Effect of orthodontic forces on levels of enzymes in gingival crevicular fluid (GCF): A systematic review

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Objective: Orthodontic force application releases multiple enzymes in gingival crevicular fluid (GCF) for activation, resorption, reversal, deposition of osseous elements and extracellular matrix degradation. The current systematic review critically evaluated all existing evidence on enzymes in orthodontic tooth movement. Methods: Literature was searched with predetermined search strategy on electronic databases (PubMed, Scopus, Embase), along with hand search. Results: Initial search identified 652 studies, shortlisted to 52 studies based on PRISMA. Quality assessment further led to final inclusion of 48 studies (13 moderately and 35 highly sensitive studies). Primary outcomes are significant upregulation in GCF levels of enzymes-aspartate aminotransferase (AST), alkaline phosphatase (ALP), matrix metalloproteinases (MMPs), lactate dehydrogenase (LDH), β-glucuronidase (βG), tartrate resistant acid phosphatase (TRAP), acid phosphatase (ACP) and down regulation in cathepsin B (Cb). Site specificity is shown by ALP, TRAP, AST, LDH, MMP9 with levels at compression site increasing earlier and in higher quantities compared with tension site. ALP levels are higher at tension site only in retention. A positive correlation of LDH, ALP and AST is also observed with increasing orthodontic force magnitude. Conclusions: A strong evidence of variation in enzymes (ALP, AST, ACP TRAP, LDH, MMPs, Cb) in GCF is found in association with different magnitude, stages and sites of orthodontic force application.

Keywords: Tooth movement. Gingival crevicular fluid (GCF). Enzymes. Systematic review.

Objetivo: a aplicação da força ortodôntica libera múltiplas enzimas no fluido crevicular gengival (FCG), desencadeando a ativação, realimentação, reversão, deposição de elementos ósseos e degradação da matriz extracelular. A presente revisão sistemática avaliou criticamente toda a evidência disponível sobre os níveis de enzimas durante a movimentação ortodôntica. Métodos: utilizando-se estratégias predeterminadas, foram realizadas buscas em bases de dados eletrônicas (PubMed, Scopus, Embase), sendo também feitas buscas manuais. Resultados: a busca inicial identificou 652 estudos e, com base na diretriz do PRISMA, foram selecionados 52 estudos. A avaliação qualitativa resultou na inclusão final de 48 estudos (13 estudos com moderada sensibilidade e 35 com alto nível de sensibilidade). Os desfechos primários foram o aumento significativo dos níveis no FCG das enzimas aspartato aminotransferase (AST), fosfatase alcalina (FA), metaloproteinases de matriz (MMPs), lactato desidrogenase (LDH), β-glucuronidase (βG), fosfatase ácido-resistente ao tartarato (TRAP), fosfatase ácida (FAC) e baixa regulação de catépsina B (Cb). Especificidade quanto ao local foi mostrada para FA, TRAP, AST, LDH e MMP9 com os níveis no lado de compressão aumentando mais rápido e em maiores quantidades, quando comparado ao lado de tensão. Os níveis de FA foram maiores no lado de tensão somente no período de contenção. Uma correlação positiva de LDH, FA e AST também foi observada à medida que a magnitude de força ortodôntica aumentou. Conclusões: há fortes evidências indicando que as variações nas enzimas (FA, AST, FAC, TRAP, LDH, MMPs, Cb) presentes no FCG estão associadas a diferentes magnitudes, estágios e locais de aplicação da força ortodôntica.

Palavras-chave: Movimento dentário. Fluído crevicular gengival (FCG). Enzimas. Revisão sistemática.

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INTRODUCTION

Orthodontic forces cause an initial inflammatory response followed by alterations in the vascular and neural envelope and perpetual bone and tissue remodelling accompanied by paracrine release of bioactive mediators.\(^1\)\(^-\)\(^3\) During orthodontic tooth movement (OTM), host-derived enzymes are released at various stages of activation, resorption, reversal and deposition of osseous elements and degradation of the extracellular matrix.\(^4\) Some of these enzymes have been identified in the periodontal (pdl) tissue of orthodontically moved teeth.\(^5\) Gingival crevicular fluid (GCF) is however a better choice for assessing biomolecules or mediators as sample collection is simple, sensitive, convenient, repetitive and non-invasive.\(^6\) Thus, the quantitative estimations of mediators in GCF reflect biochemical mechanisms associated with OTM. A systematic review (SR) by Kapoor et al\(^6\) in 2014 studied variation in GCF level of cytokines with type and magnitude of orthodontic forces and growth status of patients. It established a positive correlation of GCF activity index IL1RA (interleukin receptor antagonist)/IL-1\(\beta\) with intensity of pain and velocity of OTM and a negative correlation with growth status of patients. Besides cytokines, numerous other mediators also alter GCF during OTM, comprehensively reviewed in SR by Alhadlaq\(^7\) in 2015. This SR highlighted working mechanisms of multiple mediators but heterogeneity of studies precluded attainment of concrete conclusions. Hence, the present SR aims to assess only a single family of mechanisms of multiple mediators but heterogeneity of studies precluded attainment of concrete conclusions. Hence, the present SR aims to assess only a single family of mechanisms of multiple mediators but heterogeneity of studies precluded attainment of concrete conclusions. Hence, the present SR aims to assess only a single family of mediators, enzymes, to establish their clinical correlations on sequential release in different phases of OTM and varying magnitude of orthodontic forces.

Soluble enzymes like lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) present in cytoplasm are known to release in GCF only after cellular necrosis or hyalinization with heavy orthodontic forces.\(^4\) Tartrate-resistant acid phosphatase (TRAP) and alkaline phosphatase (ALP) exhibit osteoclastic and osteoblastic activity, respectively,\(^7\) and are identified in areas of tension (TS) or compression (CS) of teeth undergoing OTM. Heavy orthopedic forces of rapid maxillary expansion accompanied by paracrine release of bioactive mediators.\(^1\)\(^-\)\(^3\) The evidence on enzymes in OTM is plenty but scattered and lacks critical appraisal. Hence, the current SR is conducted to establish associations of enzymes in GCF to the site of application, magnitude and type of force, patient’s growth status and the type of archwire ligation.

MATERIAL AND METHODS

Protocol and registration

The protocol for SR was registered in PROSPERO (www.crd.york.ac.uk/prospero, CRD42015017496) with a predetermined search strategy (Fig 1). It comprised of MeSH terms, Boolean terminology and free text terms with the keywords "enzyme" "protease", "orthodontic tooth movement" and "gingival crevicular fluid", together with several key enzymes. This search strategy was applied to key databases PubMed, Scopus and Embase in February 2018 with no language restrictions. Additional publications were identified through reference tracking and hand search of journals (Sains Malaysiana, Orthodontic Waves, Journal of Applied Sciences, APMC). The search was performed by two reviewers, followed by a cross-check by a third reviewer, in conformity with PRISMA, as shown in Figure 2.

Evaluation of risk of bias/quality of individual studies

The risk of bias, subjective to the included studies was measured by a customized Quality Assessment Instrument (QAI)\(^6\) based on QUADAS. This was objectively scored as minimally (scores of 1-12), moderately (13-20) and highly (21-29) sensitive, summarized in Table 1. No minimally sensitive studies were included in the review.

RESULTS

Were identified 102 articles in Pubmed, 460 in Scopus, 84 in Embase and 6 from hand search, in the initial search. Strict inclusion and exclusion criteria (Table 2) were applied after removing duplicates, resulting in 41 relevant articles. Five studies were further excluded: three studies whose full texts were not retrieved despite contacting the authors repeatedly through mail and academic social networking sites; one was a review on MMPs, and one had sample size smaller than inclusion criteria. Additional exclusion of three studies was done: two with QAI score smaller than 13, and one with a cross-sectional study design (Fig 2).
PRISMA finally resulted in 48 publications in total, with consensus among all reviewers. The QAI of these studies indicated 13 moderately sensitive and 35 highly sensitive studies.

Data extraction of shortlisted studies7-54 (for participant characteristics and study design are as follows (Table 3):

- Sample size: Sample size was categorized in three groups, ≤15 (n=22), 15-20 (n=15), ≥21 (n=10) and one study each having sample of five subjects27 and 99 subjects.21
  - Sex predilection: Forty-one studies mentioned sex distribution in the sample, two of which had female subjects only,24,36 and five had equal numbers of male and female subjects.10,19,23,29,43
  - Age predilection: Studies used age as either range or mean with standard deviation in all studies; one study considered two separate age groups of adolescents and adults.15

Figure 1 - Search strategy applied on databases for inclusion of studies in the review.

Figure 2 - PRISMA flow diagram for inclusion of studies in the systematic review.
Table 1 - Inclusion and Exclusion criteria applied for inclusion of studies in the systematic review.

| Criteria          | Sub criteria                  | Inclusion                      | Exclusion                                      |
|-------------------|-------------------------------|--------------------------------|------------------------------------------------|
| Participants/     | Type of sample                | Human studies                  | Animal studies, in vitro studies               |
| population        | Age groups                    | if specified                   | Not mentioned                                  |
|                   | Male to female ratio          | if specified                   | Not mentioned                                  |
|                   | Controls                      | present (either internal /external) | No controls                                    |
|                   | Sample size (sample size, not number of teeth studied) | ≥5 | <5 |
| Intervention(s), | Mediators studied             | enzymes (AST, MPO, ALP, βG, LDH, | Other than enzymes (cytokines/ hormones/PGs)  |
| exposure(s)       | Exposure                      | CatB, Cs, cAMP RII, MMPs)      | Studied in periodontal inflammation/ root resorption/ not related |
|                   | Orthodontic mechanics         | Specified                      | Not specified                                  |
|                   | Oral hygiene regimen          | Mentioned                      | Not mentioned                                  |
|                   | Use of antibiotic/anti-inflammatory drugs | Not used                  | Not mentioned/ used                             |
|                   | Medium of study               | GCF                            | Other than GCF/ peri-implant fluid/ saliva     |
|                   | GCF sample collection instrument | Periopaper/micropipette/ endodontic paper | Not mentioned                                   |

AST: aspartate transaminase, MPO: myeloperoxidase, ACP: acid phosphatase, ALP: alkaline phosphatase, βG: β glucuronidase, LDH: lactate dehydrogenase, CatB: cathepsin B, Cs: caspase, cAMP RII: cyclic adenosine monophosphate (AMP)-dependent protein kinase subunit (RII), PGs: prostaglandins, MMPs: matrix metalloproteinases.

» Number of studies reporting enzymes: Alkaline phosphatase was evaluated in maximum number of studies (n=17), closely followed by AST in 10, matrix metalloproteinases (MMPs) in eight, LDH in six, MPO in five and TRAP in four and acid phosphatase (ACP) in three studies. Two studies studied βG, cathepsin (Cp) and tissue inhibitor of MMPs (TIMPs) each. Single studies evaluated cystatin (Cys) and thrombospondin1 (TSP1). Additionally, granulocyte-macrophage colony-stimulating factor (GMCSF), epidermal growth factor (EGF), macrophage inflammatory protein-1β (MIP-1 β), methyl-accepting chemotaxis protein-1 (MCP-1), chemokine RANTES (Regulated on activation normal T cells expressed and secreted) were evaluated as secondary outcomes.

