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Bacterial Lysine Decarboxylase Influences Human Dental Biofilm Lysine Content, Biofilm Accumulation and Sub-Clinical Gingival Inflammation

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Abstract

Background—Dental biofilms contain a protein that inhibits mammalian cell growth, possibly lysine decarboxylase from *Eikenella corrodens*. This enzyme decarboxylates lysine, an essential amino acid for dentally attached cell turnover in gingival sulci. Lysine depletion may stop this turnover, impairing the barrier to bacterial compounds. The aims of this study were to determine biofilm lysine and cadaverine contents before oral hygiene restriction (OHR), and their association with plaque index (PI) and gingival crevicular fluid (GCF) after OHR for a week.

Methods—Laser-induced fluorescence after capillary electrophoresis was used to determine lysine and cadaverine contents in dental biofilm, tongue biofilm and saliva before OHR and in dental biofilm after OHR.

Results—Before OHR, lysine and cadaverine contents of dental biofilm were similar and 10-fold greater than in saliva or tongue biofilm. After a week of OHR, the biofilm content of cadaverine increased and that of lysine decreased, consistent with greater biofilm lysine decarboxylase activity. Regression indicated that PI and GCF exudation were positively related to biofilm lysine post-OHR, unless biofilm lysine exceeded the minimal blood plasma content in which case PI was further increased but GCF exudation was reduced.

Conclusions—After OHR, lysine decarboxylase activity seems to determine biofilm lysine content and biofilm accumulation. When biofilm lysine exceeds minimal blood plasma content after OHR, less GCF appeared despite more biofilm. Lysine appears important for biofilm accumulation and the epithelial barrier to bacterial proinflammatory agents.

Clinical Relevance—Inhibiting lysine decarboxylase may retard the increased GCF exudation required for microbial development and gingivitis.

Keywords

Gingival crevicular fluid; Gingivitis; Oral hygiene; Pathogenesis of periodontal disease(s); Microbiology

Within a healthy oral cavity, muscular movements and saliva limit bacterial colonization except at teeth surfaces¹. Commensal bacteria, mostly viridans streptococci and actinomyces, but also small amounts of *Eikenella corrodens* and *Capnocytophaga gingivalis*, adhere to clean teeth² and form dental biofilms³. If oral hygiene is abolished, an inflammatory exudate (gingival crevicular fluid, GCF) appears and its content of blood plasma proteins and small molecules provides better nutrients than saliva for the gingival region of dental biofilms^{4, 5}. The sulcular dental biofilm composition shifts from a gram positive, mostly saccharolytic commensal microbiota to a gram negative, mostly proteolytic successor microbiota^{6, 7}.

In periodontally healthy adults, this shift does not appear until after a week of oral hygiene restriction and clinical evidence of inflammation requires an additional 1 – 2 weeks⁸. Dental biofilms may initiate GCF exudation by possessing toxins⁹ that affect the viability of the junctional epithelial attachment¹⁰. Assays of saline extracts from dental biofilms have identified two groups of toxins that inhibit mammalian cell growth in culture. One is a heat-stable mixture of short chain fatty acids (SCFA, average mol wt 225 Da) subsequently related to gingivitis severity^{11–14} and the other is a heat-sensitive protein (mol wt 160 kDa)¹⁵.

Previous studies have reported that mice immunized with biofilm extracts provided growth inhibitor-neutralizing monoclonal antibodies (Mabs)¹⁶. These Mabs identified antigens on the cell surface of *Streptococcus sanguis*, *Streptococcus mitis* biovar 2, and *Eikenella corrodens*, all isolated from dental biofilm¹⁷. Of these bacteria, only cell surface extracts of *E. corrodens* inhibited mammalian cell growth. On western immunoblots of such extracts, the Mabs detected a mixture of antigens. The largest antigen was an 80 kDa polypeptide¹⁸, whose tryptic digest provided peptides homologous to lysine decarboxylases from gram negative bacteria. The encoded amino acid sequence was then obtained from *E. corrodens* genomic DNA¹⁹, and modeling suggested a lysine decarboxylase whose catalytic center lay at the intersection of 80 kDa homodimers. Thus, the 160 kDa growth inhibitor in extracts of dental biofilm was possibly also a lysine decarboxylase¹⁹.

