (10^3 cells/μl), baseline $CD34^+$ cell count and Δsites% with PD ⩾ 4 mm were all the independent variables associated with the change of circulating $CD34^+$ cell count (Table 4). There were no adverse events during the trial.

Discussion

Emerging evidence shows that periodontal treatment could significantly improve endothelial function (Blum et al. 2007, Tonetti et al. 2007, Piconi et al. 2009), but the level of improvement does not correlate with the change of inflammatory biomarkers like hs-CRP and interleukin (IL)-6 (Tonetti et al. 2007, Higashi et al. 2008). This implies that assessment and monitoring of inflammatory biomarkers could not fully explain the effect of periodontal therapy on endothelial function. The present clinical trial confirms the significant effect of periodontal treatment on the count of circulating $CD34^+$ progenitor cells, a surrogate marker for cumulative cardiovascular risk and endothelial function (Fadini et al. 2006, Fadini et al. 2010). The effect observed is independent of systemic inflammation measured by lymphocyte count and hs-CRP level. In this respect, our finding provides novel insight into current knowledge on the association of periodontal infections with endothelial function beyond inflammatory load.

A recent study may shed light to our investigation. It shows that a subpopulation of $CD4^+$ T lymphocytes, which are involved in the rolling and adhesion of lymphocytes to the endothelia and participate in the early inflammation of atherosclerosis, decreases significantly 1 or 6 months after periodontal treatment (Piconi et al. 2009). It may be biologi-

Table 1. Baseline demographic data and clinical characteristics of patients

Variables (mean ± SD)	Control group ($n = 25$)	Treatment group ($n = 25$)	p-Value
Age (years)	59.7 ± 10.3	58.6 ± 11.6	0.744
Male, number (%)	13 (52.0)	10 (40.0)	0.395
Education level, number (%)			0.914
Low	6 (26)	7 (29)	
Middle	7 (30)	6 (25)	
High	10 (41)	11 (46)	
Body mass index (kg/m^2)*	23.2 ± 3.6	24.9 ± 3.7	0.133
Waist–hip ratio	0.87 ± 0.06	0.86 ± 0.09	0.403
Mean DBP (mmHg)	84.0 ± 12.0	84.9 ± 12.4	0.824
Mean SBP (mmHg)	142.6 ± 24.3	135.7 ± 19.1	0.304
Serum total protein (mmol/l)	79.1 ± 3.7	80.0 ± 4.1	0.437
Albumin (mmol/l)	43.8 ± 2.3	43.8 ± 2.7	0.965
Fasting glucose (mmol/l)	5.4 ± 0.6	5.3 ± 0.8	0.476
Serum urea (mmol/l)	5.2 ± 0.9	5.2 ± 1.9	0.874
Serum creatinine (mmol/l)	71.0 ± 16.3	70.7 ± 18.8	0.956
Total cholesterol (mmol/l)	5.2 ± 0.6	4.9 ± 0.6	0.118
Triglycerides (mmol/l)	1.4 ± 0.8	1.4 ± 0.9	0.857
Low-density lipoprotein (mmol/l)	3.2 ± 0.7	2.9 ± 0.6	0.083
High-density lipoprotein (mmol/l)	1.3 ± 0.3	1.4 ± 0.4	0.363

*Body mass index is calculated by the weight in kilograms divided by the square of the height in metres.
DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 2. Circulating progenitor cells and endothelial function at baseline and 3-month follow-up