» Study duration: The duration of studies ranged from 8 hr to 24 weeks (wk) to the maximum of one year (y). One study each was done for 8hr, 1wk, 5month (m) and 1y duration, two studies for 6m, three for 2m, five each for 2wk and 3m, eight for 3wk, 15 for approximately 1m. One study did not specify duration — only completion of alignment.

» Observation intervals for GCF collection: Studies had GCF collection at repeated observation time points (OTP) ranging from 2 times²⁸ to 31 times (each day of the month).²⁹ Six OTPs were taken in 16 studies, closely followed by 4 OTPs in 15 studies, 9 OTPs in nine studies, 3 and 10 OTPs in two studies each, 2, 7, 8 and 31 OTPs in single study each.

» Site for GCF collection: Forty one studies specified mesial or distal or buccal site for GCF collection while seven studies mentioned the tooth but not the site for sample retrieval. The technique by Lamster et al.⁵⁵ utilizing six sites was used in four studies.¹⁰,¹⁹,³³,⁴⁴,⁴⁷

» Mechanics of force: Studies used continuous force both for tooth retraction (26 studies) and leveling of arches (13 studies). Retraction involved 19 studies using NiTi coil spring, two using steel ligature lacesacks, three using NiTi push coil spring, and one study each for V loop and NiTi open coil spring. Besides, nine studies used intermittent orthodontic/orthopaedic forces, employing elastomeric chain for retraction in five, Hyrax for expansion in three, and TMA spring for intrusion in one study.

» The level of force: Only 33 studies mentioned force levels for OTM. The level of forces ranged from 50g, 50-75g, 100-150g, 16N/turn, 1-1.5N, 200cN, 400g in one study each, 125g in three, 100g in six, 250g in eight and 150g in seven studies. Few studies had different treatment groups employing variable magnitudes of force.⁹,¹¹,³⁴,³⁵,³⁶

Oral hygiene regimen and gingival health assessment (Table 4)

Professional oral prophylaxis was done before treatment in 34 studies and at every OTP in 16 studies, but was not mentioned in 12 studies. Verbal edification for oral hygiene maintenance was done in 33 studies.
Nine studies advocated chlorhexidine mouthwash and two studies, benzydamine hydrochloride; but six studies refrained the use of any mouthwash during study period. Gingival and pdl health evaluation was done before treatment in 31 studies and at every OTP in 24 studies using "Quigley Hein Index" for visual plaque or its Turesky modification, Eastman interdental bleeding score, generalized probing depths <3 mm, radiographic evidence of pdl bone loss, gingival recession, full-mouth plaque score or full-mouth bleeding score (<20%).

**Table 2** - Quality Assessment Instrument (QAI) customized from QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool for assessment of risk of bias for inclusion of studies in the review.

| S. No. | Criteria (29)                                                                 | Yes | Response | No | Unclear |
|--------|-----------------------------------------------------------------------------|-----|----------|----|---------|
| 1      | Objective: objective clearly formulated                                      |     |          |    |         |
| 2      | Sample size: considered adequate                                              |     |          |    |         |
| 3      | Spectrum of patients representative of patients receiving the test in practice |     |          |    |         |
| 4      | Ethical clearance mentioned                                                   |     |          |    |         |
| 5      | Selection criteria: clearly described                                         |     |          |    |         |
| 6      | Randomization: stated                                                         |     |          |    |         |
| 7      | Baseline characteristics: clearly defined                                    |     |          |    |         |
| 8      | Control: clearly defined                                                      |     |          |    |         |
| 9      | Orthodontic mechanics explained in sufficient detail to permit replication of experiment |     |          |    |         |
| 10     | Orthodontic force: clearly specified                                          |     |          |    |         |
| 11     | Description of execution of index test: sufficient to permit replication of test |     |          |    |         |
| 12     | Absence of time difference between index test & control: mentioned           |     |          |    |         |
| 13     | Index test executed at specified time and environmental conditions           |     |          |    |         |
| 14     | Use of proper indices for assessment of gingival & periodontal status (pre-treatment) |     |          |    |         |
| 15     | Use of proper indices for assessment of gingival & periodontal status (at each observation time) |     |          |    |         |
| 16     | Oral hygiene regime: mentioned                                               |     |          |    |         |
| 17     | Prophylaxis done (pre-treatment)                                             |     |          |    |         |
| 18     | Prophylaxis done (at each observation time)                                  |     |          |    |         |
|        | **II. Study measurements**                                                     |     |          |    |         |
| 1      | GCF handling characteristics: explained                                       |     |          |    |         |
| 2      | Measurement method: appropriate to the objective                              |     |          |    |         |
| 3      | Reliability: adequate level of agreement                                      |     |          |    |         |
|        | **III. Statistical analysis**                                                 |     |          |    |         |
| 1      | Dropouts: dropouts included in data analysis                                  |     |          |    |         |
| 2      | Statistical analysis: appropriate for data                                    |     |          |    |         |
| 3      | Confounders: confounders included in analysis                                 |     |          |    |         |
| 4      | Statistical significance level: P value stated                                |     |          |    |         |
| 5      | Confidence intervals provided                                                 |     |          |    |         |
|        | **IV. Study results and conclusions (3)**                                      |     |          |    |         |
| 1      | Index test compared to baseline                                               |     |          |    |         |
| 2      | Index test compared to control                                                |     |          |    |         |
| 3      | Conclusions: specific                                                         |     |          |    |         |

*Index test: Refers to collection of GCF at each observation interval in treatment teeth.

**GCF characteristics (Table 5)**

- GCF collection: GCF was collected by Peripaper (OraFlow, Plainview, New York, NY, USA) in 32 studies, micropipette in seven, filter paper in two, paper point in two and endodontic paper strip in five studies. Time of sample collection, room temperature and humidity conditions were specified in three studies each.
- GCF handling: Depth of Peripaper insertion was 1mm in 21 studies, 1-2mm in two, and 2mm in one study. Duration of GCF collection was 30 seconds (s) in 21 studies, 60s in 13 studies and 10s, 3 minutes (min)
### Table 3 - Participant and study characteristics table.

| Reference no. | Sa | M/F  | Age  | Me       | lx T                | cT/gp  | Site  | Rn | ml |
|---------------|----|------|------|----------|---------------------|--------|-------|----|----|
| 7             | 9  | 5M/4F| 10-18y | IL-1β, βG | 1st Mo, 1st PM, CI  | NM     | MB   & MB | N | IRE |
| 8             | 14 | 5M/9F| 12.5 ± 1.7y | MPO | Single root T | NM     | MB & DB | NM | NM |
| 9             | 12 | 5M/7F| 16-20y (17.5 ± 2.4y) | ALP | Mx C & ct C 1st Mo | MS C & D | 1st Mo | Y | Class I |
| 10            | 20 | 10M/10F| 15-25y | ALP | Mx C ct C | MB, MB, DB, MP, MiP, DP | Y | Class I bimax |
| 11            | 19 | 5M/14F| 16-28y | LDH, AST, TRAP, ALP | Mx C | NM     | NM | Y | 1st PM Ec |
| 12            | 20 | 5M/15F| 19.1 ± 3y | MPO | Md I | NM     | MB, DDB | N | crw (severe & minim) |
| 13            | 16 | 6M/10F| 13-17y (14 ± 16 7y) | TSPI, MMP9/NGAL | Mx C ct C D | NM     | 1st PM Ec |
| 14            | 20 (10CIF/10 non CIF) | Cif gp: 7M/3F | Non Cif gp: 5M/5F | 15-25y (19.75 ± 2.93y) | ALP, ACP, AST | Mx I, Mo of same q | NM | NM | NM |
| 15            | 20 (10 adol, 10 Ad) | ado – 3M/7F Ad - 4M/6F | ado 14.4 ± 143y Ad 28.5 ± 783y | MMP-9, RANKL, IL-1, IL-1RA | Mx I | Md I | DB | N | Class I minor crw |
| 16            | 40 (4gps) | 19M/21F | 12-18y | LDH | 4.1, 4.3 & 4.5 | 11, 1.3 & 1.5 | Bu | NM | Class I Md crw |
| 17            | 19 | 9M/7F | 177y | (MMPs)-1, -2, -3, -7, -8, -12, -13 | Mx C | ct C | Ms & D | Y | 1st PM Ec |
| 18            | 21 | NM   | 12-20y | GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNFα, MMP-9, TIMP-1 & 2, RANKL, OPG | Mx C | 2nd Mo | MB & DOP | NM | 1st PM Ec |
| 19            | 20 | 10M/10F | 15-25y | LDH | Mx C | NM | MB, MB, DB, MP, MiP, DP | NM | Class I bimax |
| 20            | 14 | 3M/11F | 12-28y (18.8 ± 4.8 y) | MMP-3, MMP-9, MMP-13, MMP-1β, MCP-1, RANTES | Mx C | NM | MiB | NM | Mix 1st PM Ec |
| 21            | 99 | 3 gpsi | 1st: Non ortho (35M/9F) 2nd:C re (3M/14F) 3rd: (13M/25F) | gp 1: 22 ± 10m gp 2: 24 ± 10m gp 3: 32 ± 20m | C p, cys gp2: Mx C | gp1 | D | NM | gp2: 1st PM Ec |
| 22            | 11 | 8F/3M | 13-15y (13.9y) | MMP-1, MMP-2 | L Mx C Ag Mx C | MB & DB | N | 1st PM Ec |
| 23            | 10 | 5M/5F | 13-15y (13.9y) | M - 22.5 ± 2.8y, F - 23.4 ± 3.9y | p-TAP & PAI -2 | M X C | ct & Ag C | D | NM | 1st PM Ec |
| 24            | 10 | 8F | 12-21y | ALP | Mx C | ct C | Ms & D | N | 1st PM Ec |
| 25            | 9  | 4M/5F | 14.76 ± 2.08y | ALP | Mx 1st PM | NM | MB, DB, P | N | 1st PM Ec |
| 26            | 17 | 9F/8M | 11-22y, 16.1 ± 3.8 y | LDH | 1st Mx Mo | Ag & ct 1st Mx Mo | Ms & D | N | Mo dst |
| 27            | 5  | 3F/2M | nov/36 | MMP-1 & 16β | Mx & Md CI/ Mx C | Mx & Md CI | NM | NM | |
| 28            | 21 | 11F/10M | 11.2-22.5y, 171 ± 3.3 y | ALP & AST | Mx C | Ag & ct C | Ms & D | N | 1st PM Ec |
| 29            | 10 | 5M/5F | 22.5 ± 3.9y | C p B | C | ct & Ag C | D | NM | 1st PM Ec |

