Effects of conservatively treated diseased cementum with or without EMD on *in vitro* cementoblast differentiation and *in vivo* cementum-like tissue formation of human periodontal ligament cells

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Abstract

Objectives: The present study aimed to evaluate the effects of conservatively treated diseased cementum on *in vitro* cementoblast differentiation and *in vivo* cementum-like tissue formation of human periodontal ligament cells (hPDLs), and observe differential effects of enamel matrix derivative (EMD) on *in vivo* cementum formation by hPDLs.

Materials and methods: Forty-eight cementum slices and 48 dentin slices were prepared from periodontitis compromised teeth, and hPDLs were inoculated on to all root slices. Twenty-four co-cultured root slices of each group were used for mRNA expression of cementum attachment protein and CEMP1. With application of EMD, 24 co-cultured root slices (divided into groups C, D, C+E, D+E) were transplanted subcutaneously into nude mice. All root fragments were reviewed by histological analysis and immunohistochemical staining for bone sialoprotein.

Results: mRNA expressions of cementum attachment protein and cementum protein - 1 from hPDLs on cementum slices were statistically higher than those of dentin slices. Seven specimens of group C and 10 specimens of group C+E revealed a layer of cementum-like tissue (NFC) on surfaces of pre-existing cementum. NFC was thicker in group C+E than in group C. All NFCs were positively stained for bone

sialoprotein, however, there was no NFC formation on dentin slices.

Conclusion: Conservatively treated diseased cementum promoted *in vitro* cementoblast differentiation and *in vivo* cementum-like tissue formation by hPDLs, and the *in vivo* effect was enhanced by the presence of EMD.

Introduction

Cementum, a thin mineralized connective tissue, constitutes the integrity of periodontium, its major function being the site of attachment for principal collagen fibres. Beside the majority of collagens, extracellular matrix (ECM) of cementum is a rich pool of various growth factors and non-collagen proteins, which compose the unique microenvironment of the cementum (1,2). There is accumulating evidence to show that preservation of root cementum may favour periodontal regeneration, especially for formation of new cementum (3,4). A previous study of our laboratory also found that preserved cementum from healthy root surfaces increased gene expression for cementum-specific proteins, such as cementum attachment protein (CAP) and cementum protein - 1 (CEMP1), in human periodontal ligament cells (hPDLs) and yielded more cementum-like tissue formation compared to dentin surface *in vivo* (5), indicating that pre-existing cementum may promote hPDLs to differentiate into cementoblasts. However, during the process of periodontitis, root cementum becomes exposed to periodontal pockets. In addition to contamination by bacterial plaque and endotoxin (6,7), cementum suffers loss of active substances due to periodontal inflammation (8,9). For example, bone sialoprotein (BSP) and osteopontin, both rich in healthy cementum

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matrix, were found to be absent in cementum exposed to periodontal pockets (8). Fibronectin showed variation in its distribution and fibrillar structure in pocket cementum compared to healthy cementum (9). Undoubtedly, these pathological changes of diseased cementum may influence cementum regeneration and connective tissue attachment to root surfaces. However, whether diseased cementum could be restored to biocompatibility by conservative treatment rather than complete removal, and conservatively treated diseased cementum could promote cementum regeneration remained to be answered.

Enamel matrix derivative (EMD) is an ECM that contains proteins similar to that derived from Hertwig's epithelial root sheath, and application of EMD in periodontal defects mimics events that take place during root development (10). Promoted cementum regeneration and new attachment formation by application of EMD have been demonstrated in some animal experiments and clinical investigations (11–13). However, whether the effect of EMD on cementum regeneration is influenced by different root surfaces has not been investigated.

Based on published data and biological effects of EMD on periodontal ligament cells, this study has aimed to evaluate effects of conservatively treated diseased cementum on *in vitro* cementoblast differentiation and *in vivo* cementum-like tissue formation, by hPDLs, and to observe differential effects of EMD on *in vivo* cementum formation on root cementum and dentin surfaces.

