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Changes in transforming growth factor-β1 in gingival crevicular fluid following periodontal surgery

L. Kuru[1,2], G. S. Griffiths[2], A. Petrie[3] and I. Olsen[2]

[1]Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey; Departments of [2]Periodontology and [3]Biostatistics Unit, Eastman Dental Institute for Oral Health Care Sciences, University College London, UK

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Abstract

Objectives: Growth factors play a major part in wound healing, including in the periodontium. However, the presence of growth factors in gingival crevicular fluid (GCF) in humans during periodontal wound healing has not yet been determined. Our hypothesis is that such factors are present in GCF and that changes in their levels might be of value as a prognostic marker of wound-healing activity and therapeutic progress following periodontal surgery. The aim of this study was therefore to measure transforming growth factor-β1 (TGF-β1) in GCF collected from sites that have undergone guided tissue regeneration (GTR) and conventional flap (CF) surgery and to compare these with GCF collected from unaffected healthy sites.

Materials and Methods: GCF samples were collected, using filter paper strips, at baseline (pre-surgical) and then at intervals up to 26 weeks from 16 patients undergoing GTR and from 11 patients undergoing CF surgery. After elution and acid treatment, TGF-β1 levels were measured by ELISA.

Results: Treatment of periodontal defect sites significantly reduced the mean probing pocket depth (PPD) and improved the mean lifetime cumulative attachment loss (LCAL). Average GCF volumes also significantly increased at all sites at 2 weeks post-surgery and thereafter declined to baseline levels, except at the GTR test sites that were still elevated at 7 weeks. TGF-β1 could be detected in almost all GCF samples, and 2 weeks after surgery, the average levels increased two-fold at the surgically treated but not at the control sites, which remained unchanged.

Conclusion: TGF-β1 is readily detectable in GCF and increases transiently following periodontal surgery. This suggests that changes in the levels of this growth factor in GCF might be useful for monitoring the progress of periodontal repair and regeneration.

Key words: flap surgery; gingival crevicular fluid; guided tissue regeneration; transforming growth factor-β1

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Guided tissue regeneration (GTR) (Cortellini et al. 1993a, b, 1995, 1996a, b, Gottlow 1993, Tonetti et al. 1993) is now considered to be a substantial clinical improvement over conventional flap (CF) surgery. However, developing more effective and more predictable treatment modalities for the repair and regeneration of the periodontal ligament depends on a more precise understanding of the cellular components and changes in gene activity that underly periodontal wound healing in humans (Wakabayashi et al. 1996, 1997, Kuru et al. 1999, 2001). A number of studies have shown that growth factors regulate a wide range of essential cellular functions, including activation, proliferation, migration and the synthesis of extracellular matrix (ECM) components (Sporn & Roberts 1991, Kiritsy et al. 1993). It is thus likely that these factors may also play a major part in mediating the re-building of healthy periodontal ligament (Terranova & Wikesjö 1987).

Transforming growth factor-β (TGF-β) is produced by both activated macrophages and neutrophils that are present during the initial phases of wound healing (Igarasi et al. 1993). The biological effects of this factor in vitro are highly diverse and include chemotactic and mitogenic activity towards gingival and periodontal ligament cells (Postlethwaite et al. 1987, Oates et al. 1993, Dennison et al. 1994, Nishimura & Terranova 1996, Anderson et al. 1998) and up-regulation of the ECM components collagen, fibronectin, tenascin and proteoglycans (Lynch et al. 1989, Matsuda et al. 1992). Although TGF-β has also been found to have

some degree of clinical efficacy in promoting periodontal regeneration in vivo (Selvig et al. 1994, Tatakis et al. 2000) and the cell surface receptors of TGF-β have been reported to be elevated in the regenerated periodontal tissues formed beneath GTR membranes (Parkar et al. 2001), the precise role of TGF-β in periodontal wound healing remains unclear. Since gingival crevicular fluid (GCF) is considered to reflect ongoing cellular activities in the surrounding periodontal tissues, the constituents of GCF are likely to comprise the components involved in tissue formation and remodelling as well as the products of tissue inflammation and destruction (Offenbacher et al. 1993, Embery & Waddington 1994). However, TGF-β has not yet been examined in GCF in humans during periodontal repair and regeneration. The present study has, therefore, for the first time, measured the levels of this growth factor in GCF samples collected from periodontal sites that have undergone GTR and CF surgery and compared these with TGF-β levels at unaffected healthy periodontal sites.