A - article, f-force, t/o-type of, mc-mechanics, md/mc-mode of mechanics, tm-time, a-appliance, re-re-activation, to-total, du-duration, n-number, ob-observation, B-baseline, min-minutes, g-grams, Ir-Irreupted, Cn-Continuous, Im-intermittent, Rt-retraction, sg-segmented, sp-spring, Ech-elasticomeric chain, NITI-nitinol, c-control, NM-not mentioned, y-year, d-day, m-month, h-hour, lv-leveling, se-separator, ac-activated, HG-headgear, NHG-non-headgear, bu-buccal, la-labial, RME-rapid maxillary expansion, HR-hybrid retractor, RCD-rapid canine distaliser, Sa-Sample, M/F/male/female, E-enzyme, Me-mediator, T-tooth, sc-specification, rm-randomisation, ml-malocclusion, HS-Handsearched, P-Pubmed, S-Scopus, GS-Google scholar, N-No, Y-yes, Mx-Maxilla, Md-Mandible, H-history, Is-loss, gv-gingival, if-inflammation, PD-probing depth, wk-week, R-right, L-left, C-canine, PM-premolar, M-molar, Ci-central incisor, l-lower, Ag-Antagonistic, ct-Contralateral, ip-irreproducible, op-opposing, Ex-Experimental, c-Control, aj-adjacent, Exs-Experimental site, Ec-Extraction, Ms-Mesial, D-Distal, rq-required, q-quadrant, OTM-orthodontic tooth movement, sf-surface, ado-adolescent, AST-asterisk transimanses, TRAP-phosphatase, ALP-Alkaline Phosphatase, JG-beta glucuronidase, MMP-matrix metalloproteinase, LDH-lactate dehydrogenase, Cp-Cathepsin, MPO-myeloperoxidase, CK-creatine, NO-Nitric oxide, IL-Interleukin.
| F       | t/o f | mc    | md/mc | re    | to du | No. / ob | tm/ob | B       |
|---------|-------|-------|-------|-------|-------|----------|-------|---------|
| NM      | Im F  | RME   | Hyaax | Y     | 81d   | 10       | 0, 14, 25, 32, 33, 39, 46, 53, 60, 81 d | 14d    |
| NM      | Cn Lv | Arch wi | N     | 14d   | 4     | -7d, 0, 2h, 7, 14d | 0       | Y       |
| Cn Rt  | NiTi sp | NA   | 3 wk  | 4     | 0,1wk, 2wk, 3wk | 0       | N       |
| 100/150g | Cn Rt | NiTi sp | NA   | 5 wk  | 6     | 0, 1, 2, 3, 4, 5 wk | 0       | Y       |
| NM      | Cn Lv | Arch wi | N     | 2 wk  | 4     | 0, 2h, 7d, 14d | 0       | Y       |
| NM      | Cn Rt | LB    | NA    | 2 wk  | 8     | -1h, +1h, 4, 8, 24, 72, 1wk, 2wk | -1h    | N       |
| 150cN   | Cn Lv | NiTi wi | NA   | 30d   | 5     | 0, 3, 9, 15, 30d | 0       | Y       |
| NM      | Cn Lv | NiTi wi | Y     | 20 wk | 4     | 0, 3, 6, 18, 20 wk | 0       | Y       |
| NM      | Cn Lv | NiTi & thm wi | Y | 42d   | 6     | -2wk, 0, 1h, 7, 28, 42d | -2wk   | N       |
| 150g    | Cn Rt | NiTi sp | N    | 21d   | 6     | 0, 1, 24, 7, 14, 21d | 0       | N       |
| 100g    | Cn Rt | NiTi sp | N    | 42d   | 4     | -10wk, 0, 4h, 7d, 42d | 0       | N       |
| 125g    | Cn Rt | NiTi sp | N    | 21d   | 5     | 0, 1n, 7, 14, 21d | 0       | Y       |
| 150g    | Cn Rt | V- loop & NiTi sp | N | 87d   | 7     | -7d, 0, 1h, 24h, 14, 21, 40d | 0       | Y       |
| 100-150g| Imf Rt | E Ch  | NM    | 1m    | 4     | 0, 1d, 1wk, 1m | 0       | N       |
| 150g    | Cn Rt | NiTi sp | N    | 8 h   | 5     | 0, 1n, 2h, 3n, 4h, 8h | 0       | N       |
| 250g    | Imf Rt | E Ch  | NM    | 168h  | 4     | 0, 1, 24, 168h | 0       | N       |
| 100g    | Cn Rt | NiTi sp | N    | 21d   | 6     | -1, 0, 1, 7, 14, 21d | -1      | N       |
| 50g     | Imf Intr | TMA sp | Y    | 28d   | 5     | 0, 1, 24, 168h, 22d, 28d reac-21d | 0       | Y       |
| 250g    | Cn Rt | NiTi sp | N    | 21d   | 4     | 0, 7, 14 6 21d | 0       | N       |
| NM      | Cn ain | NM    | N     | 30d   | 31    | 0,1-30d,once/d for 1 m | 0       | N       |
| 150g    | Cn Rt | NiTi sp | N    | 28d   | 2     | 0, 28d | 0       | N       |
| 250g    | Imf Rlt | E ch  | NM    | 168h  | 4     | 0, 1n, 24h, 168h | 0       | N       |

CRP- C Reactive Protein, hrm-humidity, sc-specification, ins-insertion, MB-Mesio-buccal, ML-Mesio-lingual, DP-Disto-palatal, DB- Disto-buccal, df-differentiation, gp-group, cmp-compression, kPa-kilopascal, mx-maximum, gw-growth, Oc-osteoclast, lx- Index, Bu Tp-buccal tipping, C-canine, Clf-cleft, I-incisor, NA-not applicable, wk-week, crw-crowding, mm-minimum, bimax-bimaxillary, wi-wire, lig-ligature, Ad-adult, RANKL-receptor antagonist nuclear kappa ligand, OPG-osteo-protegerin, Il- _1ra-interleukin 1 receptor antagonist, therm-thermoplastic, t-PA-plasminogen, TNFα-tumour necrosis factor, TIMP-Tissue inhibitor metalloproteinase, MCP- Methyl-accepting chemotaxis protein, MPO-myeloperoxidase, ortho-orthodontic, cys-cysteine, CN-centinewton, TSP-thrombospondin 1, NGAL-neutrophil gelatinase-associated lipocalin, ACP-acetyl carrier protein, CS-chondroitin sulphate, GM-CSF- Granulocyte-macrophage colony-stimulating factor, IFNγ-Interferon gamma, MIP-Macrophage inflammatory protein, jG-beta globulin, PAI-plasminogen activator inhibitor, EGF-Epidermal growth factor, dst-distalisation, Intr-intrusion, aln-alignment, cst-constriction, AL-after loading, BL-before loading, M contr- Maxillary constriction, Exp- Expansion, Hyr- Hyrax, LB-laceback, TB-Tie back, SE-superelastic NiTi, HANT- heat-activated NiTi, MSSS- multistranded stainless steel.
Table 3 - continuation - Participant and study characteristics table.

| Ref No. | Sa  | M/F  | Age                  | Me                                  | Ix T                  | cT/gp                      | Site       | Rn      | ml     | gp  |
|---------|-----|------|----------------------|-------------------------------------|-----------------------|----------------------------|------------|---------|--------|-----|
| 30      | 16  | 10F/6M| 11-21y, 15.5±3.5y   | ALP                                | Mx 1st Mo             | ct bAg 1st Mo             | Ms & D     | NM      | Nost  |     |
| 31      | 9   | 5M/4F| 10-18y               | IL-1β, IL-6, TNF-α, EGF, β2-µG     | Mx 1st Mo, 1st PM & CI | NM                        | MB EMP     | NM      | Mx cst |     |
| 32      | 12  | 3M/9F| 14.4±0.9 y          | IL-1β, IL-6, TNF-α, EGF, β2-µG     | C                     | Ag C/cD C                 | D          | NM      | 1st PM Ec |     |
| 33      | 9   | NM   | 13-17 y             | TRAP5b, IL-10, TNF-α              | Mx & MdC              | ct C                      | MB, MIB, MP & DB, MIP, DP | NM      | 1st PM Ec |     |
| 34      | 19  | 13F/6M| 16 – 28y            | ALP, AST, TRAP                     | R & L Mx C            | NM                        | D          | NM      | Mx 1st PM Ec |     |
| 35      | 12  | NM   | 14–24 y             | LDH                                | Mx C                  | BS                        | Ms & D     | NM      | Class II C |     |
| 36      | 12  | 11F/1M| 14-24y               | TRAP                              | Mx C                  | BS                        | Ms & D     | NM      |       |     |
| 37      | 14  | 4M/10F| 15-27y              | ALP                                | Mx C                  | NM                        | Ms & D     | NM      |       | crw (4-8mm) |
| 38      | 10  | 8F/2M| 15-27y              | ALP                                | Mx C                  | NM                        | Ms & D     | NM      | 1st PM Ec |     |
| 39      | 13  | NM   | 14.4±3.7y, 23.3±4y  | AST                                | Mx C                  | ct C                      | Ms & D     | NM      | 1st PM Ec |     |
| 40      | 13  | NM   | 14.4±3.7y, 23.3±4y  | AST                                | Mx PM                 | Ag PM                     | NM         | 1st PM Ec |       |     |
| 41      | 22  | 12F/10M| 13-22y              | AST                                | Mx C                  | NM                        | Ms & D     | Y       |       |     |
| 42      | 12  | 7F/5M| 14±2y               | ACP, ALP                          | Mx C                  | Ag C, ct C                | Ms & D     | Y       |       |     |
| 43      | 10  | 5F/5M| 15-20y              | ALP                                | MxC, Mx 2nd PM        | NM                       | D of C & Ms of 2nd PM | NM      | 1st PM Ec |     |
| 44      | 23  | 15F/8M| 9±1.4y              | ALP                                | Mx rt & lt 1st M      | Ag ist M                 | MB, MIB, DB, MP, MIP, DP | NM      | Mx constr |     |
| 45      | 10  | 7F/3M| 14 - 27 y           | ALP                                | Rt Mx C               | Lt Mx C                  | Ms         | D       | 1st PM Ec |     |
| 46      | 7   | 5F/2M| 14 - 27 y           | ALP                                | Rt Mx C               | Lt Mx C                  | Ms         | D       | 1st PM Ec |     |
| 47      | 20  | 9F/11M| 12- 25 y            | LDH                                | Mx C                  | ct C                      | MB, MIB, DB, MP, MIP, DP | NM      | 1st PM Ec |     |
| 48      | 18  | 10F/8M| nov/22              | AST                                | Max 1st Mo            | ct & Ag 1st Mo           | Ms & D     | N       |       |     |
| 49      | 20  | 6F/4M| 20.6 ± 3.2y         | ALP                                | Mx C, Md C            | BS                        | D          | Y       | 1st PM Ec |     |
| 50      | 55  | 28F/ 27M| 15.1 (1.7)         | Adiponectin, Leptin, Resistin, MPO, CRP, MMP 9, TIMP1, MMP/ TIMP1, MMP9/ TIMP1, RANKL | Mand 6 anterior teeth | Normal weight children | D          | NM      | Non Ec |     |
| 51      | 22  | 14F/8M| 11-21y              | ALP                                | Max 1st - M rt & Lt   | Mand 1st - M rt & Lt    | NM         | MB      | Exp    | 4-6mm mand | crow |
| 52      | 60  | 41F/19M| 18 ± 1.5            | MPO                                | Mand Cl               | BS                        | NM         | NM      | 4-6mm mand | crow |
| 53      | 45  | NM   | 6.25, 5.6, 6.10     | MPO                                | Mand I                | BS                        | NM         | Y       |       |     |
| 54      | 30  | NM   | 9-15y               | AST                                | Rt Mx PM              | Lt Mx PM                 | NM         | NM      |       |     |