Materials and methods

Preparation of root slices

Tooth collection. With informed consent of each human subject, 56 teeth extracted due to periodontitis, were collected, according to the Ethics Committee of Shandong University. Inclusion criteria were: (i) premolars without root furcation, or anterior teeth; (ii) no history of subgingival scaling or root planning; (iii) gingival recession ≤ 2 mm at any site and probing depth ≥ 6 mm at any site. Markers were made by pencil both at the level of 3 mm apical to cemento-enamel junction and at the bottom level of periodontal pockets.

Scanning electron microscopy observation of root surfaces. To observe which method is better at preserving root cementum, root slices debrided by ultrasonic scalers or hand instruments were evaluated by scanning electron microscopy (SEM). Eight premolars with almost similar amounts of calculus on both medial and distal sides, were chosen. Each tooth was ultrasonically scaled using a slim-

line (EMS, Herrliberg, Switzerland), mid-power frequency, on one side (ultra-group) and manual scaling using a Gracey curette (NO. 5/6; Hu-Friedy, Chicago, IL, USA) on the other side (manual-group). Instrument-cleaning was performed by the same experienced operator until root surfaces were adequately debrided and planed. Following trimming by cutting down portions beyond coronal or apical markers, roots were then longitudinally dissected to obtain symmetrical root fragments. After internal pulp was cleared, all root slices were then dehydrated through a series of graded ethanol solutions, coated with gold and finally analysed using SEM (Hitachi, SU-70, Analytical scanning electronic microscope, Tokyo, Japan).

Root specimen preparation and treatment. Based on the SEM results, the rest of the 48 teeth were root debrided using the EMS ultrasonic scalers to prepare specimens for the following experiments. Ninety-six root slices were harvested by longitudinally dividing every root. Then one symmetrical slice of each root was further scaled to remove root cementum, and was allotted to the D (dentin) group. The other symmetrical slice was allotted to the C (cementum) group in which the cementum was preserved *in situ*. All slices were conditioned with 24% ethylene diamine tetraacetic acid (EDTA) gel (pH 6.5–7.2) (Straumann AG, Basel, Switzerland) for 2 min, washed in copious quantities of distilled water and immersed in phosphate-buffered saline (PBS) supplemented with 10% penicillin and streptomycin, for 24 h at 4 °C; then these specimens were stored in PBS at –80 °C for the following experiments.

In vitro experiments of CAP and CEMP1 mRNA expression of hPDLs grown on root slices

Inoculation of hPDLs on the root slices. Root slice chips, ~ 4 mm $\times$ 4 mm $\times$ 1 mm, were placed in 48-well plates (Corning, Corning, CA, USA) one chip per well, dentin or cementum surface oriented upwards. hPDLs from 6 periodontally healthy premolars were isolated and identified as described previously (5). Second passage hPDLs was harvested and cells suspended in DMEM culture medium, 1×10^6 /ml. 20 μ l cell suspension was sparsely seeded on each root slice, followed by addition of 0.5 ml DMEM culture medium to each well. Identical cell suspension was also seeded on 48-well plates as control (group B). Cells and root slices were co-cultured under incubation, medium changed every 3 days.

CAP and CEMP1 mRNA expression by real-time reverse transcription polymerase reaction. After 7 days co-culture, total cell RNA of each group was extracted using RNAprep pure Micro Kit (Tiangen

Table 1. Primers for real time RT-PCR

Gene	Primer sequence	T_m (°C)	Size	Accession number
CAP	5'-CCTGGCTCACCTTCTACGAC-3'	59.87	156	AY455942
	5'-CCTCAAGCAAGGCAAAATGTC-3'	60.78		
CEMP1	5'-GGCGATGCTCAACCTCTAAC-3'	59.84	158	NM_001048212
	5'-GATACCCACCTCTGCCTTGA-3'	60.07		
β -actin	5'-GATGAGATTGGCATGGCTTT-3'	59.36	160	NM_001101
	5'-CACCTTCACCGTTCAGTTT-3'	60.24		

Biotech, Beijing, China) according to the manufacturer's instructions. Total RNA was then transcribed into cDNA with RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD, USA). Primers for CAP and CEMP1 (Boya Corporation, Shanghai, China) and β -actin for internal control are listed in Table 1. Real-time PCR was performed with LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics Corp, New York, NY, USA). Standard curves were analysed using LightCycler software 4.0 (Roche Diagnostics Corp) and relative amounts of transcripts were quantified, after standardization with respect to amount of β -actin mRNA.