Materials and Methods

Subject populations

The study population represented a cohort of patients undergoing different forms of periodontal surgery, and the objective was to examine changes in the production of TGF-β1. The patient population included 27 systemically healthy subjects (10 males and 16 females; mean age 38.1 years, 95% confidence intervals (CIs) = 34.6–41.6 years) with moderate-to-severe periodontal disease who were referred to the Periodontology Clinic at the Eastman Dental Hospital. After initial periodontal therapy, a decision was made, at re-assessment, regarding the potential value of surgical intervention. The need for, and type of, surgery was decided purely on clinical grounds by the clinician responsible for treatment. Subjects were instructed about the nature and purpose of the study and informed consent forms were obtained. Subject inclusion was based on the presence of at least one tooth with a probing pocket depth (PPD) of $\geqslant$5 mm, a lifetime cumulative attachment loss (LCAL) of $\geqslant$5 mm and radiographic evidence of bone loss after completion of initial periodontal therapy comprising oral hygiene instruction, scaling and root planing. The protocol was approved by the Eastman Dental Institute and Hospital Joint Research and Ethics Committee.

Clinical measurements and surgical procedures

Although not the primary objective of this study, measurements of PPD, LCAL and recession of marginal gingiva (REC) were nevertheless carried out in order to ensure that the clinical outcomes of the different types of periodontal surgery were comparable with previous studies. The clinical measurements were recorded to the nearest millimetre, by a single investigator (L.K.) using an EN15 periodontal probe, immediately before the surgery and for 6 months post-operatively, although not all measurements were carried out on every visit.

A total of 16 interproximal defects (of six males and 10 females patients; mean age 39.6 years, 95% CI = 34.5–44.7 years) were treated using the principles of GTR surgery. Following sulcular incisions, full thickness mucoperiosteal flaps were raised, granulation tissue removed and the exposed root surfaces instrumented. Expanded polytetrafluoroethylene (ePTFE) membranes (Gore-Tex periodontal material) (W.L. Gore and Associates, Flagstaff, AZ, USA) of appropriate size and shape were placed on the defect area and adapted to the teeth by Teflon sutures, after which the flaps were repositioned and sutured. Following surgery, the subjects were instructed to rinse with 0.2% chlorhexidine twice daily for a period of 2 weeks. The sutures were removed 2 weeks after surgery and professional tooth cleaning was performed at 2-week intervals for the first 6 weeks. The membranes were removed 6 weeks after placement and the sutures removed 1-week later and the subjects recalled monthly for professional tooth cleaning up to 6 months after the initial surgery.

A total of 11 interproximal defects in four males and seven females patients (mean age 36.5 years, 95% CI = 31.1–41.9 years) were treated by CF surgery. Following reverse bevel incisions, mucoperiosteal flaps were raised, granulation tissue removed and root instrumentation performed. The flaps were replaced to their original position and secured with sutures. Subjects were advised to rinse with 0.2% chlorhexidine twice daily for a period of 2 weeks. The sutures were removed 2 weeks after surgery and the subjects recalled monthly for professional tooth cleaning up to 6 months after the initial surgery.

Site selection and GCF collection

The defects in the patients undergoing GTR surgery were localised, but in order to gain adequate access the surgical field had to be extended to include healthy adjacent tissue. Consequently, the subjects were divided into three groups for GCF collection and analysis, as follows: (i) the GTR test group comprising three to four GCF sites per subject immediately adjacent to the infrabony defect and treated by GTR using the PTFE membrane; (ii) a GTR control group of the same subjects, comprising two to four GCF sites per subject within the same surgical field as the GTR test group but at least one complete unit away from the defect and therefore not associated with GTR treatment and (iii) the CF group, of four to six GCF sites per subject adjacent to the periodontal defect that was treated by CF surgery. These groups thus comprised the same 16 GTR subjects that provided the GTR test and GTR control samples and 11 different subjects who provided the CF samples. The GCF samples of the GTR test and GTR control groups were collected immediately before surgery and at 2, 4, 6, 7, 12 and 26 weeks post-surgery, whereas the GCF samples of the CF group were collected before surgery and at 1, 6, 12 and 26 weeks post-surgery. However, it was not possible to obtain all clinical measurements and laboratory samples at all time periods from all of the patients, particularly at week 4 post-surgery. This is indicated in the results by an n value of less than 16 and 11 for the number of GTR and CF samples, respectively.