A- article, f-force, t-type of, mc-mechanics, md/mc-mode of mechanics, tm- time, a-appliance, re-reactivation, to-total, du-duration, n-number, ob-observation, B-baseline, min- minutes, g- grams, Ir- Interrupted, Cn- Continuous, Rt-retraction, sg-segmented, sp-spring, Ech-elasticometric chain, NITI-nitinol, c-control, NM-not mentioned, y-year, d-day, m-month, h-hour, lv-levelling, se-separator, ac-activated, HG-headgear, NH- non-headgear, bu-buccal, la-labial, RME-rapid maxillary expansion, HR-hybrid retractor, RCD- rapid canine distaliser, Sa-Sample, M/F-male/female, E- enzyme, Me- mediator, T-tooth, sc-specification, m-randomisation, ml-malocclusion, HS-Handsearched, P-Pubmed, S-Scopus, G5- Google scholar, N-No, Y–yes, Mx-Maxilla, Md-Mandible, H-history, ls-loss, gvingival, if-inflammation, PD-probing depth, wk-week, R-right, L-left, C-canine, PM-premolar, Mo-molar, CI-central incisor, I-incisor, Ag- Antagonistic, ct- Contralateral, ip-interproximal, op-opposing, Ex- Experimental, c- Control, aj-adjacent, Es-Experimental site, Ec- Extraction, Ms- Mandible, D- Distal, rq-required, q- quadrant, OTM-orthodontic tooth movement, sf- surface, ado-adolescent, AST-aspartate transaminase, TRAP-Acid phosphatase, ALP-Alkaline Phosphatase, βG- beta glucuronidase, MMP-matrix metalloproteinase, LDH-lactate dehydrogenase, Cp-Cathepsin, MPO-myeloperoxidase, CK-creatinine, NO-Nitric oxide, IL-Interleukin,
| Ref No. | Age | Sex | Site | Rn | ml | No. / ob | tm/ob | B | B=c |
|---------|-----|-----|------|----|----|----------|-------|---|----|
| 40      | 13  | F/M |       |    |    |          |       |   |    |
| 48      | 18  | F/M | nov/22 |      |   |          |       |   |    |
| 49      | 20  | F/M |       |    |    |          |       |   |    |
| 50      | 55  | F/M |       |    |    |          |       |   |    |
| 38      | 10  | F/M |       |    |    |          |       |   |    |
| 36      | 12  | F/M |       |    |    |          |       |   |    |
| 43      | 10  | F/M |       |    |    |          |       |   |    |
| 34      | 19  | F/M |       |    |    |          |       |   |    |
| 32      | 12  | M/F |       |    |    |          |       |   |    |
| 52      | 60  | F/M |       |    |    |          |       |   |    |
| 53      | 45  | F/M |       |    |    |          |       |   |    |
| 35      | 12  | F/M |       |    |    |          |       |   |    |
| 47      | 20  | F/M |       |    |    |          |       |   |    |
| 37      | 14  | M/F |       |    |    |          |       |   |    |
| 41      | 22  | F/M |       |    |    |          |       |   |    |
| 54      | 30  | F/M |       |    |    |          |       |   |    |
| 15.5±3.5y |     |     |     |    |    |          |       |   |    |
| 23.3±4y |     |     |     |    |    |          |       |   |    |
| 23.3±4y |     |     |     |    |    |          |       |   |    |
| β       |     |     |     |    |    |          |       |   |    |
| α       |     |     |     |    |    |          |       |   |    |
| IL-1    |     |     |     |    |    |          |       |   |    |
| β       |     |     |     |    |    |          |       |   |    |
| IL-6    |     |     |     |    |    |          |       |   |    |
| TNF-α   |     |     |     |    |    |          |       |   |    |
| Leptin  |     |     |     |    |    |          |       |   |    |
| Resistin|     |     |     |    |    |          |       |   |    |
| TIMP1   |     |     |     |    |    |          |       |   |    |
| MMP8    |     |     |     |    |    |          |       |   |    |
| MMP9    |     |     |     |    |    |          |       |   |    |
| RANKL   |     |     |     |    |    |          |       |   |    |
| Adiponectin |   |     |     |    |    |          |       |   |    |
| IL-10   |     |     |     |    |    |          |       |   |    |
| TNF-β   |     |     |     |    |    |          |       |   |    |
| EGF     |     |     |     |    |    |          |       |   |    |
| 2-µG    |     |     |     |    |    |          |       |   |    |
| G       |     |     |     |    |    |          |       |   |    |
| MPO     |     |     |     |    |    |          |       |   |    |
| CRP     |     |     |     |    |    |          |       |   |    |
| MIP-1α  |     |     |     |    |    |          |       |   |    |
| TF-β    |     |     |     |    |    |          |       |   |    |
| MCP-1   |     |     |     |    |    |          |       |   |    |
| MPO      |     |     |     |    |    |          |       |   |    |
| PAI      |     |     |     |    |    |          |       |   |    |
| EGF      |     |     |     |    |    |          |       |   |    |
| TSP      |     |     |     |    |    |          |       |   |    |
| NGAL     |     |     |     |    |    |          |       |   |    |
| CRP      |     |     |     |    |    |          |       |   |    |

CRP- C Reactive Protein, hm-humidity, sc-specification, ins-insertion, MB-Meso-buccal, ML-Meso-lingual, DP-Disto-palatal, DB- Disto-buccal, df-differentiation, gp-group, cmp-compression, kPa-kilopascal, mx-maximum, gw growth, Oc-osteolast, lx- Index, Bu Tp-buccal tipping, C-canine, Clf-cleft, I-incisor, NA-not applicable, wk-week, crw-crowding, mnm-minimum, bimax-bimaxillary, wi-wire, lig-ligature, Ad-adult, RANKL-receptor antagonist nuclear kappa ligand, OPG-osteoprogerin, IL-1Ra-interleukin 1 receptor antagonist, therm-thermoplastic, t-PA-plasminogen, TNF-α-tumour necrosis factor, TIMP-Tissue inhibitor metalloproteinase, MCP- Methyl-accepting chemotaxis protein, MPO-myeloperoxidase, orth-orthodontic, cys-cysteine, ch-centinewton, TSP-thrombospondin 1, NGAL-neutrophil gelatinase-associated lipocalin, ACP-acyl carrier protein, CS-chondroitin sulphate, GM-CSF- Granulocyte-macrophage colony-stimulating factor, IFN- Interferon gamma, MIP-Macrophage inflammatory protein, iG-beta globulin, PAl-plasminogen activator inhibitor, EGF-Epidermal growth factor, dst-distalisation, Intr-intrusion, aln-alignment, cstr-constriction, AL-after loading, BL-before loading, Mx constr- Maxillary constriction, Exp- Expansion, Hyr- Hyrax, LB-Retraction upset, cN-centinewton.
Table 4 - Oral hygiene regimen.

| Ref No. | Oral px (Pre t/t) | Oral px (Every ob po) | Oral hy instr/ motiv | Mw | fq/o mw/d | asm for gv pd in (pre t/t) | At every ob po |
|---------|------------------|-----------------------|----------------------|----|-----------|---------------------------|----------------|
| 7       | Y                | NM                    | Y                    | Cx glu | 2         | Y                         | Y              |
| 8       | Y                | NM                    | NM                   | NM   | NM        | Y                         | NM             |
| 9       | NM               | NM                    | Y                    | 0.15% Benz HCL/d | 1/d | NM | NM |
| 10      | Y                | NM                    | Y                    | 0.5 oz of 0.2% Cx glu | 2/d | NM | NM |
| 11      | Y                | NM                    | NM                   | NM   | NM        | Y                         | NM             |
| 12      | NM               | NM                    | NM                   | NM   | NM        | Y                         | NM             |
| 13      | Y                | Y                     | Y                    | NM   | NM        | NM                        | NM             |
| 14      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 15      | Y                | NM                    | NM                   | NM   | NM        | Y                         | Y              |
| 16      | Y                | Y                     | Y                    | NM   | NM        | Y                         | Y              |
| 17      | Y                | Y                     | Y                    | 0.5 oz of 0.2% Cx glu | 2/d | Y  | Y  |
| 18      | NM               | NM                    | NM                   | NM   | NM        | Y                         | Y              |
| 19      | Y                | Y                     | Y                    | 0.5 oz of 0.2% Cx glu | 2/d | Y  | Y  |
| 20      | Y                | Y                     | Y                    | 0.12% Cx glu | 2/d for 4 wk | NM | NM |
| 21      | NM               | NM                    | Y                    | NM   | NM        | Y                         | NM             |
| 22      | NM               | NM                    | Y                    | NM   | NM        | Y                         | NM             |
| 23      | Y                | Y                     | NM                   | NM   | NM        | Y                         | Y              |
| 24      | Y                | Y                     | Y                    | Cx glu | 2/d | Y  | NM |
| 25      | NM               | NM                    | NM                   | Benz HCL | NM | NM | NM |
| 26      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 27      | NM               | NM                    | NM                   | NM   | NM        | Y                         | NM             |
| 28      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 29      | NM               | NM                    | NM                   | NM   | NM        | Y                         | Y              |
| 30      | NM               | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 31      | Y                | NM                    | Y                    | Cx glu | NM | Y  | Y  |
| 32      | NM               | NM                    | NM                   | NM   | NM        | Y                         | Y              |
| 33      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 34      | NM               | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 35      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 36      | Y                | NM                    | NM                   | NM   | NM        | Y                         | Y              |
| 37      | Y                | Y                     | Y                    | N     | N         | Y                         | Y              |
| 38      | Y                | Y                     | Y                    | N     | N         | Y                         | Y              |
| 39      | Y                | Y                     | Y                    | N     | N         | Y                         | Y              |
| 40      | Y                | Y                     | Y                    | N     | N         | Y                         | T              |
| 41      | Y                | Y                     | Y                    | N     | N         | Y                         | Y              |
| 42      | NM               | NM                    | Y                    | Cx glu | 2/d | Y  | Y  |
| 43      | Y                | NM                    | Y                    | (against it) | N  | Y  | NM |
| 44      | Y                | Y                     | Y                    | 0.012% Cx glu | 2/d | Y  | Y  |
| 45      | Y                | Y                     | Y                    | Cx glu | NM | Y  | Y  |
| 46      | Y                | Y                     | Y                    | NM   | NM        | Y                         | Y              |
| 47      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 48      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 49      | NM               | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 50      | NM               | NM                    | NM                   | NM   | NM        | Y                         | Y              |
| 51      | Y                | Y                     | NM                   | NM   | NM        | Y                         | Y              |
| 52      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 53      | Y                | NM                    | Y                    | NM   | NM        | Y                         | Y              |
| 54      | Y                | NM                    | NM                   | NM   | NM        | Y                         | Y              |

A—article, Mw—mouth wash, fq/o—frequency of, d—day, px—prophyaxis, t/t—treatment, ob—observation, po—point, asm—assessment, gv—gingival, pd—periodontal, in—inflammation, cx glu—chlorhexidine gluconate, Y—yes, NM—not mentioned, N—no, h—hour, Benz HCL—benzydamine hydrochloride, wk—week, hy—hygiene, instr—instructions, motiv—motivation.
Table 5 - GCF characteristics.