Saline extracts of *E. corrodens* were further reported to inhibit growth by depriving the medium of lysine, which was converted to cadaverine (Fig. 1a)¹⁹. Lysine is essential for all mammalian cells, which cannot synthesize this amino acid *de novo*. A lack of lysine causes autophagy; cells self-digest until apoptosis develops and the cell is phagocytosed^{20, 21}. At the base of gingival sulci, oral epithelium is attached to teeth surfaces by junctional epithelial basal cells that continually proliferate to remain attached²². The underlying gingival stroma maintains the healthy dental attachment by providing interstitial fluid, a protein-free transudate containing lysine, other amino acids and small molecules (Fig. 1b). It was previously suggested that lysine decarboxylase converts so much lysine to cadaverine (and carbon dioxide) if oral hygiene is restricted that the epithelial attachment becomes impaired¹⁹.

Lysine decarboxylase is present in about 82% of *E. corrodens* strains and about 8% of *Capnocytophaga gingivalis* and *ochracea* strains²³. *E. corrodens* and *C. gingivalis* adhere

quickly to newly cleaned teeth, but increase markedly from levels found during oral hygiene maintenance after 2 days of oral hygiene restriction^{3, 8}. We propose that lysine decarboxylase activity should increase correspondingly. Cadaverine, which is absent from blood plasma or interstitial fluid in the human body, appears at the expense of lysine and dentally attached (DAT) cells become lysine deprived. The lysine deprived cells may release proinflammatory cytokines which increase the permeability of subepithelial blood vessels, or undergo autophagy allowing biofilm to access the gingival stroma and release cytokines. Either or both mechanisms would activate GCF exudation^{6, 19}. If oral hygiene remains restricted, biofilm colonization by the successor microbiota provides SCFA that directly inhibits DAT cell turnover and further impairs the epithelial barrier irrespective of lysine content²⁴. Indeed, the most coronal layer of DAT cells disappears from gingivitis sites (Fig. 1c)²⁵.

Lysine decarboxylase is induced from *Escherichia coli* and other enterobacteria by acids in the stomach or from bacterial carbohydrate metabolism in the small intestine²⁶. Most enterobacteria are also proteolytic; they obtain lysine from peptides or proteins in the diet. Cadaverine is a strong base that prevents the pH from becoming too acidic locally and therefore improves bacterial viability²⁷. Cadaverine also enhances bacterial growth by inhibiting bacterially stimulated leukocytes from secreting oxidative enzymes, specifically superoxide and peroxynitrite which are bactericidal^{28, 29}, and also by being oxidized to piperidine³⁰ which inhibits leukocyte migration and oxidative enzyme synthesis³¹.

Cadaverine is increased in saliva from subjects with oral halitosis^{32, 33}. In addition, both cadaverine and lysine are increased in the GCF from inflamed compared with healthy sulci³⁴, as determined by chromatographic elution position and mass spectral ion signature³⁵. The recovery and ionization efficiency of cadaverine differs from that of lysine, requiring a set of absolute quantitation measures which were not available. The ratio of cadaverine to lysine in each sample was therefore not determined³⁴. In addition, the piperidine product of cadaverine oxidation was absent from these same GCF samples (Dr Lining Guo, Metabolon Inc., E-mail communication to M Levine, Aug 17th 2010).

The aims of our study were to determine biofilm lysine and cadaverine contents before oral hygiene restriction and their association with plaque index and gingival crevicular fluid exudation after oral hygiene restriction for a week.

MATERIALS AND METHODS

Participant Selection and Examination

The study was conducted in Hungary and Dr Lohinai was Principal Investigator. The study was monitored in accordance with the ICH note for guidance on Good Clinical Practice and the Helsinki declaration. The human protocol was reviewed and approved by the Ethics Committee of the Hungarian Medical Research Council (Approval # 11878-1/2006-1017EKL). Prior to participation, the purpose and risks of the investigation were fully explained to all participants. Subjects were entered into the study only after having given written consent.