The selected sites were isolated with cotton rolls, saliva removed using a fine-bore high-power suction tip and supragingival plaque, if present, was removed using a periodontal probe to prevent saliva and/or plaque contamination (Griffiths et al. 1992). Pre-cut strips of Whatman 3 MM chromatography paper (Whatman Lab. Sales Ltd, Maidstone, UK) (2 mm $\times$ 10 mm) (Griffiths et al. 1988) were placed at the entrance of the crevice and left in position for 2 min. The volumes of GCF collected were measured by weighing the papers,

before and after sample collection, using a Cahn 25 microbalance (Cahn Instruments Inc., Cerritos, CA, USA) accurate to within 0.01 mg. The weight of the fluid was converted to volume by assuming that the density of GCF was 1.0 (Cimasoni & Giannopoulou 1988). For each subject in each group, the multiple strips obtained from the separate sites were pooled at the time of sampling, and the data are thus the pooled patient data for the combined similar sites, with the 16 GTR subjects providing the GTR test and the GTR control samples, the other data being derived from the pooled sites of each of the 11 CF patients. Samples were stored at -70°C prior to use.

Strips with any visible sign of saliva or blood contamination were discarded, so the number of paper strips differed from one subject to another and also from one time point to another for each individual subject. Since the samples from several sites of similar status in each subject were pooled, the average GCF volume per site was calculated as the total GCF volume divided by the total number of paper strips.

Elution of GCF samples and measurement of TGF-β1

The GCF-blotted paper strips were allowed to thaw at room temperature for 30 min. For elution of the GCF samples, 50 μl of phosphate-buffered saline (PBS) was added to each tube containing the multiple strips and centrifuged at 11,000 r.p.m. for 15 min (Griffiths et al. 1988). This step was repeated, the eluates combined and the total volume of 100 μl stored at 4°C for up to 24 h prior to use.

The levels of TGF-β1 in the GCF samples were determined using a commercially available Colorimetric Sandwich ELISA Kit (Quantikine DB100, R&D Systems, Minneapolis, MN, USA). An aliquot of 40 μl of each sample was transferred to a polypropylene tube and the latent TGF-β1 activated by adding 40 μl of 2.5 M acetic acid/10 M urea. After mixing and incubation at room temperature for 10 min, the acidified sample was neutralised by adding 40 μl of 2.7 M NaOH/1 M HEPES buffer. The activated GCF samples were further diluted fourfold according to the manufacturers instructions.

An aliquot of 200 μl of known concentrations (0, 31.2, 62.5, 125, 250, 500, 1000 and 2000 pg/ml) of the activated recombinant human TGF-β1 standard (R&D Systems) and of the activated samples of GCF were added to the ELISA plate, which had been precoated with a recombinant human soluble receptor II that specifically binds human TGF-β1. The plate was covered with an adhesive strip and incubated at room temperature for 3 h. Each well was aspirated and washed three times with 400 μl of the wash buffer provided, after which 200 μl of the detecting antibody (horseradish peroxidase-conjugated polyclonal antibody against TGF-β1, supplied by R&D Systems) was added, the plate again covered with an adhesive strip and incubated at room temperature for 1.5 h. After washing three times, 200 μl of the substrate solution (tetramethylbenzidine containing H_2O_2) was added to each well and incubated at room temperature for 20 min. Following the addition of 50 μl of 2 M H_2SO_4 to stop the reaction, the absorbance at 450 nm (A_{450}) was measured using a spectrophotometer (Titertek Multiskan Plus, Helsinki, Finland). The absorbance was also measured at 570 nm to determine any optical imperfections between the wells, and this value was subtracted from the A_{450}.

Statistical analysis

The data are derived from two groups of patients, one of which (the GTR patients) underwent different types of healing (the GTR test and GTR control samples). Together with the CF surgery, which was performed in different subjects, this resulted in a total of three groups for analysis. Thus, the data from the GTR surgery and from the CF patients are independent, but the GTR test and GTR control data are not independent of each other. Consequently, analysis between groups took this into account and was performed in two ways, with comparisons between the GTR test and GTR control groups analysed as data within the same subjects and comparisons between either the GTR test or GTR control and the CF patients analysed as data between different subjects.