In vivo experiments, formation of cementum-like tissue by hPDLCs on different root slices with or without EMD

EMD treatment of hPDLCs on root slices. Twenty-four cementum slices and 24 dentin slices were divided into four groups with 12 slices in each group, according to differences of root slice and culture media. 20 μ l hPDLC suspension, 1×10^6 /ml, was inoculated on all root slices, then different culture media were added. Group C (cementum slices) and group D (dentin slices), cells were cultured with DMEM supplemented with 10% FBS; group C+E and D+E, cells were cultured with DMEM supplemented with 10% FBS and 100 mg/l of EMD (Straumann AG).

Transplantation of root slices into nude mice. This animal experiment was conducted in accordance with the regulations and approval of the Institutional Animal Care and Use Committee of Shandong University. Twelve BALB/C nude mice (4 weeks, 20–24 g, Beijing Laboratory Animal Research Center) were used. On the eighth day of hPDLCs co-culture, 12 root slices of each group were rinsed with PBS and wrapped in sterilized expanded polytetrafluoroethylene (ePTFE) (Shanghai Plastic Research Center, China). Mice were anaesthetized by intraperitoneal injection of 50 mg/kg chloramine. Incisions were made in dorsal skin and wrapped root slices were inserted beneath.

Wounds were closed by suturing. Each mouse received four slices, with one from each group.

Histological and immunohistochemical analysis. All mice were sacrificed by overdose anaesthesia 3 weeks post-surgery. Transplanted root slices were separated from surrounding tissue, and fixed in 4% paraformaldehyde for 24 h. Then specimens were decalcified in 15% EDTA solution ahead of preparation of paraffin wax blocks and sections. Longitudinal 5 μ m serial sections were cut and processed for haematoxylin and eosin staining (H&E) and immunohistochemistry; BSP staining was performed as previously described (5). Histological observation was performed using an Olympus CX71 microscope (Olympus, Tokyo, Japan). Specimens with newly formed cementum-like tissue (NFC) in each group were counted and numbers were compared between groups. Furthermore, for each specimen with NFC, mean width of NFC (W-NFC) from three H&E-stained sections was measured at midpoint of NFC by an examiner, blinded with respect to grouping, using image-analysis software (Image-Pro Plus 6.0; Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis

Differences in CAP and CEMP1 expression between groups were evaluated by one-way ANOVA, and mean value of each group was compared using the Student–Newman–Keuls (SNK) test ($P = 0.05$) with SPSS 16.0 (SPSS, Chicago, IL, USA). In the nude mice transplantation experiment, differences in number of specimens with NFC were analysed using Fisher's exact test ($P = 0.05$) with SAS 9.1 (SAS Institute, Cary, NC, USA); the W-NFC of each group was compared by t -test ($P = 0.05$) using SPSS 16.0.

Results

SEM observation of root slices

Periodontal scaling and root planing included thorough calculus removal, but complete cementum removal was not a goal of periodontal therapy. As shown in Fig. 1,

Figure 1. Representative scanning electron micrograph of root surfaces debrided by different methods of scaling. (a) Manual-group showed a rough surface with many pits. (b) Ultra-group showed a relatively smooth surface without pits. (c) High magnification of Manual-group showed obvious cementum loss. (d) High magnification of Ultra-group showed a clean and smooth surface without cementum loss.

root slices of the ultra-group had less and shallower pits compared to root surfaces of the manual-group. Our results agreed with previous studies which demonstrated that ultrasonic (power-driven) instruments can be used effectively to accomplish definitive root detoxification, without over-instrumentation of the root and without extensive cementum removal (14). Based on SEM observation, all root slices used in this study were debrided by ultrasonic scalers (EMS).

CAP and CEMP1 mRNA expression of hPDLCs grown on root surfaces

Both CAP and CEMP1 expression by hPDLCs was significantly higher on cementum surfaces (group C) than on dentin surfaces (group D), and expression of both proteins in group C or in group D was significantly higher than that in control group B, as shown in Fig. 2.