Sixteen subjects, 7 men and 9 women were enrolled and completed the project: 12 aged 19 to 24 years and 4 (2 men and 2 women) aged 29, 30, 36 and 38 years. Inclusion requirements were at least 26 teeth, no existing or prior medical conditions and no drug therapy or smoking tobacco. Dental exclusion categories were partially erupted wisdom teeth, cavities requiring treatment, a pocket with a probing depth >2 mm, >10% of teeth with gingival sulci that bled on probing, and the presence of gingival recession or calculus.

The protocol is summarized in Fig. 2. At admission, saliva was collected into a sterile 10 ml centrifuge tube for 5 min. Biofilm from the upper surface of the tongue or from the gingival surfaces of teeth was collected with a plastic tongue scraper or curette as appropriate (admission samples). After a thorough professional cleaning, an upper right or left quadrant was randomly selected. An impression was taken and a bioguard was fabricated to restrict oral hygiene in this quadrant³⁶. The participants were then given a new medium toothbrush and toothpaste^{††}, instructed to brush thrice-daily at home, and to return after 3 days without cleaning their teeth that morning.

At that visit, saliva, tongue and dental biofilm samples were collected a second time (baseline samples). The teeth were again professionally cleaned and the participants practiced using the bioguard which they were instructed to use when tooth cleaning. They were also told to avoid all supplementary oral hygiene procedures such as flossing or chewing gum. Plaque index and GCF exudation rate were not measured at these visits.

At the return visit after 7 days, all subjects were examined by the same experienced clinician (ZL) to reduce inter-examiner variability in clinical measurement. The bioguarded region was isolated from saliva with cotton rolls, gently air-dried, and the buccal and mesio-buccal surfaces of the upper second incisor, first premolar, and first molar scored for plaque index³⁷. Each subject rinsed their mouth with water and the bioguarded region was air-dried and isolated for GCF exudation measurement by the method of Loe & Holm-Pedersen⁴ because a Periotron was not available. The examiner gently inserted a paper strip (2 mm wide)^{‡‡} into the gingival sulci at each of the three buccal surfaces from which plaque index was obtained. The strip was left undisturbed for exactly 3 min to ensure adequate fluid uptake and the wetted length immediately measured with a caliper to avoid fluid loss. The wetted length of the paper strip was multiplied by its width and liquid load (0.22 $\mu\text{g}/\text{mm}^2$) and divided by 3 to obtain the GCF exudation volume in $\mu\text{L}/\text{min}$. Dental biofilm was then collected from the bioguarded region and the teeth were professionally cleaned.

Measurement of Cadaverine and Lysine

Cadaverine and lysine were determined as described previously by our group³⁸. Biofilm samples were placed in pre-weighed micro-centrifuge tubes and weighed using an analytical balance. Biofilm and saliva samples were diluted with 10 times their wet weight of 65 mM NaCl. The saliva samples were centrifuged (10,000 g, 10 min, 4°C), but the biofilm samples were first homogenized in the micro-centrifuge tubes using a pestle^{§§}. A homolog of cadaverine and putrescine (1,3-diaminopropane) was added to each supernatant to 25 μM (internal standard), followed by a double volume of acetonitrile to precipitate protein. After centrifugation, the supernatants were made to 50 mM borate buffer pH 8.0. Ethanolic 4-fluoro-7-nitrobenzofurazan (NBD-F) was added to 0.05 mg/mL and incubated at 65°C for 15 min. An equal volume of water was added and the solutions were stored at 4°C.

Samples were introduced into 20 cm fused silica capillaries^{||||} under pressure (5 sec, 0.5 psi, 15°C) and separated at 70 μA in 0.03 M phosphate buffer, pH 2.2. NBD-derivatives were separated using a capillary electrophoresis system controlled by 32 Karat software version 5.0^{¶¶} coupled to an argon-ion, laser-induced fluorescence detector and identified by emission at 520 nm after excitation at 488 nm. The amine derivatives (0 – 50 μmol) were used to construct linear calibration curves. Losses were adjusted by internal standard

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^{||||}Polymicro Technology, Phoenix, AZ, USA

^{¶¶}P/ACE MDQ controlled by 32 Karat software version 5.0, Beckman Coulter Inc., Fullerton, CA, USA

recovery. Assay accuracy and precision were ~6% as reported in detail previously³⁸. In addition, because lysine is the sole source of cadaverine which is stable in the oral cavity³⁹, totaling the cadaverine plus lysine content of dental biofilm indicates lysine converted to cadaverine plus lysine remaining ('total' lysine content). The cadaverine fraction (CF) of this 'total' indicates lysine decarboxylase activity.