| Ref No. | Time | tp | hm | mt/o cl | ins (in mm) | du/o mm | rep mm | i/o mm | mt/o mm | pt of st | mt/o al | pr cc |
|---------|------|----|----|---------|-------------|---------|--------|--------|---------|----------|---------|-------|
| 7       | NM   | 30%| 21°C| PP      | NM          | 30s     | NM     | NM     | PT6000  | NM       | ELISA   | pg / 30-s |
| 8       | NM   | NM | NM   | PP      | NM          | 30s     | 4      | NM     | NM      | -70°C    | SP      | PMNs/µl |
| 9       | NM   | NM | NM   | PP      | 1mm        | 1min    | 3      | 1min   | NM      | NM       | SP      | IU/1 µl |
| 10      | NM   | NM | NM   | PP      | NM          | 1min    | NM     | NM     | PT 8000 | -70°C    | SP      | IU/L   |
| 11      | NM   | NM | NM   | PP      | 1-2mm      | 1min    | 3      | NM     | NM      | -20°C    | ELISA   | LDH, AST-mIU/ml, TRAP-ALP-ng/ml |
| 12      | NM   | NM | NM   | PP      | NM          | 30s     | 4      | NM     | NM      | -70°C    | SP      | U/100 ml |
| 13      | NM   | NM | NM   | PP      | NM          | 30s     | 3      | 1min   | PT 8000 | -20°C    | SP      | µg/ml  |
| 14      | NM   | NM | NM   | µP      | NM          | 5min    | NM     | NM     | NM      | -70°C    | SP      | µg/ml  |
| 15      | NM   | NM | NM   | PP      | NM          | 1min    | NM     | NM     | NM      | -80°C    | QAK     | pg/ml  |
| 16      | NM   | NM | NM   | PP      | 1mm        | 30s     | NM     | NM     | NM      | -30°C    | SP      | µg / ml |
| 17      | NM   | NM | NM   | PP      | NM          | 30s     | NM     | NM     | -70°C   | IA       | pg/site |
| 18      | NM   | NM | NM   | PP      | NM          | 30s     | NM     | NM     | PT 8000 | NM       | LMAT    | pg/ml  |
| 19      | NM   | NM | NM   | PP      | NM          | 1min    | 5      | NM     | NM      | NM       | SP      | µmol/U/L |
| 20      | NM   | NM | NM   | PP      | 1mm        | NM      | NM     | NM     | PT 8000 | -80°C    | SP      | mb-IA   |
| 21      | NM   | NM | NM   | PP      | NM          | 30s     | NM     | NM     | PT8000  | -80°C    | SP      | cp µL/ µl, Cys, ng/µl |
| 22      | 20°C | 40%| PP   | NM      | NM          | 30s     | NM     | NM     | NM      | -70°C    | SP      | WB     |
| 23      | NM   | NM | PP   | 1mm     | 1min      | 2       | 1min   | PT8000 | -30°C   | ELISA    | µg/µl  |
| 24      | NM   | NM | NM   | PP      | NM          | 1min    | 2      | 5s     | NM      | -80°C    | ELISA   | µmol/mg |
| 25      | NM   | NM | NM   | PP      | 1mm        | 30s     | NM     | NM     | -80°C   | SP       | mL     |
| 26      | NM   | NM | NM   | PP      | NM          | 3min    | NM     | NM     | -20°C   | WB       | µg/l   |
| 27      | NM   | NM | NM   | PP      | 1mm        | 10s     | NM     | NM     | NM      | SP       | µL/sample |
| 28      | NM   | NM | NM   | PP      | 1mm        | 1min    | 1      | 30s    | NM      | -30°C    | WB      | µL/µl |
| 29      | NM   | NM | NM   | PP      | 1mm        | 30s     | NM     | NM     | NM      | SP       | µL/sample |
| 30      | NM   | NM | NM   | PP      | 1mm        | 30s     | NM     | NM     | NM      | SP       | µL/sample |
| 31      | NM   | 30%| 21°C| PP      | NM          | NM      | 30s    | PT6000 | -70°C   | ELISA    | pg/µg  |
| 32      | NM   | NM | PP   | 1mm     | 1min      | 1       | 30s    | PT     | -30°C   | ELISA    | pg/µL  |
| 33      | NM   | NM | NM   | PP      | NM          | NM      | 30s    | PT8000 | -20°C   | ELISA    | pg/µL  |
| 34      | NM   | NM | NM   | PP      | 1-2mm      | 1min    | 2      | 1min   | NM      | NM       | SP      | µmol/ min |
| 35      | NM   | NM | NM   | PP      | 1mm        | NM      | NM     | NM     | NM      | SP       | µL/mg  |
| 36      | NM   | NM | NM   | PP      | 1mm        | 1min    | 3      | 1min   | NM      | NM       | SP      | µL/mg  |
| 37      | NM   | NM | NM   | end PP  | 1mm        | 30s     | 3      | 90s    | NM      | -40°C    | SP      | µmol/min |
| 38      | NM   | NM | NM   | end PP  | 1mm        | 30s     | 3      | 90s    | NM      | -40°C    | SP      | µmol/min |
| 39      | NM   | NM | NM   | end PP  | 1mm        | 30s     | 3      | 90s    | NM      | -40°C    | SP      | µmol/min |
| 40      | NM   | NM | NM   | end PP  | 1mm        | 30s     | 3      | 1min   | NM      | -4°C     | SP      | µmol/min |
| 41      | NM   | NM | NM   | µP      | 2mm        | NM      | NM     | NM     | NM      | -70°C    | SP      | U/mg   |
| 42      | NM   | NM | NM   | PP      | 1mm        | 30s     | NM     | NM     | NM      | -20°C    | SP      | NM     |
| 43      | Y    | NM | NM   | FP      | NM          | 1min    | NM     | 5s     | NM      | -80°C    | PNPP kin | NM     |
| 44      | NM   | NM | NM   | FP      | NM          | 30s     | NM     | NM     | -80°C   | SP       | µL/sample |
| 45      | NM   | NM | NM   | µP      | NM          | NM      | NM     | NM     | NM      | SP       | U/L    |
| 46      | NM   | NM | NM   | µP      | NM          | NM      | NM     | NM     | NM      | SP       | U/L    |
| 47      | NM   | NM | NM   | µP      | NM          | NM      | NM     | NM     | NM      | SP       | µmol units(L) |
| 48      | NM   | NM | NM   | µP      | NM          | NM      | NM     | NM     | NM      | SP       | µL/sample |
| 49      | NM   | NM | NM   | µP      | NM          | NM      | NM     | NM     | NM      | SP       | µL/sample |
| 50      | Y    | NM | NM   | PP      | 1mm        | 30s     | NM     | NM     | NM      | SP       | µL/sample |
| 51      | NM   | NM | NM   | end PP  | 1mm        | 30s     | NM     | NM     | NM      | -30°C    | SP      | µL/sample |
| 52      | NM   | NM | NM   | PP      | 1mm        | 30s     | NM     | NM     | -70°C   | SP       | units/100 µL |
| 53      | NM   | NM | NM   | PP      | 30s       | 2       | 30s    | NM     | -70°C   | SP       | units/100 µL |
| 54      | NM   | NM | NM   | pp      | 1mm        | 1min    | NM     | NM     | NM      | SP       | µL/sample |

A: article, tp: temperature, hm: humidity, mt/o cl: method of collection, sp: specification, ns: insertion, mm: millimeter, du/o: duration of, rep: repeated, i/o: interval of, st: storage, al: analysis, pr: protein, cc: concentration, NM: Not Mentioned, N- No, Y: Yes, PP: Periopaper, PT- Periotron, WB: Western Blot, ELISA: Enzyme linked immunosorbent assay, IA: Immunoassay, RIA: Radio IA, meas: measurement, pg: picogram, µg: microgram, ml: millilitre, µL: microlitre, GCF: gingival crevicular fluid, tot: total, g: gram, ng: nanogram, s: second, min: minutes, °C: degree Celsius, SP: spectrophotometry, Ar-array, As-assay, mb: multiplex bead, IL-international units, L: litre, LDH-lactate dehydrogenase, AST-aspartate transaminase, TRAP-ACid phosphatase, ALP- Alkaline Phosphatase, PMNs – polymorphonucleosides, Cp -cathepsin, Cys -cysteine, Tot -total, pmol –picomol, flr-flurometery, QA- Quantibody assay, PNPP kin- para nitrophenyl phosphate kinetic, pp- paper point.
Table 6 - Differential expression of enzymes in GCF.

| Ref No. | sts al ap | cf | Drop outs | Up / down rg | Pk |
|---------|-----------|----|-----------|---------------|----|
| 7       | 1-tailed paired Student t | Y  | NM        | βG: inc       | M-010 |
| 8       | ANOVA, paired t test      | Y  | NaM       | Inc at 2h, bas in 7d | 2h   |
| 9       | (ANOVA) Kolmogorov–Smirnov test, Paired-samples test | Y  | N         | Inc           | 2wk   |
| 10      | Kolmogorov and Smirnov (ANOVA) & Tukey’s post-hoc test | Y  | N         | Inc           | 14d   |
| 11      | Paired t test Pearson’s cr | Y  | N         | LDH inc at 2, 3; 4 wk(100 g) & 1, 2, 3 wk (150 g); AST inc at 4 & 5 wk (100 g) & 3 & 4 wk (150 g); TRAP inc at 5 wk (100 g) | AST: 1wk, TRAP: 2wk |
| 12      | Friedman test for interg & intrago, Wilcoxon test for related samples, Kruskal-Wallis test for independent samples in both gps | Y  | N         | inc           | 2h   |
| 13      | Intra gp: Friedman’s test, Wilcoxon test Inter gp: Mann-Whitney U-test, Pearson’s test | Y  | N         | Inc           | MMP9: 8h, MMP9/NGAL: 72h |
| 14      | Intergp: Mann Whitney U test. Intra gp: Students unpaired t-test | Y  | N         | Inc           | ACP: 3d ALP, AST: 15d |
| 15      | SAS version 9.2 proc mixed subroutine | Y  | N         | No sts sn change | NM   |
| 16      | MedCalc software Intergp: Student’s t-test, ANOVA. | Y  | N         | SL+ NITI wi: inc SL+ thrm wi: dec | No sts sn change |
| 17      | Luminex analysis | Y  | N         | MMP1,3, inc, pk at 24h MMPB: pk at 14d | 24h   |
| 18      | Paired non-parametric Kruskall–Walls. Spearman Rank Sum anal | Y  | NM        | Inc           | Exp-TIMP, MMP-9.4h, cmp: TIMP-1, MMP-9.4h, TIMP-2: 7d |
| 19      | GraphPad®Instat, ANOVA, Friedman | Y  | NM        | Inc           | 14d, 21d |
| 20      | Friedman, Mann–Whitney | Y  | NM        | Inc           | 1h   |
| 21      | ANOVA | Y  | NM        | Cp: dec Cys:inc | 1d |
| 22      | ANOVA | Y  | NM        | Inc           | MMP1-1h, MMP2-1h, 8h |
| 23      | Mann-Whitney U-tests | N  | NM        | Inc at 24h at lx t >CT | 24h   |
| 24      | ANOVA BLSD | Y  | NM        | Inc           | 14d   |
| 25      | Friedman test | Y  | NM        | Dec           | 1d   |
| 26      | Friedman and Bonferroni-corrected, Wilcoxon paired signed rank tests | Y  | NM        | Inc           | 14d   |
| 27      | NM | Y  | NM        | Lvl of MMP-8 inc in lx t>c | NM   |
| 28      | Friedman & Bonferroni-corrected, Wilcoxon paired signed rank tests | Y  | NM        | Inc           | 28d   |
| 29      | Friedman & Bonferroni-corrected, Wilcoxon paired signed rank tests | Y  | NM        | Inc           | tn:7d |
| 30      | Mann Whitney U-test | Y  | N         | Inc           | 24h   |
| 31      | Bonferroni-corrected, 1-way repeated measures ANOVA, paired Student t test | Y  | NM        | Inc           | 14d   |
| 32      | One-tailed paired Student t test | Y  | N         | βG -25d IL-1β | 1β- |
| 33      | Student’s t test | Y  | N         | IL-1β, IL-6, TNF-α, EGF, β2-μG | inc   |