	Control group	Treatment group	Treatment effect (95% CI)	p-Value
$CD34^+$ (cells/μl)				
Baseline	84.00 ± 30.62	80.47 ± 34.95		0.705
3-month follow-up	103.05 ± 47.54	71.95 ± 33.68		**0.010**
Change*	19.04 ± 52.07	− 8.52 ± 36.36		**0.035**
Change†	20.19 ± 40.00	− 9.66 ± 40.00	− 29.85 (− 52.62 to − 7.08)	**0.011**
$CD133^+$ (cells/μl)				
Baseline	11.37 ± 6.60	11.58 ± 6.54		0.908
3-month follow-up	12.10 ± 6.29	12.25 ± 11.07		0.955
Change*	0.60 ± 8.49	1.16 ± 13.07		0.856
Change†	0.49 ± 9.05	1.27 ± 9.05	0.78 (− 4.38 to 5.94)	0.763
$CD34^+/KDR^+$ (cells/μl)				
Baseline	14.40 ± 6.47	12.18 ± 4.92		0.178
3-month follow-up	14.54 ± 5.40	12.56 ± 6.29		0.254
Change*	− 0.09 ± 8.73	− 0.10 ± 6.55		0.995
Change†	0.87 ± 6.03	− 1.06 ± 6.03	− 1.93 (− 5.39 to 1.53)	0.267
$CD133^+/KDR^+$ (cells/μl)				
Baseline	7.59 ± 4.83	7.24 ± 4.04		0.779
3-month follow-up	8.59 ± 4.78	6.83 ± 4.26		0.177
Change*	1.00 ± 6.43	− 0.40 ± 5.58		0.415
Change†	1.16 ± 4.55	− 0.56 ± 4.55	− 1.72 (− 4.31 to 0.87)	0.189
PAT (ratio)				
Baseline	2.22 ± 0.50	2.41 ± 0.71		0.280
3-month follow-up	2.18 ± 0.46	2.22 ± 0.62		0.769
Change*	− 0.04 ± 0.69	− 0.19 ± 0.87		0.515
Change†	− 0.13 ± 0.55	− 0.10 ± 0.55	0.03 (− 0.29 to 0.35)	0.852

*Without adjustment for baseline value.
†With adjustment for baseline value.
The bold data indicate the statistically significant difference between the Control group and Treatment group.
PAT, pulse amplitude tonometry.

Table 3. Lipids, inflammatory biomarkers and periodontal conditions at baseline and 3-month follow-up

	Control group	Treatment group	Treatment effect (95% CI)	*p*-Value
Apolipoprotein A1 (g/l)				
Baseline	1.34 ± 0.22	1.43 ± 0.24		0.172
3-month follow-up	1.42 ± 0.21	1.42 ± 0.22		0.984
Change*	0.08 ± 0.16	− 0.01 ± 0.12		**0.033**
Change†	0.07 ± 0.13	0.002 ± 0.13	− 0.07 (− 0.14 to − 0.01)	0.090
Apolipoprotein B (g/l)				
Baseline	0.97 ± 0.25	0.86 ± 0.18		0.074
3-month follow-up	1.20 ± 1.23	0.87 ± 0.17		0.187
Change*	0.23 ± 1.19	0.01 ± 0.13		0.365
Change†	0.23 ± 0.90	0.02 ± 0.90	− 0.21 (− 0.73 to 0.30)	0.413
Lipoprotein (α)				
Baseline	321.33 ± 288.20	232.44 ± 323.97		0.316