Tissue formation on transplanted root surfaces

Wound healing in mice was uneven yet all transplanted root slices (except for one in group C) were successfully harvested and processed for histological observation. There was no newly formed cementum-like tissue (NFC) either in group D or in group E+D, a thin fibrous tissue in group D (Fig. 3a), but thick, dense fibrous tissue (Fig. 3b) in group D+E was frequently observed. Wide gaps between fibrous tissue and dentin surface were invariably seen. Seven specimens in group C and 10 specimens in group C+E had a layer of NFC on the

surface of pre-existing cementum. There was no statistically significant difference between these two groups regarding number of specimens with NFC, while W-NFC of group C+E was statistically higher than that of group C (Table 2). NFCs of these groups were less dense but more cellular compared to that beneath old cementum, and there was no gap between NFC and old cementum (Fig. 3c,d). However, with the combination of preserved cementum and EMD application, newly formed fibrous tissue of group C+E usually seemed to insert into the NFC (white arrow, Fig. 3d), this is similar to structure of new attachment. Immunohistological results demonstrated that all NFCs stained positive for BSP (Fig. 4), and stain was mainly found around cells embedded in NFC and along cement lines (Fig. 4b). However, in old cementum and dentin, there was no visibly positive staining for BSP.

Discussion

In development of periodontitis, the chronic inflammatory process extends from gingival collagen fibres to root surfaces. Once attachment loss occurs, root cementum comes to be exposed to periodontal pockets and pathological changes of cementum are irreversible. The most significant change may be deposition of endotoxins, believed to interfere with periodontal wound healing. Thus, complete elimination of diseased cementum by root planing is general practice during periodontal regeneration therapy (15–17). However, experiments exist showing that endotoxins adhere to surfaces of roots

Figure 2. Regulation of gene expression in human periodontal ligament cells (hPDLs) after growing on root slices for 7 days. (a) The mRNA expression of CAP in group C was significantly higher than that in group D. (b) The mRNA expression of CEMP1 in group C was significantly higher than that in group D. The differences of CAP and CEMP1 expression between the groups were evaluated by one-way ANOVA and the mean value of each group was compared with Student–Newman–Keuls (SNK) test. *denoted statistically different to group B, $P < 0.05$; **denoted statistically different to group C, $P < 0.05$.

Figure 3. Tissue formation on root slices *in vivo*. (a) Tissue formation in group D, the representative specimen revealed the formation of a thin layer of fibrous tissue, and a wide gap was observed between the NF and dentin surface. (b) A representative specimen in group C showed a thin layer of newly formed cementum closely attached to the old cementum while there was a gap between NFC and fibrous tissue. (c) A representative specimen in group D+E revealed a thick fibrous tissue, which was much denser on the nearside of dentin surface, and a gap was also observed between the NF and dentin surface. (d) A representative specimen in group C+E showed that there was a thick layer of NFC formed on the surface of old cementum, and fibrous tissue was inserted into the NFC (white arrow). M, membrane; OC, old cementum; D, dentin; NF, new fibrous-like tissue; NFC, newly formed cementum. Magnification: $\times 100$.

rather than to penetrate into the cementum (18). Biocompatibility of diseased root surface can be achieved simply by polishing it (7,19); moreover, preserved root cementum exerts important effects on improving periodontal regeneration (3–5). Thus, a more gentle approach to diseased cementum has been proposed. In this study, effects of conservatively treated root surface on cementoblast *in vitro* differentiation of PDLs, and

capacity of cementum-like tissue *in vivo* formation by PDLs were investigated, and differential effects of EMD on potential of cementum-like tissue formation *in vivo*, on cementum and dentin surfaces by PDLs were evaluated.

Periodontal ligament cells are considered to be the most important precursor cells of cementoblasts (20). CAP and CEMP1 are both putative molecular markers

Table 2. Comparison of NFC formation in groups C and C+E

Group	Total specimens	Number of specimens with NFC	W-NFC (μm)
C	11	7	45.35 $\pm$ 12.04
C+E	12	10	76.79 $\pm$ 13.80 ^a

Difference in number of specimens with NFC was analysed by Fisher's exact test ($P = 0.05$) using SAS 9.1. W-NFC of each group was compared using the t -test ($P = 0.05$) with SPSS 16.0.

