A pilot study of the gingival response when smokers switch from smoking to vaping

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In brief

Provides an update of the latest scientific background and regulations concerning e-cigarettes/vaping.	Provides the reader with the results of the first trial considering the effects of vaping on gingival health.	Suggests future work to encourage further research in this field.

Introduction Tobacco smoking is one of the most important risk factors for periodontitis as it alters the host response to plaque. Although the prevalence of tobacco smoking has declined in recent years, the use of electronic-cigarettes (vaping) has increased. The effect of vaping on the gingiva is unknown and an evidence-base needs to be established before providing dental advice about the use of these products. **Objective** To compare the gingival health of a group of established smokers before and after substituting vaping for smoking tobacco. **Design** Pilot. **Setting** Guy's Dental Hospital (England) from April–December 2015. **Materials and methods** Twenty established smokers (all staff members at Guy's Hospital) with mild periodontal disease replaced their regular smoking habits with the use of e-cigarettes for two weeks. **Main outcome measure** The primary outcome measure of gingival inflammation was bleeding on probing. Levels of selected pro-inflammatory cytokines in GCF, saliva and serum samples were also determined. **Results and conclusions** There was a statistically significant increase in gingival inflammation when tobacco smokers switched from smoking to vaping for two weeks. However, this result must be interpreted with extreme caution since this is only a pilot study. Nonetheless, this study should provide a stepping stone to encourage further investigation of the effects of vaping on periodontal health.

Background and objectives

Periodontal diseases are multi-factorial, resulting from the interplay between microbial plaque and the host, driving an increased host immune-inflammatory response.[1] This leads to alterations in the local inflammatory cytokine profile causing clinical inflammation in the gingiva characterised by increased bleeding on probing (BOP) and increased gingival crevicular fluid (GCF) flow. Local pro-inflammatory cytokines are largely responsible for changes which occur. These mediators, which may be found in GCF, saliva and serum, might therefore serve as potential diagnostic or prognostic markers for the progression of periodontitis.[2] For example, site-specific increases of IL-1β have been observed in both gingivitis and untreated periodontitis,[3,4] while treatment of periodontitis results in a dramatic decrease in GCF levels.[5,6] IL-6 has also been implicated in periodontitis as it activates osteoclast formation, facilitates bone resorption and T-cell differentiation.[7,8] IL-8 is involved in the selective recruitment and activation of neutrophils,[9,10] but alterations of its levels during the pathogenesis of periodontal disease have been equivocal.[11–13]

One of the most significant independent risk factors for periodontitis that can affect the host immune-inflammatory response is tobacco smoking.[14–18] Although more susceptible to destructive disease, smokers display less overt gingival inflammation[19] and gingival bleeding[20,21] than non-smokers because of the perturbed inflammatory response. In addition, gingival blood flow, BOP and GCF flow increase as early as three to five days following smoking cessation.[22,23]

Smoking has also been shown to impair inflammatory cytokine production.[24] Although there are numerous studies investigating the effects of smoking on IL-1β in saliva or GCF, the findings are conflicting with several suggesting elevated levels,[25–28] some implying decreased levels[29,30] and others finding no difference.[31,32] The limited studies on IL-6 find a decrease or no significant difference in GCF IL-6 levels of smokers compared to non-smokers with periodontitis.[33,34] Studies on the effects of smoking on IL-8 in local fluids appear to vary depending on the type of periodontitis.[34–36] Studies in periodontal patients on the effects of smoking on systemically circulating inflammatory cytokines are limited and the findings equivocal.[37,38]

In recent years, electronic (e)-cigarettes have been gaining popularity with over two million Britons now regularly vaping.[39] E-cigarettes provide nicotine for inhalation in a vapour generated by heating a solution containing water, nicotine, propylene glycol and vegetable glycerine. Since e-cigarettes became available in the UK in 2007, their safety

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and use as a substitute for tobacco smoking have been surrounded by medical and public controversy. However, a recent report by the Royal College of Physicians concluded that e-cigarettes are likely to be much safer than smoking.[40] A change in the law proposed in May 2016 also means that e-cigarettes containing up to 20 mg/ml of nicotine are likely to be regulated by the Tobacco Products Directive as consumer products and those containing over 20 mg/ml may require a medicinal licence.[41]

The effect of e-cigarette use on the gingival condition and inflammatory biomarkers has not yet been investigated. A pilot study would be the first necessary step in exploring this, before robust large-scale studies. An evidence-base needs to be established before we can provide any dental advice to our patients about their use, and a potential change in the law warrants investigation as a measure of good practice. This pilot study aimed to compare the gingival health of a group of established smokers before and after substituting their regular smoking habits with vaping for two weeks. The null hypothesis was that there would be no change in their gingival health.