Clinical Measurement Variability

For each participant, plaque index was the mean from six bioguarded teeth surfaces and GCF the mean from three such surfaces. The standard deviation about each mean indicated the variability of the respective measurements within each participant. Because these standard deviations were not normally distributed, an estimate of measurement variability was obtained by converting each standard deviation to a percentage of its respective mean and identifying the median, 35.9% for plaque index and 36.5% for GCF.

Statistical Analyses^{##}

Differences in mean cadaverine and lysine contents and in cadaverine fraction of dental biofilm, saliva and tongue biofilm between admission and baseline and, independently, in dental biofilm from baseline to the end of oral hygiene restriction, were compared using non-parametric tests (Table 1).

Relationships between lysine and cadaverine within dental biofilm, saliva and tongue biofilm at baseline, and of lysine and cadaverine in dental biofilm to plaque index and GCF after oral hygiene restriction were explored. Spearman's ranked coefficients of determination squared (r^2) and significance (p) were obtained initially. Significant relationships ($p < 0.05$) were explored further using least squares multiple and non-linear regressions as appropriate. Standardized residuals were checked for normality to ensure that assumptions were not violated by the non-normal distribution of measurements in the population. Spearman correlations had a subjects-to-independent variable ratio that was 16:1, close to optimum for a search for unknown relationships. (The Princeton University Data and Statistical Services: <http://dss.wikidot.com/assumptions-of-regression>).

Parametric least squares regressions used no more than 2 variables and had a subjects-to-independent variable ratio that was greater than the accepted minimum of 5:1. Squared correlation coefficients (R^2) were adjusted for number of variables and data points in the model⁴⁰.

RESULTS

Prior to oral hygiene restriction, admission and baseline measurements of cadaverine fraction in dental biofilm were strongly correlated ($r^2 = 0.79$, $p < 0.001$). Cadaverine content was less correlated ($r^2 = 0.61$, $p < 0.001$) and lysine content was not correlated ($r^2 = 0.07$, not significant). At baseline, the cadaverine content of dental biofilm was correlated with that of saliva ($r^2 = 0.507$, $p < 0.01$) or tongue biofilm ($r^2 = 0.316$, $p < 0.03$), but the lysine content or the cadaverine fraction were not correlated ($p > 0.05$). Essentially the same results were observed at admission (data not shown). Also at baseline, the mean cadaverine and lysine contents of dental biofilm were similar to each other and the lysine in venous blood plasma, mean $0.17 \mu\text{mol/mL}$; range 0.11 to $0.23 \mu\text{mol/mL}$ ⁴¹, about 10-times greater than lysine and cadaverine in saliva or tongue biofilm (Table 1).

^{##}Statistical programs were XLSTAT 2011 Version 2 (Addinsoft, Inc., Brooklyn, NY 11201) and/or Graphpad Prism (GraphPad Software, Inc. La Jolla, CA 92037).

After oral hygiene restriction for a week, the mean cadaverine content of dental biofilm was increased, mean lysine content was decreased, but 'total' lysine was unchanged (Table 1). At this time, mean plaque index was 1.8 (0.3 standard deviation, s.d.) and mean GCF exudation was 0.20 $\mu\text{L}/\text{min}$ (0.10 s.d.). Biofilm cadaverine fraction was increased in all participants, but its extent of increase was greater if the cadaverine fraction was low at baseline (Fig. 3a) and if the biofilm lysine content was low after oral hygiene restriction (Fig. 3b).