NFC, newly formed cementum-like tissue; W-NFC, mean width of NFC.

^a $P < 0.01$ versus group C, t -test.

Figure 4. Immunohistochemical staining for bone sialoprotein (BSP) in group C and group C+E. (a) BSP stain in group C, the stain for NFC was moderate while there was no positive stain for old cementum and dentin. The most positive stain in NFC was around the embedded cells (white arrow). (b) BSP stain in group C+E, the NFC was moderately stained with BSP, the embedded cells in NFC (white arrows) were strongly positive for BSP and the stratification lines (black arrows) were clearly seen with moderate stain. All the membranes were washed away in the process of BSP stain. OC, old cementum; NFC, newly formed cementum. Magnification: $\times 100$.

synthesized by cementoblasts, and are particularly expressed in cementum and cementoblast subpopulations and progenitor cells of the human periodontal ligament (21,22). In the *in vitro* study here, statistically lower gene levels of CAP and CEMP1 were detected in PDLCs inoculated on culture plates than those on root slices after 7 days culture, implying that the differentiatonal potential for PDLCs to cementoblasts may be affected by the root microenvironment. Furthermore, the cementum surface upregulated higher gene levels of both CAP and CEMP1 the hPDLCS compared to dentin surfaces, suggesting that cementum is a better microenvironment for PDLCs to differentiate into cementoblasts than dentin, and biocompatibility of periodontitis-exposed cementum can be fulfilled by conservative approaches, such as ultrasonic scaling and EDTA treatment.

To further verify this finding, the *in vivo* experiment, aiming to detect whether more new cementum could be formed on cementum root slices, was conducted in nude mice. In this experimental model, root slices were co-cultured with hPDLCS, enclosed in ePTFE membrane and transplanted beneath the skin of the animals. The ePTFE membrane is supposed to form an enclosed root slice chamber, preventing endogenous cells from entering and providing an environment for PDLCs to grow and differentiate. This model has been proved to be a

simple and effective method to study differentiatonal activity of PDLCs on the root surface (23). Our experiment showed that almost all specimens had cell cementum-like matrix formation when the cementum was preserved. Further immunohistological reaction disclosed that cementum-like matrix was positively stained for BSP. BSP, believed to play an important role in differentiation from cementoblast progenitor cells to cementoblasts (24), is a major non-collagenous protein of cementum. Positive staining for BSP implicated that NFC was mineralized tissue rather than fibrous connective tissue. Results of the *in vivo* study further indicated that preservation of diseased cementum promoted differentiation of hPDLCS into cementoblasts and thus induced cementum formation. Our results were in keeping with studies conducted by Goncalves *et al.* (3,25), who demonstrated (through histomorphometric studies in dogs) that preservation of 'diseased' root cementum could modulate periodontal regeneration.

The cause of different differentiatonal capability of PDLCs on cementum and dentin remains unclear. It is reasonable to postulate that it is the difference of ECM between cementum and dentin that is responsible for it on these two kinds of surface. Cementum has recently been reported to contain more proteins involved in cell communication and signal transduction compared to alveolar bone (26). A number of non-collagenous

proteins have been isolated from cementum, dentin and alveolar bone; they express qualitative similarities, but significant quantitative differences. Most notable among these differences is highest levels of CAP. CAP, a protein mainly in cementum matrix rather than dentin matrix, may play an important role in cementogenesis (21). Also, it has been reported that over-expression of CEMP1 in periodontal ligament cells enhances cementoblast differentiation (27). However, differences in other components, as well as how PDLCs interact with cementum and dental hard tissues need to be elucidated by further research.

Enamel matrix derivative has been widely studied in animal experiments and in clinical applications and its periodontal regeneration potential has been identified (11–13). However, whether EMD has its differential effect on cementoblast differentiation of PDLCs on root cementum and dentin surfaces has not been investigated up to now. In this study, group C+E yielded higher quantities of newly formed cementum-like tissue, indicating that EMD enhanced cementum regeneration on pre-existing cementum. However, surprisingly, there was no NFC formation on dentin slices, whether they had been treated with EMD or not. This is not consistent with some previous studies in which EMD induced new cementum formation on dentin surfaces following GTR (13,28). In our previous investigation on healthy root slices, it was also found that there was NFC formation on dentin slices, although the number of specimens with NFC formation was much lower than that on cementum slices (5). The reason for this discrepancy remains to be determined.