3-month follow-up	353.72 ± 297.16	265.64 ± 325.19		0.322
Change*	32.37 ± 65.64	33.20 ± 99.31		0.972
Change†	32.95 ± 88.15	32.65 ± 86.35	− 0.30 (− 50.25 to 49.65)	0.990
hs-CRP (mg/l)				
Baseline	3.00 ± 3.86	2.08 ± 2.40		0.319
3-month follow-up	2.13 ± 3.36	2.40 ± 2.80		0.760
Change*	− 0.87 ± 2.33	0.32 ± 2.30		0.078
Change†	− 0.73 ± 2.20	0.19 ± 2.15	0.92 (− 0.32 to 2.16)	0.143
LY (10^3 cells/μl)				
Baseline	1.51 ± 0.54	1.61 ± 0.32		0.410
3-month follow-up	1.71 ± 0.49	1.53 ± 0.46		0.197
Change*	0.20 ± 0.65	− 0.08 ± 0.41		0.076
Change†	0.17 ± 0.46	− 0.05 ± 0.46	− 0.21 (− 0.47 to 0.05)	0.117
WBC (10^3 cells/μl)				
Baseline	5.02 ± 1.16	5.20 ± 1.25		0.592
3-month follow-up	5.12 ± 0.99	4.89 ± 1.05		0.423
Change*	0.10 ± 1.10	− 0.31 ± 1.00		0.166
Change†	0.06 ± 0.85	− 0.27 ± 0.85	− 0.32 (− 0.81 to 0.16)	0.189
Number of missing teeth				
Baseline	3.60 ± 3.08	4.28 ± 4.35		0.527
3-month follow-up	3.60 ± 3.08	4.68 ± 4.79		0.348
Change*	0 ± 0	0.40 ± 0.76		**0.012**
Change†	0.02 ± 0.50	0.38 ± 0.50	0.36 (0.08 to 0.64)	**0.014**
Sites with detectable plaque (%)				
Baseline	46.29 ± 18.40	50.47 ± 18.43		0.436
3-month follow-up	39.66 ± 22.69	23.50 ± 22.99		**0.016**
Change*	− 4.98 ± 15.78	− 29.08 ± 18.50		**0.000**
Change†	− 5.50 ± 17.00	− 28.50 ± 17.00	− 23.00 (− 32.70 to − 13.20)	**0.000**
Sites with bleeding on probing (%)				
Baseline	45.61 ± 20.57	44.94 ± 18.63		0.904
3-month follow-up	33.32 ± 18.21	21.74 ± 14.03		**0.015**
Change*	− 12.29 ± 12.34	− 23.20 ± 16.01		**0.010**
Change†	− 12.10 ± 12.00	− 23.30 ± 12.00	− 11.20 (− 17.90 to − 4.50)	**0.002**
Sites with probing pockets ⩾4 mm (%)				
Baseline	10.35 ± 10.34	13.31 ± 14.02		0.401
3-month follow-up	6.65 ± 5.56	3.44 ± 3.89		**0.022**
Change*	− 3.70 ± 9.06	− 9.86 ± 12.76		0.055
Change†	− 4.90 ± 4.50	− 8.60 ± 4.50	− 3.70 (− 6.20 to − 1.20)	**0.004**
Sites with attachment loss ⩾5 mm (%)				
Baseline	43.75 ± 20.66	49.06 ± 29.05		0.460
3-month follow-up	45.33 ± 27.87	43.13 ± 32.33		0.798
Change*	1.58 ± 29.02	− 5.93 ± 16.71		0.267
Change†	1.00 ± 23.50	− 5.30 ± 23.50	− 6.30 (− 19.60 to 7.00)	0.344