Also after oral hygiene restriction, plaque index was strongly and positively correlated with biofilm lysine content (Fig. 4a), but not cadaverine content, cadaverine fraction or cadaverine fraction increase. Biofilm lysine contents $<0.11 \mu\text{mol}/\text{g}$ (i.e. less than the minimal content of blood plasma⁴¹) were also positively correlated with GCF exudation, whereas greater lysine contents were associated with a decrease in GCF exudation, resulting in a quadratic curve (Fig 4b). In the 13 participants whose biofilm lysine content was less than $0.11 \mu\text{mol}/\text{g}$ after oral hygiene restriction, the GCF exudation rate was collinearly correlated with biofilm lysine content and plaque index (Variance Inflation Factor = 77.2; $R^2 = 0.47$, $p < 0.01$).

DISCUSSION

Trombelli et al.³⁶ reported partial oral hygiene restriction to be better tolerated than full mouth restriction. They also reported plaque index and GCF measurements after the first week of oral hygiene restriction similar to those in our study. The mean lysine content of whole saliva in our study resembled that reported by others for parotid⁴² or whole saliva⁴³. The whole saliva study⁴³ also reported that dietary lysine is cleared from the oral cavity within 10 min. We found that the lysine content of dental biofilm at baseline was an order of magnitude greater than in saliva and resembled that of blood plasma. Lysine apparently reaches the biofilm from blood plasma via interstitial fluid in health or the GCF exudate in inflammation, not from saliva.

In our study, the mean lysine content of dental biofilm fell because of increased lysine decarboxylase activity after a week of oral hygiene restriction, but it was still greater than in saliva at baseline. Cadaverine fraction (i.e. lysine decarboxylase activity) was increased because 'total' lysine content (see Methods) was unchanged. The extent of this increase, which occurred in all subjects, was inversely proportional to cadaverine fraction at baseline and the lysine content after oral hygiene restriction. We speculate that differences in baseline cadaverine fraction may indicate diversity of the oral microbiota and variations in oral hygiene efficacy between individuals before entering the study. In the subjects whose cadaverine fraction was smallest prior to the study, the cadaverine fraction increased the most and their biofilm lysine content tended to be small after oral hygiene restriction.

Our results further indicate that differences in lysine content at this time associated strongly and positively with plaque index, a measure of biofilm accumulation³⁷. The survival and colonization of many bacteria is inhibited by a lack of lysine⁴⁴ or by lysine analogues that inhibit lysine uptake⁴⁵. Nevertheless, some mixtures of oral bacteria form luxuriant biofilms when grown on whole saliva diluted four-fold with water^{46–48}. Although we cannot find any published evidence for oral bacteria synthesizing lysine *de novo*, they do synthesize arginine *de novo* in dental biofilms⁴⁹. The low lysine content of biofilm from most of our healthy subjects confirms their lack of proteolytic (successor) microbiota after a week of oral hygiene restriction⁸ and also a lack of *de novo* lysine-synthesizing bacteria. On the other hand, the high lysine content of biofilm in a few subjects, perhaps those who had the least efficient oral hygiene prior to entering the study, suggests faster re-colonization by the successor microbiota⁸ or perhaps *de novo* lysine synthesis.

It was previously suggested that lysine decarboxylase reduces the amount of lysine available to maintain the sulcular epithelial attachment. Dentally attached cells that are lysine depleted may release proinflammatory cytokines. Subepithelial vasodilation and permeability¹⁹ increase, causing the appearance and exudation of GCF. According to this proposal, low biofilm lysine content should associate with greater GCF exudation, but this was not confirmed in our study. GCF exudation was low when biofilm lysine was low and increased collinearly with plaque index relative to GCF as long as the biofilm lysine content remained below the minimal amount in plasma ($<0.11 \mu\text{mol/g}$).

At biofilm lysine concentrations only slightly less than this minimal amount, GCF increased along with the lysine content and plaque index despite the less-starved epithelial attachment. This suggests that lysine starvation likely altered epithelial permeability to biofilm agents and induced GCF in proportion to the biofilm accumulated (i.e. plaque index). Conversely, as lysine contents increase above the minimal plasma content, DAT cells are no longer lysine starved and biofilm molecules are sealed from the gingival interior. Consequently, GCF exudation decreased despite a still greater biofilm lysine content and plaque index as illustrated in Fig. 4.