Hand instrumentation and ultrasonic tools are used most commonly in sub-gingival scaling and root planing. To explore in a preliminarily test which method would better preserve cementum to the maximum, we compared by SEM roughness of root surfaces after manual and ultrasonic scaling respectively. Smoother root surfaces with less cementum pits were observed on ultrasonic scaler-treated roots; this was consistent with other studies by Ru'hling *et al.* (29) and Marda *et al.* (30). The present findings show that biocompatibility of diseased root surface with preservation of cementum can be achieved by conservative methods such as ultrasonic scaling and EDTA conditioning, which leads to a suggestion that ultrasonic scaling has advantages over manual scaling on cementum regeneration.

In conclusion, a biocompatible cementum surface of periodontitis-exposed teeth can be acquired by ultrasonic scaling and EDTA conditioning. This diseased cementum surface has up-regulated expression of CAP and CEMP1 genes in hPDLCs and yielded more newly formed cementum after transplantation into nude mice, compared to a dentin surface. It can be confirmed that

periodontally diseased cementum promoted differentiation of hPDLCs into cementoblasts and this effect was enhanced by the presence of EMD. Further elucidation the precise mechanisms of differential differentiation of hPDLCs on cementum and dentin surfaces and exploration of the better treatment protocol for periodontitis-exposed root surfaces, both preserving cementum as much as possible and satisfying requirements of biocompatibility, will have important impact on periodontal regeneration strategies.

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References

- Grzesik WJ, Narayanan AS (2002) Cementum and periodontal wound healing and regeneration. *Crit. Rev. Oral Biol. Med.* **13**, 474–484.
- Margarita ZD (2006) Regeneration of periodontal tissues: cementogenesis revisited. *Periodontol.* **2000** **41**, 196–217.
- Goncalves PF, Gurge BC, Pimentel SP, Sallum EA, Sallum AW, Casati MZ *et al.* (2006) Effect of two different approaches for root recontamination on new cementum formation following guided tissue regeneration. *J. Periodont. Res.* **41**, 535–540.
- Goncalves PF, Lima LL, Sallum EA, Casati MZ, Nociti FH Jr (2008) Root cementum may modulate gene expression during periodontal regeneration: a preliminary study in humans. *J. Periodontol.* **79**, 323–331.
- Song A, Cai J, Pan K, Yang P (2012) Pre-existing root cementum may promote cementoblast differentiation of human periodontal ligament cells. *Cell Prolif.* **45**, 249–258.
- Polson AM (1986) The root surface and regeneration; present therapeutic limitations and future biologic potentials. *J. Clin. Periodontol.* **13**, 995–999.
- Nyman S, Westfelt E, Sarhed G, Karring T (1988) Role of “diseased” root cementum in healing following treatment of periodontal disease. A clinical study. *J. Clin. Periodontol.* **15**, 464–468.
- Lao M, Marino V, Bartold PM (2006) Immunohistochemical study of bone sialoprotein and osteopontin in healthy and diseased root surfaces. *J. Periodontol.* **77**, 1665–1673.
- Komboli MG, Kodovazenitis GJ, Katsorhis TA (2009) Comparative immunohistochemical study of the distribution of fibronectin in healthy and diseased root surface. *J. Periodontol.* **80**, 824–832.
- Fong CD, Hammarstrom L (2000) Expression of amelin and amelogenin in epithelial root sheath remnants of fully formed rat molars. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **90**, 218–223.
- Hammarström L, Heijl L, Gestrelus S (1997) Periodontal regeneration in a buccal dehiscence model in monkeys after application of enamel matrix proteins. *J. Clin. Periodontol.* **24**, 669–677.