For the clinical data, one site per patient with the deepest baseline PPD was chosen as the unit of measurement for the GTR test sites and for the CF sites. For the GTR control sites, one value per patient (the mean value of two to four sites) was used as the unit of measurement. For each clinical variable, a three-way analysis of variance was performed on the GTR test and control groups. The assumptions of Normality and constant variance were fulfilled in these analyses. If there was a significant time effect in these analyses, paired *t*-tests and a more stringent significance level ($p < 0.025$) to adjust for multiple testing were subsequently used to determine whether, at each time, there was a significant effect of treatment. Since the CF patients were different from those having undergone GTR surgery, a hierarchical repeated measures analysis of variance was used for each clinical variable to investigate the effects of treatment and time on the clinical variables. Again, the assumptions of Normality and constant variance were satisfied. Each of these analyses, if producing significant effects of time, was followed by a one-way analysis of variance, with post hoc Bonferroni comparisons to compare the three treatment groups, separately for the pre-surgery values, for the post-surgery values and using the change from baseline to 6 months for each patient as the unit of measurement for each clinical variable.

The logarithm of the two variables comprising the laboratory data (GCF volume and total TGF-β1) were used for the analyses of these data in order for the assumptions of the analyses to be satisfied. Medians are used to summarise the raw data and, for each laboratory variable, a three-way analysis of variance was performed on the GTR test and control group. If there was a significant time effect in these analyses, two-way analyses of variance, followed by post hoc Dunnett's tests were subsequently used to determine whether, within each GTR group, there was a significant difference in response between the baseline pre-surgery value and the values at different weeks post-operatively. Since the CF patients were different from those having undergone GTR surgery, a hierarchical repeated measures analysis of variance was used for each variable to investigate the effects of treatment and time, but excluding the results from weeks 4 and 7. These analyses, if producing significant effects of time, were followed by a repeated measures analyses of variance for the CF group, with post hoc Dunnett's comparisons, of pre-surgery versus each week post-surgery. In order to limit the amount of data included in

this study, analysis was not carried out for the changes in TGF-β1 concentration but only for the GCF volumes and absolute amounts of the growth factor, from which the concentrations can be calculated.

Results

Clinical characteristics

The results in Table 1 show the clinical measurements of the three groups at the selected sites immediately before and 6 months after surgery. The baseline PPD was found to be reduced by an average of 4.40 mm at the GTR test sites ($p < 0.001$), while significant mean reductions were also observed at both the GTR control and CF sites (0.63 and 3.30 mm, respectively). Although GTR and CF surgery also resulted in a statistically significant mean gain in LCAL (3.60 and 2.40 mm, respectively), the mean LCAL remained the same at the GTR control sites. The mean REC at all sites showed a small but significant change ($p = 0.05$) 6 months after surgery, as shown in Table 1.

Comparison of the PPD, LCAL and REC levels of the different groups showed that there were no significant differences in means between the GTR test and the CF sites, either before or 6 months after surgery. Furthermore, comparison of the changes in these characteristics between baseline and 6 months post-surgery also showed that some differences in means between the GTR test and CF sites were large but none was statistically significant (mean differences between the changes in PPD, LCAL and REC values being 1.10, 1.20 and 0.10 mm, respectively, with *p*-values of 0.11, 0.13 and 1.0, respectively).

Changes in GCF volume

Based on the total of 253 GCF samples collected from the test and control sites of the 16 GTR subjects and 11 CF subjects, the results in Table 2 show that the average GCF volumes of all three groups increased significantly 2 weeks after surgery (*p*-values of < 0.001, 0.002 and 0.003 for the GTR test, GTR control and CF groups, respectively). However, by 6 weeks the GCF volumes at the GTR control and CF sites had decreased to levels that were not significantly different from their respective baseline volumes, as shown in Table 2. In contrast, the average GCF volume at the GTR test sites remained elevated during the 6-week period of membrane retention and was also larger at 7 weeks, 1 week after membrane retrieval, although by 12 and 26 weeks the average GCF volume of these samples also declined to a level, which was not significantly different from the baseline volume. Comparison of the different groups shows that the average GCF volume prior to surgery was the same at both the GTR test and CF sites (0.26 μl), although the range of volumes was substantially larger among the former, as indicated by the 25/75th percentiles. Notably, the average GCF volume at the GTR control sites

Table 1. Mean clinical measures at pre-surgery and at 6 months after guided tissue regeneration (GTR) and conventional flap (CF) surgery