Materials and methods

Study population

There are no published studies investigating the effects of vaping on periodontal clinical parameters. However, the effect of quitting smoking on gingival health is known and believed to be of clinical significance. Hence in the absence of current data about vaping, we used the change in gingival health which occurs when smokers quit as the basis for a prospective power calculation to estimate the number of subjects that would be needed to detect similar gingival changes should they occur when smokers switch to vaping. Previously, a group of smokers were found to have a mean 15.7% (± 7.7%) of sites bleeding after probing and this increased to 31.9% (± 8.7%) when they quit smoking.[23] If the smokers enrolled in this study had similar levels of inflammation, 13 subjects would be needed to detect a change in bleeding, half the size detected after quitting, at an α-level of 0.05 with 90% power. Since the cohort of enrolled smokers did not intend to quit smoking, but were prepared to attempt to substitute smoking with the use of e-cigarettes for two weeks, a high drop-out rate was anticipated and a sample size of 20 was proposed.

Subjects were 18–65 years old, systemically healthy, smoked at least ten cigarettes per day for five years, had at least 24 natural teeth (excluding third molars) and had no probing pocket depths over 4 mm at any site. Only smokers who did not wish to quit were enrolled because vaping is not yet a proven nicotine replacement therapy to assist quitting. It was also thought inappropriate to invite non-smokers to start using an addictive product like nicotine. Participants were excluded if they had a systemic condition known to exacerbate or modulate periodontitis (for example, diabetes), antibiotics had been taken in the previous three months, anti-inflammatory drugs or other medication likely to affect the periodontal tissues were taken routinely or if they were pregnant or a nursing mother. All participants gave written informed consent and the London Bridge Research Ethics Committee of the National Research Ethics Service approved the study.

Study design

Since this study was the first of its kind and the effects of vaping on the gingival condition were completely unknown, this was a pilot longitudinal study that assessed the same participants before and after substituting vaping for smoking. This design helped avoid confounding factors which occur when different groups of participants are compared with each other. Twenty out of the 22 potential subjects who were screened were eligible and agreed to participate. Clinical measurements and sampling were conducted at baseline and at a second visit two weeks later. Both appointments were at similar times of the day for each subject to account for diurnal variations.

Clinical measurements

Clinical measurements were completed for all teeth excluding third molars. A six point probing chart was recorded which included probing pocket depths and BOP. A dichotomous BOP score was considered to be the most objective method of assessing gingival inflammation. A dichotomous plaque score was also recorded indicating sites with plaque clearly visible without disclosing,[42] since changes in plaque levels could change the gingival condition.

Saliva collection

Whole mouth saliva was obtained by requesting the participant to spit into a sterile 20 ml collection tube until approximately 5 ml of saliva had been collected. The saliva samples were immediately centrifuged at 3,000 rpm for ten minutes and stored at -80 °C.

GCF collection

GCF was collected from the mesiobuccal sites of 12 mandibular teeth using Periopaper® strips (Oraflow Inc) and the volume was determined using a pre-calibrated Periotron 8000® (Oraflow Inc).[43] The 12 strips were pooled to generate a single sample at each visit. The GCF Periopaper strips were eluted by adding 250 µl phosphate buffered saline to the collected strips for a single visit. The suspension was then mixed by vortex for one minute and centrifuged at 10,000 rpm for 30 seconds at 6°C. The strips were thereafter removed from the tube to prevent the proteins from re-depositing. The eluted GCF sample were stored at -80°C for later analysis.

Serum collection

Two millilitres of peripheral venous blood was collected in a vacuum tube from venepuncture via a 21-gauge butterfly needle inserted in the ante-cubital fossa. Blood was allowed to clot; serum was separated using centrifugation and samples stored at -80 °C until use.