- 12 Sculean A, Donos N, Brex M, Karring T, Reich E (2000) Healing of fenestration-type defects following treatment with guided tissue regeneration or enamel matrix proteins. An experimental study in monkeys. *Clin. Oral. Investig.* **4**, 50–56.
- 13 Gkraniias ND, Graziani F, Sculean A, Donos N (2012) Wound healing following regenerative procedures in furcation degree III defects: histomorphometric outcomes. *Clin. Oral. Investig.* **16**, 239–249.
- 14 Drisko CH (1998) Root instrumentation. Power-driven versus manual scalers, which one? *Dent. Clin. North Am.* **42**, 229–244.
- 15 Inukai T, Katagiri W, Yoshimi R, Osugi M (2013) Novel application of stem cell-derived factors for periodontal regeneration. *Biochem. Biophys. Res. Commun.* **430**, 763–768.
- 16 Tobita M, Uysal CA, Guo X, Hyakusoku H, Mizuni H (2013) Periodontal regeneration of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. *Cytotherapy* **15**, 1517–1526.
- 17 Dan HX, Vaquette C, Fisher AG, Hamlet SM (2014) The influence of cellular source on periodontal regeneration using calcium phosphate coated polycaprolactone scaffold supported cell sheets. *Biomaterials* **35**, 113–122.
- 18 Nakib NM, Bissada NF, Simmelink JW, Goldstine SN (1982) Endotoxin penetration into periodontally healthy and diseased human teeth. *J. Periodontol.* **53**, 368–378.
- 19 Nyman S, Sarhed G, Ericsson I, Gottlow J, Karring T (1986) Role of “diseased” root cementum in healing following treatment of periodontal disease. A clinical study. *J. Periodont. Res.* **21**, 496–503.
- 20 Nagatomo K, Komaki M, Sekiya I, Sakaguchi Y, Noguchi K, Oda S *et al.* (2010) Stem cell properties of human periodontal ligament cells. *J. Periodont. Res.* **41**, 303–310.
- 21 Saito M, Iwase M, Maslan S, Nozaki N, Yamauchi M, Handa K *et al.* (2001) Expression of cementum-derived attachment protein in bovine tooth germ during cementogenesis. *Bone* **29**, 242–248.
- 22 Arzate H, Jimenez-Garcia LF, Alvarez-Perez MA, Landa A, Bar-Kana I, Pitaru S (2002) Immunolocalization of a human cementoblastoma conditioned medium-derived protein. *J. Dent. Res.* **81**, 541–546.
- 23 Song AM, Shu R, Xie YF, Song ZC, Li HY, Liu XF *et al.* (2007) A study of enamel matrix proteins on differentiation of porcine bone marrow stromal cells into cementoblasts. *Cell Prolif.* **40**, 381–396.
- 24 Saygin NE, Giannobile WV, Somerman MJ (2000) Molecular and cell biology of cementum. *Periodontol.* **24**, 73–98.
- 25 Goncalves PF, Gurgel BC, Pimentel SP, Sallum EA, Sallum AW, Casati MZ *et al.* (2006) Root cementum modulates periodontal regeneration in Class III furcation defects treated by the guided tissue regeneration technique: a histometric study in dogs. *J. Periodontol.* **77**, 976–982.
- 26 Salmon CR, Tomazela DM, Ruiz KG, Foster BL (2013) Proteomic analysis of human dental cementum and alveolar bone. *J. Proteomics.* **91**, 544–555.
- 27 Komaki M, Iwasaki K, Arzate H, Narayanan AS, Izumi Y, Morita I (2012) Cementum protein 1(CEMP1) induces a cementoblastic phenotype and reduces osteoblastic differentiation in periodontal ligament cells. *J. Cell. Physiol.* **227**, 649–657.
- 28 Al-Hezaimi K, Al-Askar M, Al-Rasheed A (2011) Characteristics of newly-formed cementum following emdogain application. *Int. J. Oral. Sci.* **3**, 21–26.
- 29 Ru'hling A, Wulf J, Schwahn C, Kocher T (2004) Surface wear on cervical restorations and adjacent enamel and root cementum caused by simulated long-term maintenance therapy. *J. Clin. Periodontol.* **31**, 293–298.
- 30 Marda P, Prakash S, Devaraj CG, Vastardis S (2012) A comparison of root surface instrumentation using manual, ultrasonic and rotary instruments: an in vitro study using scanning electron microscopy. *Indian. J. Dent. Res.* **23**, 164–170.