Sites	Probing pocket depth (mm)		Lifetime cumulative attachment loss (mm)		Gingival recession (mm)	
	pre-surgery	6 months	pre-surgery	6 months	pre-surgery	6 months
GTR test ($n = 15$)	7.73 [6.83,8.63]	3.33 [2.43,4.24]	9.00 [7.93,10.07]	5.40 [4.30,6.50]	1.27 [0.59,1.94]	2.07 [0.95,3.18]
Change	4.40 [3.49, 5.31], $p < 0.001$		3.60 [2.85,4.35], $p < 0.001$		-0.80 [-1.59, -0.01], $p = 0.05$	
GTR control ($n = 12$)	2.27 [1.89,2.65]	1.65 [1.35,1.94]	2.33 [1.87,2.79]	2.33 [1.85,2.82]	0.06 [-0.40,0.53]	0.69 [0.15,1.22]
Change	0.63 [0.33,0.92], $p = 0.001$		0.000 [-0.38,0.38], $p = 1.00$		-0.63 [-0.95, -0.30], $p = 0.002$	
CF ($n = 10$)	7.20 [6.20,8.20]	3.90 [2.86,4.94]	7.80 [6.80,8.80]	5.40 [4.09,6.71]	0.60 [0.23,0.97]	1.50 [0.66,2.34]
Change	3.30 [2.47,4.43], $p < 0.001$		2.40 [0.96,3.84], $p = 0.004$		-0.90 [-1.61, -0.19], $p = 0.02$	

The values shown are the means and 95% confidence intervals (in brackets).
The Change is the difference between the level 6 months post-operatively and the pre-surgery baseline level (95% confidence interval in brackets). The *p*-values were obtained from paired *t*-tests comparing these levels.

Table 2. Median gingival crevicular fluid (GCF) volumes (μl) at pre-surgery and at intervals over the next 6 months following guided tissue regeneration (GTR) and conventional flap (CF) surgery

Group	Pre-surgery	Time after surgery (weeks)					
	0	2	4	6	7	12	26
GTR test	0.26 [0.12,0.62]	1.44 [0.76,2.36]	1.18 [0.59,2.13]	1.15 [0.47,2.40]	1.00 [0.67,1.45]	0.21 [0.13,0.58]	0.11 [0.07,0.23]
	$n = 16$	$n = 16$	$n = 11$	$n = 16$	$n = 15$	$n = 13$	$n = 16$
		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 1.0$	$p = 0.054$
GTR control	0.12 [0.05,0.23]	0.57 [0.19,0.95]	0.16 [0.10,0.71]	0.28 [0.15,0.48]	0.20 [0.08,0.56]	0.11 [0.04,0.20]	0.06 [0.08,0.02]
	$n = 16$	$n = 16$	$n = 12$	$n = 16$	$n = 15$	$n = 13$	$n = 16$
		$p = 0.002$	$p = 0.26$	$p = 0.10$	$p = 0.71$	$p = 0.91$	$p = 0.31$
CF	0.26 [0.17,0.39]	1.09 [0.66,1.54]	–	0.37 [0.16,1.57]	–	0.19 [0.08,0.37]	0.14 [0.06,0.34]
	$n = 11$	$n = 10$	$n = 0$	$n = 9$	$n = 0$	$n = 9$	$n = 10$
		$p = 0.003$		$p = 0.35$		$p = 0.84$	$p = 0.27$

The numbers in brackets are the 25th and 75th percentiles of the raw data.
The *p*-values have been obtained by Dunnett's post hoc comparisons of the post-surgery volumes compared with the pre-surgery baseline level in each group, following a repeated measures analysis of variance using the log of the GCF volumes.

Table 3. Median values of the transforming growth factor-β1 (TGF-β1) levels (total pg) in the gingival crevicular fluid at pre-surgery and at intervals over the next 6 months following guided tissue regeneration (GTR) and conventional flap (CF) surgery