A – article, sts – statistically, al – analysis, ap – applied, cf – confounders, rg – regulation, Pk – peak, sd – secondary, oc – outcome, cr – correlation, sn – significant, Y – yes, N – no, NM – not mentioned, inc – increase, dec – decrease, fluct – fluctuated, h – hour, mon – month, d-day, wk – week, tot – total, prot – protein, conc – concentration, mg – milligram, ml – millilitre, g – gram, > – greater than, VAS – visual analogue scale, C-canine, mov-movement, b/w-between, cn-continuous, β- and, F-force, Asc-associated, gen-genetic, GCF- gingival crevicular fluid, comp–compared, B-baseline, IL– interleukin, βG-beta glucoronidase, TNFα-tumour necrosis factor alpha, SD–short duration, LD-long duration, HG– RDG–, Diff-difference, vol-volume, Rt-retraction, If-inflammation, Avg-average, cyt-cytokine, thermo-chemokine, kwn-known, MOP PI-plaque index, BOP-bleeding on probing, Exp-experimental, c-control, Avg-average, Mx-maxilla, ct-contrateral, differen-differentiation, se-separator, gp-group, cmp-compression, tr–tension, kPa-kilopascal, max-maximum, gw-growth, T-tooth, Oc-osteoclast, RDG- Rapid canine distalisation group, HG- hybrid reactor group, Rt- retraction, Aa-Actinobacillus, rd-reading, wi-wire, lg-ligature, Ad-adult, RANKL-receptor an-
| sd & oc | cr | sta sn rd |
|---------|----|----------|
| IL-1β sign inc for Mo- OS to O10 for PM-O6 to O10 | stronger F cause higher levels of IL-1β & βG | βG inc for Mo- O7 to O10 PM-O7, O8, O10 |
| For Cl-04, 06, 07, 09, 010 & dec at O2 for Mo, PM, Cl | IL-1β & βG | Cl-06, 07, 010 & dec at O2 for Mo, PM, Cl |
| Inc MPO in saliva at 2h, B in 7d | +ve cr of lvl in GCF & saliva | Inc at 2h |
| NM | In crs F: lvl pk at 2wk & GCF vol inc from 0 – 21d | In gradually inc: F, Lvl pk at 3wk |
| Sn inc at 14d | Exp si: lvl inc on 14d cr with pk in GCF vol | Exp si: lvl pk at 14d |
| In saliva AST inc at 5wk, TRAP at 2wk, ALP at 1 to 5wk | Weak cr b/w enz quantity & activity | LDH inc at 2, 3 & 4 wk (100 g) b 1, 2 & 3 wk (150 g). |
| minm & severe crw: inc from 0 at 2h, 7d, 14d in saliva | No cr of crw with change in MPO | AST inc at 4 & 5 wk (100 g) b 3 & 4 wk (150 g) |
| TSP1: inc from B at 8h to 72h, dec at 1wk | Strong & sn cr b/w MMP9/NGAL & TSP1 in IxT | TRAP inc at 5wk (100 g) |
| Exp si: in Ad, IL-1/1L-1RA dec in 3wk aftr 1st wi lig ado, RANKL/OPG pk at 6wk & 3 wk rect wi lig | No cr in GCF vol & PI | No sts sn change in MMP |
| Visual pl scr dec sts sn | No cr of MMP-9 & TIMP to speed of OTM at 4h in Exp | No cr of MMP-9 & TIMP to speed of OTM at 4h in Exp |
| +ve cr of GCF vol & PI at 0 at tn, cmp | +ve cr of MMP-9 & TIMP to speed of OTM at 4h in Exp | +ve cr of MMP-9 & TIMP to speed of OTM at 4h in Exp |
| At 24h GCF vol inc in 1d, dec at 1m | +ve cr of MMP-9 & TIMP to speed of OTM at 4h in Exp | +ve cr of MMP-9 & TIMP to speed of OTM at 4h in Exp |
| -ve cr in Cp & Cys Lvl | Inc at 7, 14, 21d | Inc at 7, 14, 21d |
| GCF vol higher in cmp than tn at 21d | MMPPS inc at 3h, dec at 24h | MMPPS inc at 3h, dec at 24h |
| GCF vol inc in 1d, dec at 1m | Inc in MMP1 (tn)-1h, (cmp) -1h | Inc in MMP1 (tn)-1h, (cmp) -1h |
| GCF vol no sn diff at 24h | Inc in MMP2 (tn) -1h; (cmp) -8h | Inc in MMP2 (tn) -1h; (cmp) -8h |
| Dpd, osteocalcin dec | Inc in ALP -7, 14 at Ms & D | Inc in ALP -7, 14 at Ms & D |
| NM | Inc from 0 to 28 d, inc on 7d | Dec from 0 to 28 d, inc on 7d |
| NM | ct gp greater than Ag gp on 14 d & 21d | ct gp greater than Ag gp on 14 d & 21d |
| NM | Lvl of MMP-8 inc 12 times in Ix cx | Lvl of MMP-8 inc 12 times in Ix cx |
| NM | Inc at 28d in Ix T as compd to CtxT & Ag T | Inc at 28d in Ix T as compd to CtxT & Ag T |
| Aa colonization inc sn on 28d in ExpT & ct gp | AST: inc in Ix T & ct T as compd to Ag T, inc in IxT as compd to CtxT on tn si on 14d & on cmp on 7d & 14d | AST: inc in Ix T & ct T as compd to Ag T, inc in IxT as compd to CtxT on tn si on 14d & on cmp on 7d & 14d |
| NM | CpxB higher at 24h in IxT | CpxB higher at 24h in IxT |
| NM | Sign inc on both M & D at 1,2,3 wks | Sign inc on both M & D at 1,2,3 wks |
| sn inc in IL-1β level at 4d 619d to 60d AL | inc in βG at 25d to 60d AL | inc in βG at 25d to 60d AL |
| 24h | Intergrp btw cont & Exp: IL-1β inc in 24h > BS, IL-6 inc in 24h > BS or 168h, TNF-α inc in 24h > BS or 168h | Intergrp btw cont & Exp: IL-1β inc in 24h > BS, IL-6 inc in 24h > BS or 168h |
| & EGF inc in 24h > BS | Integra-activity, actvn-activation, compl-completion, reactivation-reactvn, SE-superelastic Niti, HANT- heat-activated Niti, MSSS- multistranded stainless steel, vol-volume. | Integra-activity, actvn-activation, compl-completion, reactivation-reactvn, SE-superelastic Niti, HANT- heat-activated Niti, MSSS- multistranded stainless steel, vol-volume.
Table 6 - Differential expression of enzymes in GCF.

| Ref No. | sts al ap | cf | Drop outs | Up / down rg | Pk |
|---------|-----------|----|-----------|--------------|----|
| 34      | ANOVA     | Y  | NM        | Inc          | 7d |
| 35      | Shapiro-Wilk test | Y  | NM        | Inc          | 100g gp-TRAP-3wk, 150g gp-ALP & TRAP-5wk |
| 36      | Student’s paired t test | Y  | NM        | Inc          | M-4wk, D-1SN-2wk |
| 37      | Kruskal Wallis test. | Y  | NM        | Inc: at 1wk, 4wk, stabilised | 4wk |
| 38      | Paired sample t-test | Y  | NM        | Inc: at 1wk,4wk, stabilised | 4wk |
| 39      | Paired sample t-test | Y  | NM        | Inc: at 1wk, dec in next 3wk | 1wk |
| 40      | Wilcoxon signed rank test | Y  | NM        | Inc: at archwi>self lig site, inc at 1wk | 1wk |
| 41      | Wilcoxon signed rank test | Y  | NM        | Inc: at archwi>self lig site, inc at 1wk | 1wk |
| 42      | ANOVA, paired t-test using SPSS | Y  | NM        | ALP inc in 14d, 28d, ALP at Ms>D, ACP inc in Ms ED | ALP, ACP : 14d |
| 43      | ANOVA, Student’s t-test | Y  | NM        | ALP dec, D of C > Ms of 2nd PM on 1, 7, 14, 21, 28d | Dec |
| 44      | Friedman test followed by a Bonferroni-corrected Wilcoxon signed rank test | Y  | Y         | ALP inc in 3m, 6m | inc |
| 45      | ANOVA, Tukey’s HSD Post-Hoc test, Mann-Whitney U-test | Y  | NM        | ALP inc 14d, 28d | 28d |
| 46      | ANOVA, Independent Samples t-test, Mann-Whitney U-test | Y  | NM        | ACP inc both Ms & D si D si>M si at 7d, 21d | 21d |
| 47      | ANOVA, Tukey HSD | Y  | NM        | LDH inc at TT>cT 7d, 14d,21d | 28d |
| 48      | Friedman & Bonferroni-corrected Wilcoxon paired signed rank tests | Y  | N         | AST inc from BS in T/T gp from BS to 2wk followed by dec | 14d |
| 49      | One-way ANOVA was used for multiple group and Student t test for group-wise comparisons | Y  | N         | Inc in ALP b/w 21d & 28d of 200% in active TB gp, of 260% in RT screw gp | TB: 21d |
| 50      | independent t tests, _2 tests, or Mann-Whitney, intraexaminer reliability - concordance correlation coefficient (CCC) & Bland-Altman method | N  | Y         | MMP8,9, MMP8/TIMP1, MMP9/TIMP1, resistin at BS>1m>1wk>compl of Aln | NM |
| 51      | Fisher’s PLSD followed by post hoc, Bonferroni-Dunn | Y  | N         | ALP on cmp site: 0>2wk>6wk>1y | tn site: 0<2wk<4wk<1y |
| 52      | paired & unpaired T test and ANOVA | Y  | N         | MPO inc from BS to 2h in HANT, SE, MSSS gp | 2h |
| 53      | Chi-square | Y  | N         | MPO inc from BS to 2h in HANT, SE, MSSS gp | 2h |
| 54      | Student’s t-test, and one-way analysis of variance | Y  | N         | MPO inc from BS to 2h in HANT, SE, MSSS gp | 2h |

A—article, sts—statistically, al—analysis, ap—applied, cf—confounders, rg—regulation, Pk—peak, sd—secondary, oc—outcome, cr—correlation, sn—significant, Y—yes, N—no, NM—not mentioned, inc—increase, dec—decrease, fluct—fluctuated, h—hour, mon—month, d-day, wk-week, tot—total, prot—protein, conc—concentration, mg—milligram, ml—millilitre, g—gram, >—greater than,VAS—visual analogue scale, C—canine, mov—movement, b/w-between, cn—continuous, &—and, F-force, Asc—associated, gen—genetic, GCF—gingival crevicular fluid, compd—compared, B—baseline, IL—interleukin, BS—beta glucuronidase, TNFα—tumour necrosis factor alpha, SD—short duration, LD—long duration, HG—RGD, Diff—difference, vol—volume, Rt—retraction, it—inflammation, Avg-average, cyt—cytokine, chemox—chemokine, kwn-known, MOP—PI-plaque index, BOP—bleeding on probing, Exp—experimental, c—control, Avg—average, Mx—maxilla, ct—contralateral, differen—differentiation, se—separator, gp—group, cmp—compression, tn—tension, kPa—kilopascal, max—maximum, gw—growth, T—toothing, Oc—osteoclast, RDG—Rapid canine distalisation group, HG—hybrid reactor group, Rt—retraction, Asa—Actinobacillus, rd—reading, wi—wire, lig—ligature, Ad—adult, RANKL—receptor antago.
TNF-α in D & Ms sites of TT sn higher than both sites of c, also > B, inc sn at 1 h & 24h. IL-10 dec during Exp period in c & TT

TRAP5b. Level in D & Ms sites of TT were sn higher than that at both sites of c of TT compd with B values, inc was sn at 1 h & 24h.