E-cigarette usage

At visit one, subjects were given a blu PRO™ e-cigarette kit (Electric Tobacconist®), an extra bottle of blu PRO Tobacco™ e-Liquid (Electric Tobacconist) and written instructions. The e-Liquid was Classic Tobacco flavoured and contained 18 mg of nicotine (medium strength). The choice of this particular brand of e-cigarette was random and there was no commercial sponsorship from the company. The participants agreed to substitute their regular smoking habits with the use of e-cigarettes. They were asked to make a note of any cigarette smoking during the two weeks if complete abstinence was unsuccessful.

Cytokine determination

Cytokine levels in saliva, GCF and serum were determined using the Performance Fluorokine MAP cytokine multiplex kits (Bio-techne, UK), coupled with the Bioplex 200 machine (Bio-rad, UK) according to the manufacturer's protocol. Samples of eluted GCF and saliva were used undiluted. Serum samples were diluted four-fold as recommended by the manufacturer. The dynamic range of the assay for GCF and saliva was given as between 2.74-2000 pg/ml of IL-1β, 4.25-3,100 pg/ml of IL-8 and 5.42-3,950 pg/ml of IL-6. The dynamic range of the assay for serum ranged from 0.11-2400 pg/ml of human IL-1β, 0.10-4,950 pg/ml of human IL-6 and 0.11-2950 pg/ml of

human IL-8. Concentrations were calculated using the Bioplex Manager 6.1 software.

Statistical methods

BOP was the primary outcome measure and the clinical variable on which the prospective sample size calculation had been undertaken. Statistical tests are therefore only reported for this parameter. The percentage of sites with BOP before and after vaping was tested using a two-tailed Wilcoxon signed-rank test. Drop-outs were accounted for by using an intention-to-treat analysis. A P value of <0.05 was considered to be statistically significant. Other parameters were analysed using descriptive statistics.

Results

Study adherence

Eighteen out of the 20 recruited participants attended for the reassessment visit. Two participants could not attend the second appointment due to unexpected parental responsibility and a cough. None of the 20 participants reported any adverse effects while using the e-cigarettes. Four out of the 18 participants did not achieve complete smoking cessation, but they all reported smoking fewer than five cigarettes throughout the two-week study period.

Clinical status

Following the substitution of vaping for smoking, the percentage of sites with BOP increased statistically significantly (P <0.0008) even though the results suggested minimal change in the levels of plaque (Fig. 1a). GCF volume also appeared to increase when subjects switched to e-cigarette use and is consistent with the changes in BOP (Fig. 1b).

The changes in BOP remained statistically significant when the baseline data from the two drop-outs were carried forward using the intention-to-treat approach. Furthermore, statistically significant differences were still detectable when the same statistical test was performed following removal of the four individuals who had smoked during the study period (% BOP, P <0.002). The mean pocket depth was 2 mm (±0.43 mm) and there were negligible changes in mean pocket depths between the visits.

Cytokine production

Table 1 illustrates the levels of IL-1β and IL-8 in GCF, saliva and serum. There was a high level of variation between individuals and although there may be an increase in levels of IL-8 in GCF, no significant conclusions can be drawn from these results. Levels of IL-6 were below the limits of detection. Collection of blood was problematic in a number of subjects and samples were only available for 14 of the 18 participants who completed the study. One subject had far higher levels of both cytokines in their serum than the other subjects, particularly at visit 1.