Three weeks of oral hygiene restriction results in greater GCF exudation than in this study³⁶, and an associated, greater diversity of successor microbial species in saliva⁵⁰. We speculate that prior to and during the development of the successor microbiota, the integrity of the epithelial attachment is sensitive to whether the biofilm lysine content is greater or less than the minimal lysine content of blood plasma. In individuals whose dental biofilm lysine content exceeds minimal blood plasma levels, the epithelial barrier remains intact and inhibits proinflammatory stimulation; less GCF is present after oral hygiene restriction and development of the successor microbiota and gingivitis is retarded. In individuals whose dental biofilm lysine content remains below minimal blood plasma levels, the epithelial barrier is impaired. Greater proinflammatory stimulation and GCF exudation despite little biofilm may hasten the development of the successor microbiota and gingivitis.

Some individuals develop large amounts of commensal biofilm without a corresponding increase in inflammatory response. This well-documented variation in individual susceptibility to gingivitis³⁶ might be related to differences in biofilm lysine content prior to successor microbial colonization. Thus, measurements of biofilm lysine content may separate individuals who are resistant to gingival inflammation from those who are susceptible.

Other consequences of greater lysine decarboxylase activity stem from cadaverine. It enhances oral halitosis and bacterial growth (see Introduction). Cadaverine is not present in healthy human blood, but it is present in GCF³⁴. In our study, the cadaverine content of dental biofilm correlated positively not only between admission and baseline, but also with the small cadaverine contents of saliva and tongue biofilm at baseline. These results suggest that that dental biofilm is a major site of cadaverine production in the oral cavity and that it diffuses from there to saliva and tongue biofilm. Inhibiting lysine decarboxylase during oral hygiene restriction may maintain biofilm lysine at blood plasma levels, keep the epithelial attachment sealed from bacterial agents that stimulate GCF exudation and disease, and reduce cadaverine-mediated enhancement of bacterial growth.

CONCLUSIONS

We showed that dental biofilm is a major site of cadaverine production in the oral cavity due to more substrate (lysine) in interstitial or gingival fluid at the sulcus, and that the product, cadaverine, likely diffuses from biofilm into saliva and tongue biofilm. After a week of oral

hygiene restriction, lysine decarboxylase activity increases in all subjects. Biofilm lysine contents below the minimum in blood plasma associate positively with biofilm accumulation and GCF exudation, whereas greater lysine contents associate with still greater amounts of biofilm but markedly less GCF exudation. Biofilm lysine content thus appears to control both biofilm development and access of bacterial agents to subgingival tissues. Measurements of biofilm lysine content may identify individuals resistant to gingival inflammation, and lysine decarboxylase inhibitors may retard gingivitis development.

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Figure 1. Proposed role of lysine decarboxylase in generating biofilm access to the gingiva²⁵

(a) Reaction mediated by lysine decarboxylase.

(b) Healthy gingival sulcus (biofilm repeatedly removed by oral hygiene). Interstitial fluid (ISF) containing lysine keeps the dentally attached cells of the junctional epithelium (DAT cells of JE) dividing and attached to enamel at the base of a gingival sulcus.

(c) Inflamed gingival sulcus (biofilm not removed by oral hygiene). Lysine decarboxylase depletes lysine (Lys) and the coronal layer of dentally attached cells is missing, especially beneath the biofilm, impairing the dental attachment. Cadaverine (Cad) is increased and helps protect biofilm from activated leukocytes within a biofilm-induced inflammatory exudate (gingival crevicular fluid, GCF) which replaces ISF. The proteins and other nutrients in GCF promote growth of the successor microbiota. Protein metabolism by this microbiota produces short chain fatty acids that keep the attachment impaired and enhances inflammation.

Figure 2. Protocol for partial human experimental gingivitis study. See Methods for details

Figure 3. Relationships of increase in biofilm cadaverine fraction (CF) to baseline CF and biofilm lysine content after oral hygiene restriction

(a) Relationship of CF increase to baseline CF. Spearman $r^2 = 0.43$; $p < 0.02$.

(b) Relationship of CF increase to biofilm lysine content after oral hygiene restriction. Spearman $r^2 = 0.38$; $p < 0.001$.