Group	Pre-surgery	Time after surgery (weeks)					
	0	2	4	6	7	12	26
GTR test	30.17	59.50	44.61	21.35	24.33	17.15	14.41
	[13.87,59.41]	[30.62,185.96]	[33.90,133.29]	[3.26,50.22]	[14.55,67.35]	[5.73,35.41]	[11.71,33.17]
	$n = 16$	$n = 16$	$n = 10$	$n = 16$	$n = 15$	$n = 13$	$n = 16$
		$p = 0.051$	$p = 0.16$	$p = 1.0$	$p = 0.77$	$p = 1.0$	$p = 1.0$
GTR control	27.77	32.87	35.84	13.11	13.30	7.66	13.70
	[1.32,83.99]	[13.79,108.33]	[17.43,94.73]	[0.00,57.67]	[1.02,43.19]	[0.00,13.16]	[0.89,44.34]
	$n = 16$	$n = 16$	$n = 10$	$n = 16$	$n = 15$	$n = 13$	$n = 16$
		$p = 0.28$	$p = 0.39$	$p = 0.63$	$p = 1.0$	$p = 0.48$	$p = 0.99$
CF	23.95	48.86	–	11.69	–	0.00	16.75
	[0.00,444.07]	[18.81,117.42]		[3.38,33.56]		[0.00,19.35]	[0.00,21.30]
	$n = 11$	$n = 10$	$n = 0$	$n = 9$	$n = 0$	$n = 9$	$n = 10$
		$p = 0.09$		$p = 0.89$		$p = 0.15$	$p = 0.99$

Numbers in brackets are the 25th and 75th percentiles of the raw data.
The *p*-values have been obtained by Dunnett's post hoc comparisons of the post-surgery levels compared with the pre-surgery baseline amount in each group, following a repeated measures analysis of variance using the log of the TGF-β1 levels.
The changes shown are for the total amount of growth factor only and not the concentration; the latter can be calculated from the data in this table and that in Table 2.

(0.12 μl) was, prior to surgery, significantly lower than at the GTR test sites.

TGF-β1 levels in GCF

Almost all samples of GCF were found to have detectable levels of TGF-β1. In particular, TGF-β1 was detected in 100% of sites 2 weeks after periodontal surgery, regardless of the type of surgery, and only in the CF samples obtained at 12 and 26 weeks post-surgery was the proportion of TGF-β1-positive samples less than 90% of the total (229 of a total of 258; data not shown).

The average amount of TGF-β1 was found to increase at the GTR test sites at 2 weeks post-surgery (from 30.17 to 59.50 pg), although this difference was not statistically significant ($p > 0.05$) (Table 3). Nevertheless, the amount of TGF-β1 remained high at these sites until 4 weeks post-surgery, but thereafter declined to levels that were lower than baseline values. Notably, TGF-β1 also remained at this low level even at week 7, 1 week after surgical removal of the membrane from the GTR test sites. Similar changes after week 4 were also observed at the CF sites, as shown in Table 3, whereas the amount of TGF-β1 at the GTR control sites remained unchanged for the first 4 weeks following surgery and then decreased as it did at the other sites. Comparison between the groups showed that although average TGF-β1 levels were very similar at all sites prior to surgery, by 2 weeks after surgery TGF-β1 markedly increased, by approximately twofold, at the GTR test and CF sites but not at the GTR control sites (Table 3). However, because of the wide range throughout as evidenced by the 25/75th percentile values, these differences were not statistically significant.

Discussion

The results of the present study have shown that there is a statistically significant reduction in PPD and a statistically significant gain in LCAL following treatment of intraosseous periodontal defects, as reported previously for both CF surgery (Lindhe et al. 1982, Cortellini et al. 1995, 1996a) and GTR surgery (Cortellini et al. 1993a, 1995, 1996a, Sander et al. 1994). In addition, there was a marked increase in the volume of GCF (between four and five times the respective baseline volumes) at all sites, which occurred 2 weeks after surgery. This most probably reflects the early inflammatory stage of periodontal wound healing, which is accompanied by enhanced permeability of gingival blood vessels and increased fluid passage through the vasculature into the extravascular space (Egelberg 1966). Elevated GCF volumes have previously been reported 1 week following GTR surgery of periodontal Class II furcation defects (Yan et al. 2000), and have even been observed within 2 days following a single episode of non-surgical treatment of periodontal pockets (Talonpoika & Hämäläinen 1992, 1993, Talonpoika et al. 1993). Notably, the present study also found that this apparent inflammatory effect persisted for a prolonged period at the GTR test sites, since there was a statistically significant increase in average GCF volumes in the 6-week period during which the membrane was in position and also 1 week following its removal, prior to decreasing to pre-surgical levels by 12 weeks. In contrast, the GCF volumes at the GTR control and CF sites declined far more rapidly, reaching baseline values by 6 weeks.