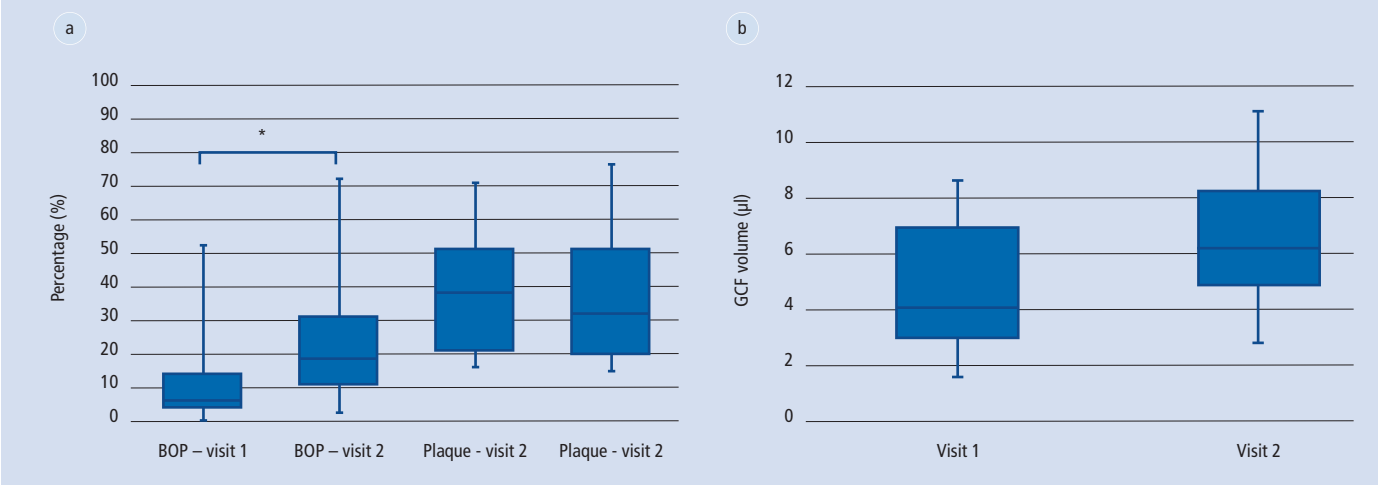
Discussion

To the best of our knowledge this is the first study to show a change in gingival health when subjects switched from tobacco smoking to vaping. We demonstrated that the percentage of sites with BOP increased significantly and in a similar direction to that which occurs when smokers quit. GCF volume, an alternative parameter which also reflects gingival inflammation appeared to increase in a comparable manner. Although we cannot draw any further conclusions beyond this, the pilot study provides an important stepping stone to further work on this relevant and important topic.

Table 1 Mean levels of IL-1β and IL-8 in the GCF, saliva & serum of smokers before (visit 1) and after substituting vaping for smoking (visit 2)

	Mean (SD) IL-1β (pg/ml)		Mean (SD) IL-8 (pg/ml)	
	Visit 1	Visit 2	Visit 1	Visit 2
GCF	331.5	442.8	507.5	697.4
	(173.2)	(222.2)	(330.2)	(352.5)
Saliva	252.1	273.4	612	456.5
	(232.5)	(257.1)	(434.8)	(290.4)
Serum	13.4	0.91	62.1	6.85
	(50.3)	(0.65)	(223.4)	(2.52)

Fig. 1 a) Percentage of sites with BOP and plaque in smokers before (visit 1) and after (visit 2) substituting e-cigarettes for smoking (N = 18). Centrality indicated by the median, the box describes the interquartile range and the whiskers the maximum and minimum values. Changes in BOP tested with Wilcoxon signed rank test (*P <0.0008). b) GCF volume (µl) in smokers before (visit 1) and 2 weeks after (visit 2) substituting e-cigarettes for smoking (N = 18)



Changes in the gingival condition are of relevance in susceptible individuals as persistent gingivitis may lead to periodontitis and smokers are at higher risk of periodontitis. The effect of vaping is particularly relevant in existing smokers as e-cigarette use in Britain is almost entirely restricted to this cohort of the population.[40] A criticism of this study design is that our results may simply reflect the effect of quitting smoking, rather than an effect of vaping. However, as nicotine is known to be addictive, we thought it was inappropriate to invite subjects to start using nicotine who were not already users. It is also useful to know that 14 of the 18 smokers who completed the study reported no use of tobacco during the two weeks of vaping.

As this is a pilot study, there were no control groups of smokers who continued to smoke or who quit during the study and the participants served as their own control to reduce confounding factors. A cross over study design had been considered in which participants were randomly assigned to switch to vaping or to continue smoking for the first two weeks followed by a washout period and then a second experimental fortnight when subjects vaped if they had not vaped during the first experimental period. This might have increased the chance that the examiners were blind to whether the subject was smoking or vaping. However, since most smokers smell of cigarette smoke, complete blinding of examiners is unlikely even with a randomly assigned cross-over design. The extended study period and need for subjects to attend more appointments would have increased the chances of more drop-outs. To help mitigate the possible biases, a dichotomous bleeding score was used as a reasonably objective measure of gingival inflammation. GCF volume was used as an additional objective indicator of gingival inflammation.