In 100 g gp, TRAP sn inc in 3-5 wk compd to TRAPB. ALP & AST slightly inc. In 150 g gp, ALP & TRAP slightly inc compd with their B. AST sn inc in 5 wk.

LDH at Ms site in 1.0 N & 1.5 N gp, inc sn on 4th wk. At D site, LDH with 1.5 N was higher than 1.0 N throughout 5 wk of TM. LDH with 1.5 NF inc at both Ms (wk 2) & D site (wk 3) with sn diff to 1.0 N F

Rate of OTM at 150g>100g 150g F at 3 & 4wk>100g f, +ve cr of lvl of TRAP & rate of OTM

150g gp, Ms si: inc at 3wk>BS At D si: inc at 4wk>BS TRAP at 150 gm>100gm F at 4wk (D site)

Inc at 1wk, 2wk from Bas Dec at 4wk

Inc at 1wk, 4wk, At D si=Ms si

Plk at 1wk in T/T>CT

Inc at archwi>self lig

ALP, ACP inc, ALP inc more on M si

Dec at D of C > Ms of 2nd PM on 1, 7, 14, 21, 28d

+ve corr of ALP lvl with time at tn si

ALP at 3m, 6m > CT

+ve corre of mechanical stress to AST levels, T/t>CC

sn inc in T/t>CC vs AC gp: 1, 2, 3, 4w

sn inc in T/t vs CC gp: 1, 2wk AST level in comp >tn on 1wk

-space closure rate, root resorption, Rt, anchorage loss with

Hycon screw were assessed

+ve corre of ALP in Hycon screw gp with actvn of screw

Sign diff in ALP on 21d & 28d b/w TB & Rt screw gp

Resistin at BS>1h>1wk>compl of Aln

CRP, RANKL inc from BS to compl of Aln

Adiponectin BS>1h>1wk>compl of Aln

Leptin dec from BS to compl of Aln

Mediators correll with Aln rate- MPO, RANKL, Leptin, Resistin

MPO at BS>1h>1d>compl of Aln

+ve corre of intermolar distance with ALP level in tn site

tn site: 0 (before actvn) < 4wk, 0<1y cmp site: 0>4wk, 0<3y, 2wk>4wk

MPO in HANT>SE>MSSS

sn diff in MPO b/w SE & MSSS: 2h, 2wk, b/w HANT & MSSS>2h, b/w SE & MSSS:1wk

MPO b/w SE & MSSniTi: 2h, 1, 2wk, b/w HANT & MSSniTi:2h

Levels greater in Exp than Cn gp at 1, 2, 3, 4wk

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Table 7 - Results of quality assessment of 48 studies for inclusion of studies in the review

| S. No. | Criteria (29)                                                                 | Yes | No | Unclear |
|--------|-----------------------------------------------------------------------------|-----|----|---------|
| 1      | Objective: objective clearly formulated                                     | 48  | -  | -       |
| 2      | Sample size: considered adequate                                             | 2   | -  | 46      |
| 3      | Spectrum of patients representative of patients receiving the test in practice | 48  | -  | -       |
| 4      | Ethical clearance mentioned                                                  | 40  | 8  | -       |
| 5      | Selection criteria: clearly described                                        | 48  | -  | -       |
| 6      | Randomization: stated                                                         | 21  | 27 | -       |
| 7      | Baseline characteristics: clearly defined                                    | 47  | 1  | -       |
| 8      | Control: clearly defined                                                     | 46  | -  | 2       |
| 9      | Orthodontic mechanics explained in sufficient detail to permit replication of experiment | 45  | 1  | 2       |
| 10     | Orthodontic force: clearly specified                                          | 35  | 12 | 1       |
| 11     | Description of execution of index test: sufficient to permit replication of test | 45  | -  | 3       |
| 12     | Absence of time difference between index test & control: mentioned           | 36  | 12 | -       |
| 13     | Index test executed at specified time and environmental conditions          | 4   | 44 | -       |
| 14     | Use of proper indices for assessment of gingival & periodontal status (Pre-treatment) | 40  | 8  | -       |
| 15     | Use of proper indices for assessment of gingival & periodontal status (at each observation time) | 17  | 29 | 2       |
| 16     | Oral hygiene regime-mentioned                                                | 32  | 3  | 13      |
| 17     | Prophylaxis done (Pre-treatment)                                             | 34  | 14 | -       |
| 18     | Prophylaxis done (at each observation time)                                  | 11  | 37 | -       |

II. Study measurements (3)

|                      | response |
|----------------------|----------|
| 1.                   | GCF handling characteristics: explained | 47 - 1 |
| 2.                   | Measurement method: appropriate to the objective | 48 - |
| 3.                   | Reliability–adequate level of agreement | 48 - |

III. Statistical analysis (5)

|                      | response |
|----------------------|----------|
| 1.                   | Dropouts: dropouts included in data analysis | 1 47 - |
| 2.                   | Statistical analysis: appropriate for data | 48 - |
| 3.                   | Confounders: confounders included in analysis | - 48 - |
| 4.                   | Statistical significance level: P value stated | 48 - |
| 5.                   | Confidence intervals provided | 48 - |

IV. Study results and conclusions (3)

|                      | response |
|----------------------|----------|
| 1.                   | Index test compared to baseline | 48 |
| 2.                   | Index test compared to control | 48 |
| 3.                   | Conclusions: specific | 40 8 |

*Index test: Refers to collection of GCF at each observation interval in treatment teeth.
and 5 min in one study each. GCF measurements were repeatedly taken in 18 studies with specified number of
intervals, interval of repeat measurements were 30s (n=8), 60s (n=7), 90s (n=3) and 5s (n=2). Storage of
samples was done at -20°C (n=5), -30°C (N=4), -40°C (n=3), -70°C (n=11) and -80°C (n=9). Retrieval of
GCF from PerioPaper was done by Periotron (Ora-
Flow, PlainView, New York, NY, USA) in 11 stud-
ies, but not mentioned in 38 studies. Enzymes levels
were estimated by ELISA (n=8), spectrophotometry
(n=30), immunoassay (n=2), Luminexmultianalyte
technology (n=1), Quantibody Array kit (n=1), western blotting (n=3), fluorometry (n=1) and para-nitro-
phenol phosphate kinetic (n=1), but omitted in one
study. Protein concentration in GCF was measured
in variable units in 38 out of 42 studies.

DISCUSSION

The findings of the current review are presented in
Table 6. It depicts various enzymes released in GCF in
a time-dependent manner and also establishes correla-
tions (if any) with levels or type of force applied. In this
review, we have tried to establish associations of enzyme
levels to magnitude or type of force in each phase of
OTM, given by Burstone66 in his classic model or four
phase time/displacement modification model.57,58

An initial upregulation in enzymes for bone resorp-
tion and matrix degradation like TRAP, ACP or MMPs
and an immediate decrease in bone formative ALP
corresponded with Burstone’s initial phases of OTM.
Different MMPs responsible for extracellular matrix
(ECM) breakdown are increased at variable times in
OTM.13,15,17,18,20,22,27,50 as early as 1hr or till completion
of alignment.50 MMP-9 increased in 4hr, peaked at 8hr
using stainless steel ligatures for canine retraction in one
study, while MMP9/NGAL ratio peaked in 72hr in an
other study.13

MMPs also varied with different magnitudes of
force as MMP-9 peaked in 4hr in a study using 100g
force for canine retraction,18 compared to another
study using 150g force in which MMP3, 9 and 13
peaked in 24hr.20 The difference in peaks of various
MMPs can be explained on the basis of difference
in their roles in bone turnover and remodeling with
orthodontic forces.59 MMP-9 is responsible for cleav-
age of denatured collagen, i.e gelatin,60 MMP-13
dissolves native fibrillar collagen; MMP-1 is an in-
terstitial collagenase hydrolyzing mainly type III col-
lagen,61 and MMP-3 is responsible for activation of
MMPs 8 and 9.62 Hence peaks of MMP8 and MMP9/NGAL ratio at 14d17 and 72hr,13 respectively, occur
subsequent to peak of MMP-3 in 1hr/24hr.17,20 In vi-
tro studies also support rise in MMPs in orthodon-
tic forces, specifically MMP-1,2 mRNA and protein
production in human gingival and pdl fibroblasts63,64
and MMP-1,2, 9 in gingival tissue of dogs.60

On the other hand, no significant change in MMP
levels were seen in control teeth where no orthodontic
force was applied.17,22 This clearly supports MMPs as key
mediators of remodeling in OTM.

MMPs are also shown to vary with site (tension and
compression) in a time-dependent manner, as supported
by in vitro models on pdl fibroblasts.63,66 Current review
showed an increase in MMP1,2 in 1-3hr on tension site
(TS) of maxillary canine after activation of NiTi spring
while in compression (CS), MMP1 increased at 1hr and
MMP2 later, at 8hr.22 MMP-9 also increased from 4hr
to 7d on compression site in another study.13 This up-
surge in levels indicate initial collagen turnover and dis-
integration of ECM on both tension and compression
sites in initial phases of OTM.