*Without adjustment for baseline value.

†With adjustment for baseline value.

The bold data indicate the statistically significant difference between the Control group and Treatment group.

WBC, white blood cells; hs-CRP, high-sensitivity C-reactive protein; LY, lymphocyte.

cally plausible that the treatment of periodontitis reduces the risk of vascular injuries and thus decreases the counts of both $CD34^+$ cells and $CD4^+$ cell types, which are involved in vascular inflammation and repair. Further investigations are required to confirm this hypothesis. In the present study, the change of circulating $CD34^+$ cell count is positively correlated with that of apolipoprotein A1, the core protein of high-density lipoprotein. This finding is consistent with the evidence that decreased concentrations of apolipoprotein A1 is associated with fewer numbers of EPCs (Tobler et al. 2010) and apolipoprotein A1 gene transfer increases circulating EPCs in mice (Feng et al. 2008, 2009).

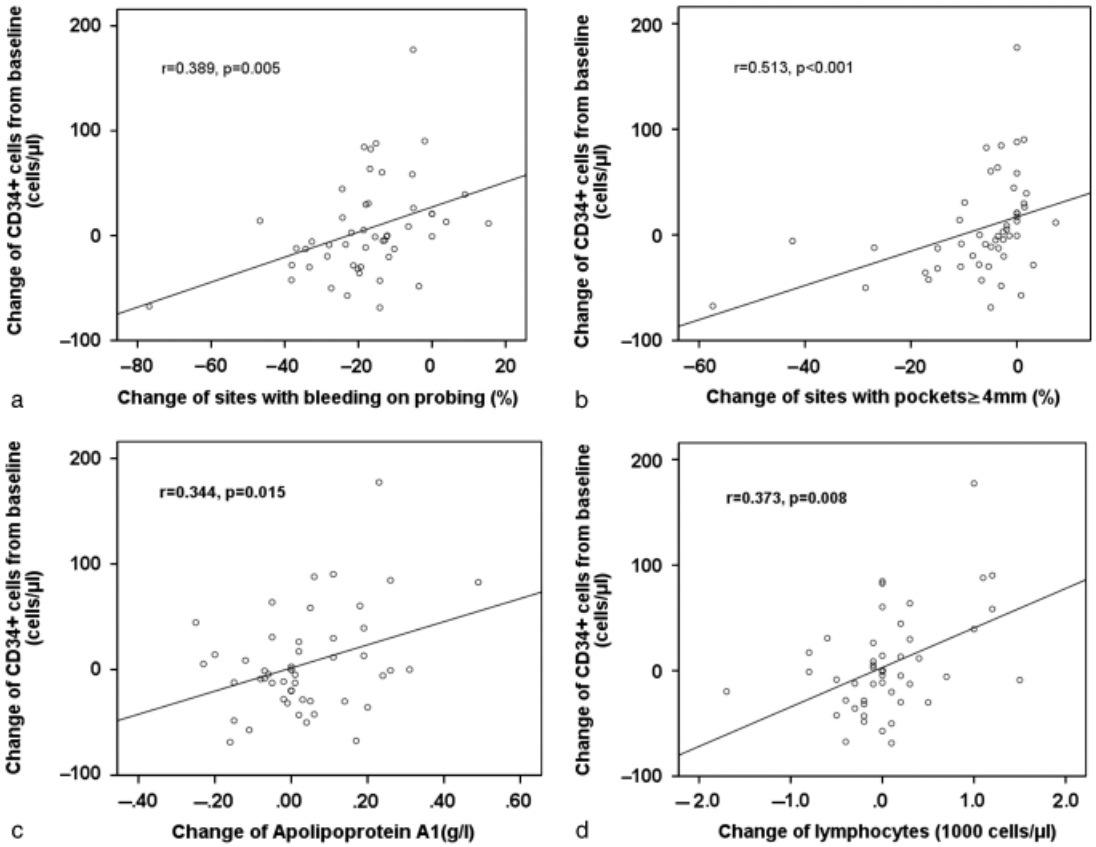

Fig. 3. (a–d) Significant linear correlation of the changes in circulating $CD34^+$ cells with changes of sites% with bleeding on probing (a) and probing depth $\geqslant 4$ mm (b), Apolipoprotein A1 (g/l) (c) and lymphocytes (1000 cells/μl) (d) after the treatment.

Table 4. Risk variables associated with the change of circulating $CD34^+$ cells (cells/μl)*

	Coefficient	95% CI	*p*-Value
ΔApolipoprotein A1 (g/l)	83.66	11.54 to 155.77	0.024
ΔLymphocyte (10^3 cells/μl)	27.32	8.65 to 46.01	0.005
ΔSites% with pockets $\geqslant 4$ mm	1.28	0.35 to 2.20	0.008
Baseline $CD34^+$ count	−0.47	−0.79 to −0.15	0.005

*Adjusted $R^2 = 45.6\%$ in the final model.

The present study shows that periodontal treatment exhibits a neutral effect on peripheral endothelial function. Whereas previous studies show that periodontal treatment could significantly improve vascular endothelial function as assessed by brachial FMD (Blum et al. 2007, Tonetti et al. 2007, Piconi et al. 2009). The duration of these studies ranges from 3 to 12 months, and the sample size in their treatment groups ranges from 13 to 61 subjects. Although in the present study the exact reasons for the neutral effect of periodontal treatment on endothelial function remain unclear, they might be to some extent due to the differences in patients' selection criteria and methods of testing endothelial function. In this study, the inclusion criteria were very strict, and CVD patients were actively screened out by investigating their medical history as well as by cardiac computed tomography scan, which may not be necessarily performed in previous studies. Indeed, the mean of PAT ratio at baseline was 2.32 ± 0.62, which was far above the level than the established definition of endothelial dysfunction at 1.67 with PAT (Yinon et al. 2006). Therefore, in these normal subjects who do not have baseline vascular endothelial dysfunction, it is unlikely to achieve an obvious and immediate benefit from control of periodontal infections. A recent study suggests that subjects with coronary calcium score <10 as those enrolled in the present study have a very low risk of cardiovascular events up to 5 years of follow-up (Detrano et al. 2008). Indeed, by use of coronary calcium score to exclude the subjects with underlying subclinical atherosclerosis, thus, the low-risk subgroup for CVD who were included in the present study might to some extent account for the negative results of endothelial function observed in the present study.