Multiple least squares regression indicated that the relationships of CF increase to both baseline CF (Fig. 1a) and biofilm lysine content (Fig. 1b) were independent and significant (Total $R^2 = 0.61$, d.f. = 2,13, ANOVA $F = 12.5$, $p < 0.001$. Partial R^2 (baseline CF) = 0.48, $p < 0.02$; partial R^2 (post-oral hygiene restriction lysine content) = 0.13, $p < 0.04$ (r^2 and R^2 are defined in the Methods).

Figure 4. Relationships of plaque index and GCF exudation rate to biofilm lysine content
 (a) Plaque index correlated with biofilm lysine content after oral hygiene restriction. Spearman $r^2 = 0.58$, $p < 0.001$. Graph shows the result of a least squares regression (see Methods) whose equation was: $PI = 1.48 + 3.75 \cdot Lys$; $R^2 = 0.61$, d.f. = 1,14, ANOVA $F = 24.1$, $p < 0.001$. $R^2 = 0.61$, $p < 0.001$. If the three high lysine values (values exceeding $0.11 \mu\text{g/mL}$) are removed, the association remains significant (Spearman $r^2 = 0.33$, $p = 0.042$).
 (b) GCF exudation rate regressed on biofilm lysine content after oral hygiene restriction. Vertical arrow indicates the minimal lysine concentration of normal blood plasma ($0.11 \mu\text{g/mL}$; see Results). A quadratic relationship was significant: least squares regression $R^2 = 0.37$, d.f. = 2,13, ANOVA $F = 5.4$, $p < 0.02$. $GCF = -18.5 \cdot Lys^2 + 4.5 \cdot Lys + 0.05$; $R^2 = 0.37$, d.f. = 2,13, Coefficients: Lys^2 't' statistic = -3.09 , $p < 0.01$; Lys 't' statistic = 3.27 , $p < 0.01$; constant 't' statistic = 1.01 , not significant.

Table 1

Mean cadaverine and lysine contents of saliva, and tongue and dental biofilms

Measure	Source ^a	Time ^b	Mn $\mu\text{mol/g} \pm \text{sd}^c$	% Mn ^d	CF ^e
Cadaverine	Saliva	Baseline	$\bar{x}_{0.008} \pm 0.008$	3.9	0.365 ± 0.137
Lysine	Saliva	Baseline	$\bar{x}_{0.013} \pm 0.008$	7.7	
Cadaverine	Tongue	Baseline	$\bar{x}_{0.012} \pm 0.014$	5.8	0.304 ± 0.189
Lysine	Tongue	Baseline	$\bar{x}_{0.028} \pm 0.020$	16.6	
Cadaverine	Dental	Baseline	$\bar{x}_{0.207} \pm 0.131$	100.0	0.519 ± 0.142
Lysine	Dental	Baseline	$\bar{x}_{0.169} \pm 0.053$	100.0	
Cadaverine	Dental	After	$\bar{x}_{0.338} \pm 0.248$	163.3	0.815 ± 0.114
Lysine	Dental	After	$\bar{x}_{0.071} \pm 0.067$	42.0	

^aSource: Tongue = Tongue biofilm, Dental = dental biofilm.

^bTime: Baseline (before oral hygiene restriction of upper quadrant for 7 days) and afterwards (After).

^cMn $\mu\text{mol/g} \pm \text{sd}$: Mean μmol cadaverine or lysine normalized per g wet weight of plaque and the standard deviation (sd).

^d%Mean: Percentage of the mean $\mu\text{mol/g}$ in dental biofilm at baseline.

^eCF: Cadaverine fraction: cadaverine content expressed as its ratio to 'total' lysine (see Introduction).

^fFriedman's statistic indicated saliva, tongue and dental biofilms means differed significantly ($p < 0.01$). Dunn's test indicated that the respective mean cadaverine, lysine, 'total' lysine, and cadaverine fraction in saliva and tongue biofilm were similar, but significantly lower than in dental biofilm ($p < 0.01$). Protocol permitted comparisons with saliva or tongue biofilm only before oral hygiene restriction.

^gWilcoxon matched-pairs signed-ranks test: 2-tailed p value significant after oral hygiene restriction compared with baseline.