Contrary to the MMPs, CS showed a significant
increase in GCF levels of MMP inhibitors, TIMP-1 at
4hr and TIMP-2 after 7d during retraction of ca-
nines, coinciding with lag phase where tooth move-
ment slows down.18,50 At TS, a significant increase in
TIMP1 and 2 levels was seen at 4hr and 42d. This
finding is in agreement with the results of a study by
Bildt et al67 where a continuous force with NiTi spring
of 150cN was applied for retraction and an increase in
MMP1 and TIMP1 was seen on pooled samples from
resorption (corresponding to compression) and apposi-
tion side (tension) but no trace of TIMP2 was found.
The mechanism of action of TIMP-1 stimulates release
of MMP1,68 an interstitial collagenase, associated with
normal tissue remodeling or stretch of pdl fibers, hy-
drolysing mainly type III collagen.64 Also, TIMP-1 in-
creases in smaller amounts on the site of compression,
while retraction due to stimulation of bone resorption
but in higher amounts on tension, it decreases bone re-
sorption.67 A study by Garlet et al.69 provided evidence
of greater expression of TIMP-1 mRNA on TS and
MMP-1 mRNA on CS and TS of experimental teeth
compared with the control.
Besides MMPs, histological studies on rats provide evidence of other enzymes for bone resorption predominant in CS in early phases of OTM followed by bone deposition in TS.\textsuperscript{70,71} In accordance, the current review also shows resorptive enzyme -ACP in initial 3-5d of tooth movement.\textsuperscript{14} Few studies on retraction with continuous forces document an initial rise in ACP both on TS and CS with a peak in 14d\textsuperscript{12} and 21d\textsuperscript{46}. Initial resorption is followed by a late phase of bone deposition (7-14d) marked by an increase in bone formative ALP levels,\textsuperscript{37,45} seen both in TS and CS of alveolar wall. Increase in ALP occurs by increasing the local concentration of phosphate ions after hydrolysis of phosphomonoester bonds, thus bone mineralisation. Highest serum ALP activity in humans has been correlated with greatest osteoblastic activity during growth spurts.\textsuperscript{72,73} The current review has 17 studies evaluating ALP in association with type, site and magnitude of force. ALP levels increased at TS in continuous retraction forces by NiTi spring as well as in gradually increasing force from 50 cN to 150cN at 2wk, showing a predisposition towards bone deposition.\textsuperscript{9} A study in rats supported osteoid deposition in the lacunae on TS in 80–120d.\textsuperscript{74} The current review shows peak in ALP levels at 2wk on continuous force application of 150cN, 100g or 150g force\textsuperscript{9,10,14,24,28,45}, with greater levels on TS compared to CS. This is followed by fall in ALP levels corresponding to hyalinised tissue removal and initiation of post lag phase.\textsuperscript{9,24} Magnitude of force was another determinant of variation in ALP. Decrease in ALP levels seen at 1hr, 1d after intrusion by TMA spring is believed to be caused by heavy forces leading to a hyalinised zone.\textsuperscript{25} Conversely, distalisation of molars with heavy cF of 250g\textsuperscript{31} showing high ALP levels at both TS and CS and ALP levels greater in 150g than 100g force,\textsuperscript{34} were attributed to extensive osteoblast recruitment on application of heavy forces.\textsuperscript{9} One study showing decreased ALP levels on both TS and CS of canine retraction with push coil spring was probably due to combination of bodily and tipping movement, which precludes pure compression and tension areas.\textsuperscript{38} ALP also varied with type of force: one study compared levels in Hycon\textsuperscript{8} screw with active tie-backs for retraction. A significant difference was seen at 3 and 4 wk of retraction with levels in Hycon screw group 260\% higher after one half turn twice weekly activation, compared with 200\% increase in active tie-back group.\textsuperscript{49} This may be ascribed to elastomeric force decay to 30-40\% of original force in 3 weeks. Another study on maxillary expansion by hyrax followed by retention noticed fall in ALP levels on CS and TS till four weeks of activation, followed by peak at 1yr on TS, thus indicating bone apposition during retention period.\textsuperscript{51}

Contrary to ALP, TRAP or ACP facilitates dissolution of bone minerals by forming a highly acidic extracellular environment and are potent osteoclast biomarkers expressed in areas of compression.\textsuperscript{74} The present review supports rise in TRAP levels at CS more than TS to reach peak at 1wk,\textsuperscript{33} 2wk\textsuperscript{11} and 4-5wk.\textsuperscript{34,36} This is supported by histochemical study by Casa et al,\textsuperscript{75} suggestive of appearance of mononuclear TRAP positive cells on application of forces at 2wk and multinucleated TRAP positive cells at 3 and 4wk. Even ACP activity was maximum at 3d, followed by its reversal, explained by natal release of enzymes from surface of osteoclasts.\textsuperscript{14} A secondary outcome of faster rate of OTM with minimal lateral and apical root resorption was noticed with higher levels of TRAP in 150g, compared with 100g force.\textsuperscript{34,36}

The consummation of bone resorption occurs by resolution of organic matrix mediated by lysosomal cysteine protease cathespin B that is increased 1d after application of 100-150g or 250g retraction force by E chain,\textsuperscript{21,30} while levels of inhibitor cystatin decreases in 1d.\textsuperscript{21} In association, plasminogen activator (t-PA) and its inhibitor (PAI) responsible for extravascular fibrinolysis, reach peak at 24hr only to fall later at 7d.\textsuperscript{23}

AST is another cytoplasmic enzyme released in extracellular environment after cell membrane lysis following necrosis\textsuperscript{76} and has been evaluated in 10 studies in the current SR. Peak levels of AST were seen at 1wk,\textsuperscript{31,40,41,54} 2wk,\textsuperscript{14,48} and 4wk.\textsuperscript{28,39} This may be explained on the basis of increase in AST activity for 14d due to hyalinization of pdl in compression zone, decreased later upon resolution of hyalinised area by macrophages.\textsuperscript{14} The formation of hyalinised zone and cellular necrosis may cause higher levels on CS than TS in retraction cases\textsuperscript{39,48} and also in 150g force, compared to 100g.\textsuperscript{31,34} But, such sporadic evidence could not be definitive for site predilection. Rather this enzyme has been associated more with destruction of gingival tissues in experimental and chronic periodontitis\textsuperscript{77} and subgingival colonization with arch wire ligation\textsuperscript{41} than orthodontic force application.
The current review has also monitored LDH, an enzyme released from cytoplasm to extracellular space after cell death in gingivitis or periodontitis as well as in orthodontic treatment. Variation in LDH levels were recorded with type, magnitude and direction of application of force. Continuous force of 125g with NiTi spring showed increase in levels at 7d to peak at 14d, but remained higher in CS than TS at 1.5 N, thus favouring its release after cell death. Timing of increase varied with force level, with an early increase seen at 2wk in heavy force of 250g applied for molar distalisation compared with rise in 3wk in 125g force. However no significant difference in LDH levels could be correlated to high friction between self-ligating brackets and thermoelastic or superelastic Niti-nol wires, as the forces produced by frictional resistance are insufficient for LDH release. One study supporting greater LDH levels in teeth undergoing retraction compared with controls was excluded from this review because of its cross-sectional study design. It supported LDH as a sensitive marker of the pdl metabolism changes during OTM.

Other inflammatory mediators like MPO and βG were also evaluated in this review. MPO released from PMNLs (polymorphonuclear leukocytes) is a sensitive marker for inflammation and pain associated to OTM and showed an early increase at 2hr. In cases of alignment, the levels of MPO increase from baseline to 1hr to 1d till completion of alignment, correlating it with inflammation caused by NiTi wire alignment. Studies on MPO also supported superelastic NiTi wires as best alignment wires, giving low continuous force and rapid tooth movement, showing higher MPO levels at 2hr, compared with heat-activated NiTi or multistranded NiTi or stainless steel wires.

Studies also mentioned increase in lysosomal enzyme, βG released from PMNLs after 14d of heavy interrupted force for mid-palatal hyrax expansion in adolescents. However, the levels remained high till 28d in retention, probably due to elastic recoil of stretched supracrestal gingival fibers.

The risk of bias assessment in QAI though indicated all studies as moderately or highly sensitive, revealed certain strengths and weaknesses of variable study designs (Table 7). While the objectives of the studies, selection criteria and orthodontic mechanics were generally clear, they strikingly lacked sample size calculation with only one study indicating the same. The authors took 5 as the sample size for inclusion, based on statistician’s advice. Randomization of experimental teeth/patients falling into study and control group have been clearly stated in only 21 out of 48 studies, suggesting substantial bias in all studies. The present SR deals with biomarker evaluation in GCF, hence the GCF handling characteristics have been adequate in all studies. However, the specification of time, temperature and humidity at the time of GCF collection was a major shortfall, with only four studies mentioning it. The statistical significance of the results, wherever applicable, have been stated in all the studies, but none of the studies mentioned dropouts or confounders, which might influence the results.

Despite the various shortcomings noticed in the study designs, the current evidence has generated ample evidence related to enzymes in OTM and has also opened new arena for future research in this direction.

Perhaps a most exciting area of research will involve biological basis of tooth movement with different ligation modes of brackets. Further studies could be conducted with LDH as marker for high frictional resistance in different combinations of brackets and wires, as only single study in this SR found no significant change in LDH in initial OTM with self-ligating brackets and superelastic or thermoactive archwire. Another split-mouth study correlating biomarker level with microbial colonization in different ligation modes showed a significantly greater level of AST in arch wire ligation than self-ligation, associated with greater microbial count.

An interesting correlation of MPO with pain was established with an early increase in MPO within 2hr of force application, coinciding with initial pain incidence in orthodontic patients. βG has been explored for its association with the most suitable wires for alignment and could be explored further in different types and magnitudes of forces.

Based on similarity between peri-implant fluid (PIMF) and GCF, the mediators studied in GCF could also be evaluated in PICF to assess stability of contemporary orthodontic anchorage devices, micro-implants, as has been suggested by study of interleukin 1β in PIMF. Despite the heterogeneity in study design and categories of enzymes studied in literature, this SR provides an essential overview of the mechanism by which en-
zymes play a role in bone apposition, resorption as well as ECM degradation. The current SR also correlates mediator levels in GCF with phases of OTM at different magnitudes and types of forces and also ligation modes. It goes a step further in suggesting the potential areas of research in this field, based on individual studies designed for associations of mediator levels with ideal orthodontic force magnitudes, method of ligation and periodontal status, thus setting a direct implication in clinical practice.

CONCLUSIONS

1. Orthodontic force induces change in levels of multiple enzymes detectable in GCF. These are:
   a) cytoplasmic enzymes released in extracellular environment after cell lysis (LDH, AST), b) Inflammatory markers released from PMNs (MPO, βG), c) enzymes involved in bone and tissue remodelling by bone resorption (TRAP, ACP), d) bone apposition (ALP) or dissolution of organic matrix (Cp, Cys, tPA, PAI) and e) various categories of MMPs responsible for degradation of ECM (MMP1, 2, 3, 8, 9, 13).

2. Compression sites showed early increase in levels of MMP1, MMP2, TIMP1, MMP9 between 1-4hr, and late peak in TIMP2, TRAP, AST after 7d, 4-5wk and 8-12wk, respectively.

3. Tension sites showed significant increase in ALP after 7d, MMP1 between 1-3hr and TIMP 1 and 2 levels at 4hr, 7d and 42d.

4. Distinction between TS and CS could be made with levels of TRAP, AST, LDH, MMP9, being greater on CS than TS, and ALP greater on TS.

5. ALP, TRAP levels were greater in 150g force than 100g force. An early rise in AST levels was seen in 150g force at 3 and 4wk, as compared to 100g force at 4 and 5 wk.

6. Mechanical stress with continuous force of NiTi spring causes increase in MMPs 1, 3 in 24hr in CS and of ALP as early as 7d in TS.

7. No significant association between levels of MMP-9 or AST and growth status could be established as adult and adolescents, gave no significant difference in levels.

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