In the present study, instead of utilizing brachial FMD, automatic Endo-PAT device was used to assess peripheral endothelial function by measurement of reactive hyperaemia in the fingertips (Bonetti et al. 2004, Hamburg et al. 2008). This PAT index was based on a computerized program to measure the change in digital blood flow, such that intra- and inter-observer variability can be minimized and it might be more sensitive to detect microvascular endothelial function associated with periodontitis. Furthermore, this method exhibits an excellent reproducibility and is less affected by recent nutritional intake than other common methods like brachial FMD (Selamet Tierney et al. 2009). PAT index has also been shown to correlate significantly with brachial

FMD and predict CVD events (Kuvin et al. 2003, Hamburg & Benjamin 2009). Meanwhile, it should be noted that the PAT hyperaemic response largely reflects vasodilator responses in digital microvessels and may not yield equivalent information to measures of conduit artery vasodilation with brachial FMD.

The present study shows that periodontal treatment does not have significant effects on systemic levels of CRP, inconsistent with some published papers (D'Aiuto et al. 2004, Seinost et al. 2005, Paraskevas et al. 2008). The discrepancy may be due to different sample size, difference in the extent and type of periodontal disease and other possible oral diseases involved, as well as different periodontal treatment protocols and their efficacy (Jastrzebski et al. 2009). A systematic review and meta-analysis points out that it cannot be concluded that non-surgical periodontal treatment may definitely modulate the level of CRP (Ioannidou et al. 2006). Furthermore, a recent study finds that there is heterogeneity of systemic inflammatory responses to periodontal treatment, and that CRP and IL-6 may increase more than 25% compared with baseline in 14–44% of subjects (Behle et al. 2009). Our recent study finds that human gingiva per se can directly produce CRP and its expression profile in gingiva may be subject dependent (Lu & Jin 2010). It has also been shown recently that circulating CRP level is subject to regulation by a common genetic variation at the CRP locus (Brull et al. 2003, D'Aiuto et al. 2005). Taken together, individual CRP level may to some extent be subject to genetic modulation. In our present study, the overall baseline level of CRP in the Treatment group was relatively low. It is therefore speculated that there may be limited potential for significant decrease after the treatment. Therefore, further long-term clinical trial with a large sample size adopting strategies to accomplish a more definitive endpoint of treatment is highly needed to achieve convincing evidence.

The present study has several limitations to be addressed. The patients recruited presented with moderate-to-severe periodontitis rather than only severe periodontitis. This may partly explain why periodontal treatment has an insignificant effect on endothelial function. In terms of outcome measures, only the counts of CPCs were assessed and monitored after the periodontal treatment, while their functional activity was not investigated. The observation period was only 3 months and the treatment effect after 12 months or a longer time was unknown. Based on previous studies (Tonetti et al. 2007, Buhlin et al. 2009a, Piconi et al. 2009), the effect of periodontal treatment after a longer period may be more obvious or even contrary. Furthermore, it should be stated that in the present study, the treatment effect on $CD34^+$ cells is largely due to an inexplicable increase of $CD34^+$ cell count in the control group at the 3-month follow-up assessment, which may be caused by other confounding factors like physical exercise, dietary change and some unknown environmental factors (Zaldivar et al. 2007, Muller-Ehmsen et al. 2008), rather than a highly meaningful reduction in the actively treated group of subjects. Further investigation is required to clarify the exact reasons for the inexplicable increase of $CD34^+$ cells count in the control subjects. It should be also noted that in the present study the subjects were relatively healthy without major systemic diseases, and the finding of the present study may be different or even converse to the ones involving systemically compromised patients. In future studies, it would be appropriated to focus on the compromised patients like those with CVD, diabetes, chronic kidney disease or subjects with subclinical endothelial dysfunction for generating more meaningful scientific data.

In conclusion, the present short-term, randomized clinical trial shows that treatment of periodontitis significantly decreases circulating $CD34^+$ cell count, while it has neutral effects on peripheral vascular endothelial function measured by PAT. This study may enhance the understanding of the plausible mechanisms accounting for the association of periodontal infections with endothelial dysfunction and atherosclerosis.