Although GCF has been shown to originate from the serum in proximal blood vessels, it is generally considered to reflect the ongoing processes in the surrounding periodontal tissues, including inflammation, turnover of connective tissue and resorption of alveolar bone (Talonpoika & Hämäläinen 1992, Embery & Waddington 1994, Giannobile et al. 1995, Griffiths et al. 1998). Thus periodontal regeneration (Wikesjö & Selvig 1999) might also be expected to generate components in GCF that are closely associated with this process, and analysis of the constituents of GCF has suggested that wound-healing mediators are indeed present (Embery & Waddington 1994). Moreover, in view of the clinical outcomes of periodontal surgery we and others have observed, it is likely that the GCF samples collected from surgically treated defect sites represent wound fluid associated with periodontal repair and regeneration whereas the GCF at healthy periodontal sites (the GTR ''control'' sites) adjacent to such defects may be considered to be derived from healthy sites undergoing normal

healing. This is consistent with previous observations that, during healing after periodontal treatment, newly synthesised connective tissue components such as collagen types I and III and fibronectin are present at elevated levels in the GCF of treated sites, as noted above (Talonpoika & Hämäläinen 1992, 1993, Talonpoika et al. 1993). Periodontal therapy has also been shown to modulate GCF levels of glycosaminoglycans (Yan et al. 2000), matrix metalloproteinases (Said et al. 1999) and their tissue inhibitors (Haerian et al. 1996), while cells of regenerated periodontal tissue express relatively high levels of matrix components collagen type I, fibronectin and tenascin (Kuru et al. 2001) and cell surface receptors specific for the growth factor TGF-β1 (Parkar et al. 2001).

TGF-β1 has previously been identified in fluids obtained from healing skin wounds (Ono et al. 1995a), blisters of partial skin burns (Ono et al. 1995b), after colorectal surgery (Wiik et al. 2001) and in bronchoalveolar lavage fluid following acute lung injury (Kolb et al. 2001). In addition, this growth factor has been found to be present in GCF in cyclosporin A-treated patients (Buduneli et al. 2001) and during orthodontic tooth movement (Uematsu et al. 1996). In the present study we have shown, for the first time that TGF-β1 is also readily detectable in GCF following periodontal surgery, with approximately 90% of samples, after acidification, giving a positive signal. The inability of a previous study to identify TGF-β1 in any GCF obtained from any of the implant sites in humans using a similar commercially available ELISA kit (Salcetti et al. 1997) may have been due to the lack of acid pretreatment of the samples, while the failure to detect TGF-β1 in a small proportion of the GCF samples in the present study is probably due to the very small volume of these particular samples. Our finding that this growth factor, which is able to stimulate the production of a number of ECM constituents (Matsuda et al. 1992), doubled 2 weeks after surgery at the GTR test and CF sites but not at the GTR control sites, compared with the pre-surgery levels that remained unchanged, suggests that the increase was more than just a response to surgery alone and may be exaggerated by the previous presence of pathology. This is supported by the observation that TGF-β1 levels remained unchanged 1 week after the surgical removal of the membrane from the GTR test sites at which periodontal healing had occurred during the previous 6 weeks. In addition, in preliminary experiments not shown here we found that platelet-derived growth factor (PDGF) was also present in GCF following periodontal surgery. Although only 17 of a total of 54 GCF samples had detectable levels of this factor, there was nevertheless a clear trend of a transient increase at the GTR test sites, similar to that observed for TGF-β1. This is consistent with the role of PDGF in wound-healing processes (Matsuda et al. 1992, Oates et al. 1993), in which even a low level of this growth factor by itself or in synergy with other factors including TGF-β1 (Matsuda et al. 1992, Rutherford et al. 1992, Cooper et al. 1994, Ono et al. 1995a, b), may be highly effective in mediating tissue regeneration. The present findings thus highlight both the multiplicity of mediators involved in the periodontal tissue rebuilding process and the potential value of utilising changes in the levels of these factors as a prognostic marker of wound-healing activity in the periodontium and for evaluating successful therapeutic progress.

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Address:
Irwin Olsen
Department of Periodontology
Eastman Dental Institute for Oral Health Care Sciences
University College London
Room RL 16 Levy Wing
256 Gray's Inn Road
London WC1X 8LD
UK
Fax: +44 20 7915 1254
E-mail: i.olsen@eastman.ucl.ac.uk