Acknowledgements

The authors are grateful to the administrative assistants, nurses, dental surgery assistants and staff hygienists from the Department of Medicine, Cardiology Division, Queen Mary Hospital and the Prince Philip Dental Hospital for their great contributions to the study.

References

Altman, D. G. & Bland, J. M. (2005) Treatment allocation by minimisation. *British Medical Journal* **330**, 843.

Behle, J. H., Sedaghatfar, M. H., Demmer, R. T., Wolf, D. L., Celenti, R., Kebschull, M., Belusko, P. B., Herrera-Abreu, M., Lalla, E. & Papapanou, P. N. (2009) Heterogeneity of systemic inflammatory responses to periodontal therapy. *Journal of Clinical Periodontology* **36**, 287–294.

Blum, A., Kryuger, K., Mashiach Eizenberg, M., Tatour, S., Vigder, F., Laster, Z. & Front, E. (2007) Periodontal care may improve endothelial function. *European Journal of Internal Medicine* **18**, 295–298.

Bonetti, P. O., Pumper, G. M., Higano, S. T., Holmes, D. R. Jr., Kuvin, J. T. & Lerman, A. (2004) Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *Journal of the American College of Cardiology* **44**, 2137–2141.

Brull, D. J., Serrano, N., Zito, F., Jones, L., Montgomery, H. E., Rumley, A., Sharma, P., Lowe, G. D., World, M. J., Humphries, S. E. & Hingorani, A. D. (2003) Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* **23**, 2063–2069.

Buhlin, K., Hultin, M., Norderyd, O., Persson, L., Pockley, A. G., Pussinen, P. J., Rabe, P., Klinge, B. & Gustafsson, A. (2009a) Periodontal treatment influences risk markers for atherosclerosis in patients with severe periodontitis. *Atherosclerosis* **206**, 518–522.

Buhlin, K., Hultin, M., Norderyd, O., Persson, L., Pockley, A. G., Rabe, P., Klinge, B. & Gustafsson, A. (2009b) Risk factors for atherosclerosis in cases with severe periodontitis. *Journal of Clinical Periodontology* **36**, 541–549.

Cairo, F., Castellani, S., Gori, A. M., Nieri, M., Baldelli, G., Abbate, R. & Pini-Prato, G. P. (2008) Severe periodontitis in young adults is associated with sub-clinical atherosclerosis. *Journal of Clinical Periodontology* **35**, 465–472.

D'Aiuto, F., Casas, J. P., Shah, T., Humphries, S. E., Hingorani, A. D. & Tonetti, M. S. (2005) C-reactive protein (+1444C>T) polymorphism influences CRP response following a moderate inflammatory stimulus. *Atherosclerosis* **179**, 413–417.

D'Aiuto, F., Parkar, M., Andreou, G., Suvan, J., Brett, P. M., Ready, D. & Tonetti, M. S. (2004) Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. *Journal of Dental Research* **83**, 156–160.

Deanfield, J. E., Halcox, J. P. & Rabelink, T. J. (2007) Endothelial function and dysfunction: testing and clinical relevance. *Circulation* **115**, 1285–1295.

Detrano, R., Guerci, A. D., Carr, J. J., Bild, D. E., Burke, G., Folsom, A. R., Liu, K., Shea, S., Szklo, M., Bluemke, D. A., O'Leary, D. H., Tracy, R., Watson, K., Wong, N. D. & Kronmal, R. A. (2008) Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *New England Journal of Medicine* **358**, 1336–1345.

Fadini, G. P., Baesso, I., Albiero, M., Sartore, S., Agostini, C. & Avogaro, A. (2008) Technical notes on endothelial progenitor cells: ways to escape from the knowledge plateau. *Atherosclerosis* **197**, 496–503.

Fadini, G. P., de Kreutzenberg, S. V., Coracina, A., Baesso, I., Agostini, C., Tiengo, A. & Avogaro, A. (2006) Circulating CD34+cells, metabolic syndrome, and cardiovascular risk. *European Heart Journal* **27**, 2247–2255.

Fadini, G. P., Pagano, C., Baesso, I., Kotsafti, O., Doro, D., de Kreutzenberg, S. V., Avogaro, A., Agostini, C. & Dorigo, M. T. (2010) Reduced endothelial progenitor cells and brachial artery flow-mediated dilation as evidence of endothelial dysfunction in ocular hypertension and primary

open-angle glaucoma. *Acta Ophthalmologica* **88**, 135–141.

Feng, Y., Jacobs, F., Van Craeyveld, E., Brunaud, C., Snoeys, J., Tjwa, M., Van Linthout, S. & De Geest, B. (2008) Human ApoA-I transfer attenuates transplant arteriosclerosis via enhanced incorporation of bone marrow-derived endothelial progenitor cells. *Arteriosclerosis Thrombosis, and Vascular Biology* **28**, 278–283.

Feng, Y., Van Craeyveld, E., Jacobs, F., Lievens, J., Snoeys, J. & De Geest, B. (2009) Wild-type apo A-I and apo A-I(Milano) gene transfer reduce native and transplant arteriosclerosis to a similar extent. *Journal of Molecular Medicine* **87**, 287–297.

Hamburg, N. M. & Benjamin, E. J. (2009) Assessment of endothelial function using digital pulse amplitude tonometry. *Trends in Cardiovascular Medicine* **19**, 6–11.

Hamburg, N. M., Keyes, M. J., Larson, M. G., Vasan, R. S., Schnabel, R., Pryde, M. M., Mitchell, G. F., Sheffy, J., Vita, J. A. & Benjamin, E. J. (2008) Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* **117**, 2467–2474.

Hewitt, C. E. & Torgerson, D. J. (2006) Is restricted randomisation necessary? *British Medical Journal* **332**, 1506–1508.

Higashi, Y., Goto, C., Jitsuiki, D., Umemura, T., Nishioka, K., Hidaka, T., Takemoto, H., Nakamura, S., Soga, J., Chayama, K., Yoshizumi, M. & Taguchi, A. (2008) Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. *Hypertension* **51**, 446–453.

Hill, J. M., Zalos, G., Halcox, J. P., Schenke, W. H., Waclawiw, M. A., Quyyumi, A. A. & Finkel, T. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *New England Journal of Medicine* **348**, 593–600.

Ioannidou, E., Malekzadeh, T. & Dongari-Bagtzoglou, A. (2006) Effect of periodontal treatment on serum C-reactive protein levels: a systematic review and meta-analysis. *Journal of Periodontology* **77**, 1635–1642.

Jastrzebski, M., Zaleska, M., Klocek, M., Stolarz, K., Wojciechowska, W., Olszanecka, A., Czarnecka, D. & Kawecka-Jaszcz, K. (2009) Should dental treatment be considered for lowering inflammatory markers in hypertensive patients? *International Journal of Cardiology* **132**, 439–441.

Komor, M., Güller, S., Baldus, C. D., de Vos, S., Hoelzer, D., Ottmann, O. G. & Hofmann, W. K. (2005) Transcriptional profiling of human hematopoiesis during in vitro lineage-specific differentiation. *Stem Cells* **23**, 1154–1169.

Kuvin, J. T., Patel, A. R., Sliney, K. A., Pandian, N. G., Sheffy, J., Schnall, R. P., Karas, R. H. & Udelson, J. E. (2003) Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *American Heart Journal* **146**, 168–174.

Lau, K. K., Chan, Y. H., Yiu, K. H., Li, S. W., Tam, S., Lau, C. P., Kwong, Y. L. & Tse, H. F. (2007) Burden of carotid atherosclerosis in patients with stroke: relationships with circulating endothelial progenitor cells and hypertension. *Journal of Human Hypertension* **21**, 445–451.

Li, X., Tse, H. F., Yiu, K. H., Jia, N., Chen, H., Li, L. S. & Jin, L. (2009) Increased levels of circulating endothelial progenitor cells in subjects with moderate to severe chronic periodontitis. *Journal of Clinical Periodontology* **36**, 933–939.

Lu, Q. & Jin, L. J. (2010) Human gingiva is another site of C-reactive protein formation. *Journal of Clinical Periodontology* **37**, 789–796.

Moher, D., Schulz, K. F. & Altman, D. G. (2001) The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet* **357**, 1191–1194.

Muller-Ehmsen, J., Braun, D., Schneider, T., Pfister, R., Worm, N., Wielckens, K., Scheid, C., Frommolt, P. & Flesch, M. (2008) Decreased number of circulating progenitor cells in obesity: beneficial effects of weight reduction. *European Heart Journal* **29**, 1560–1568.

Paraskevas, S., Huizinga, J. D. & Loos, B. G. (2008) A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *Journal of Clinical Periodontology* **35**, 277–290.

Persson, G. R. & Persson, R. E. (2008) Cardiovascular disease and periodontitis: an update on the associations and risk. *Journal of Clinical Periodontology* **35**, 362–379.

Petrie, A., Bulman, J. S. & Osborn, J. F. (2002) Further statistics in dentistry. Part 4: clinical trials 2. *British Dental Journal* **193**, 557–561.

Piconi, S., Trabattoni, D., Luraghi, C., Perilli, E., Borelli, M., Pacei, M., Rizzardini, G., Lattuada, A., Bray, D. H., Catalano, M., Sparaco, A. & Clerici, M. (2009) Treatment of periodontal disease results in improvements in endothelial dysfunction and reduction of the carotid intima-media thickness. *FASEB Journal* **23**, 1196–1204.

Seinost, G., Wimmer, G., Skerget, M., Thaller, E., Brodmann, M., Gasser, R., Bratschko, R. O. & Pilger, E. (2005) Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. *American Heart Journal* **149**, 1050–1054.

Selamet Tierney, E. S., Newburger, J. W., Gauvreau, K., Geva, J., Coogan, E., Colan, S. D. & de Ferranti, S. D. (2009) Endothelial pulse amplitude testing: feasibility and reproducibility in adolescents. *Journal of Pediatrics* **154**, 901–905.

Tobler, K., Freudenthaler, A., Baumgartner-Parzer, S. M., Wolzt, M., Ludvik, B., Nansalmaa, E., Nowotny, P. J., Seidinger, D., Steiner, S., Luger, A. & Artwohl, M. (2010) Reduction of both number and proliferative activity of human endothelial progenitor cells in obesity. *International Journal of Obesity (London)* **34**, 687–700.

Tonetti, M. S. (2009) Periodontitis and risk for atherosclerosis: an update on intervention trials. *Journal of Clinical Periodontology* **36** (Suppl. 10), 15–19.

Tonetti, M. S., D'Aiuto, F., Nibali, L., Donald, A., Storry, C., Parkar, M., Suvan, J., Hingorani, A. D., Vallance, P. & Deanfield, J. (2007) Treatment of periodontitis and endothelial function. *New England Journal of Medicine* **356**, 911–920.

Tsioufis, C., Thomopoulos, C., Soldatos, N., Syrseloudis, D., Kasiakogias, A., Silvestros, S., Stefanadi, E., Mostratou, E. & Stefanadis, C. (2010) The conjoint detrimental effect of chronic periodontal disease and systemic inflammation on asymmetric dimethyl-arginine in untreated hypertensive subjects. *Atherosclerosis* **208**, 258–263.

Yinon, D., Lowenstein, L., Suraya, S., Beloosesky, R., Zmora, O., Malhotra, A. & Pillar, G. (2006) Pre-eclampsia is associated with sleep-disordered breathing and endothelial dysfunction. *European Respiratory Journal* **27**, 328–333.

Zaldivar, F., Eliakim, A., Radom-Aizik, S., Leu, S. Y. & Cooper, D. M. (2007) The effect of brief exercise on circulating CD34+ stem cells in early and late pubertal boys. *Pediatric Research* **61**, 491–495.

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Clinical Relevance

Scientific rationale for the study: Our recent study shows that circulating EPCs are associated with periodontitis in humans. This study extended to investigate the effect of periodontal treatment on CPCs count and peripheral vascular endothelial function.

Principal findings: Periodontal treatment decreased significantly the count of circulating $CD34^+$ cells in concomitance with the improvement of periodontal conditions.

Practical implications: This study enhances current understanding of the mechanisms accounting for the association of periodontal infections with endothelial dysfunction and atherosclerosis.