At the start of the study, vaping was not considered a method of nicotine replacement to assist smoking cessation and we therefore only enrolled smokers who did not intend to quit. We expected a high drop-out rate and it was encouraging that 14 of the 18 subjects who completed the study reported complete abstinence from smoking. This is consistent with the recent claims made by the Royal College of Physicians that vaping may be a successful tool in helping to achieve smoking cessation.[40]

Studies have shown that cigarette smoking appears to have a long-term chronic effect on the periodontal tissues, leading to less BOP with impairment of the gingival vasculature[44] and to lower volumes of GCF in smokers, suggesting a suppression of the normal inflammatory response to plaque.[45–47] These findings are consistent with a diminished peripheral blood flow leading to a reduction in GCF flow.[22] The results of the current study show that when substituting cigarette smoking with vaping for two weeks, there is a significant increase in BOP and an increase in GCF volume, even when the levels of plaque were similar between visits. It was important to consider the plaque levels as any significant increase in plaque is likely to result in an increase in both BOP and GCF volume. The clinical findings from the current study are similar to those that occur following verified smoking cessation. For example, during a successful period of quitting smoking, gingival bleeding doubled from 16% to 32% in a group of 27 smokers followed for 4–6 weeks, even though there were some improvements in the subjects' plaque control.[23] Results from this study are also consistent with studies that suggest a fairly rapid recovery of the inflammatory response following smoking cessation.[48] Although with the current study design we cannot differentiate the changes due to smoking cessation from the changes due to vaping, our findings warrant further investigation.

Some early studies[49] on smoking suggested that the reduction in bleeding was due to the induction of gingival vasoconstriction by the nicotine component of cigarette smoking. However, on reviewing the literature, there appears to be very little evidence for this. The results from this study also suggest that it is unlikely to be nicotine causing gingival vasoconstriction and reduction in BOP[49] since both cigarettes and e-cigarettes provide a source of nicotine. Other features of cigarette smoking, which do not occur during vaping, such as inhalation of particulates and other chemicals or heat damage, are the more likely cause of these vascular changes. For future studies, measuring cotinine levels would allow for comparison of chronic exposure to nicotine after smoking and vaping. Monitoring exhaled carbon monoxide levels during the period of vaping would provide additional confirmation that the participants were not smoking and this could be more reliable than self-reported abstinence. However, the results of the current study would have been unlikely if participants had continued their usual smoking habits.

With the increase in gingival inflammation, concurrent changes in local cytokine levels within GCF and saliva might be expected. However, due to the limited sample size and large variation no definitive conclusions could be drawn from this dataset. Gingival changes in patients with mild periodontal disease are very localised and it was therefore unsurprising that the levels of systemically circulating cytokines within serum before and after switching to vaping appeared remarkably similar. However, the dataset is limited with high variability so no conclusions can be drawn from this. Therefore, a larger sample size is required to elucidate what the underlying biological changes are that are driving the clinical change. The range of levels of cytokines and scale of change detected in this pilot study will assist the calculation of an appropriate sample size for future studies of these materials. For future studies, it would be interesting to include other key pro-inflammatory cytokines such as the macrophage migration inhibitory factor.

Although there appeared to be negligible change in the percentage of sites with plaque, changes in the current study may be a function of the constituents of the local microbiota, where changes in the microbiota have been demonstrated in different periodontal disease states[50] and in smokers.[51] Further studies could include collection of plaque samples and consider how the microbiota changes when smokers substitute their regular smoking habits with vaping.

Supplemental *ex vivo* experiments would be useful to allow the effects of vaping to be studied in a more controlled environment. Gingival epithelial cells in either monolayer submerged cultures or organotypic models (resembling the gingiva) could be exposed to cigarette smoke extract or e-cigarette liquid extract and the levels of inflammatory cytokines that are produced compared. To determine the impact of the microbiota on these responses, levels of cytokines produced by resting cells could also be compared to the response when exposed to a bacterial stimulus (induced state). Similar lab-based studies are emerging in the medical literature and include the effects of e-cigarettes on the levels of pro-inflammatory cytokines from human lung epithelial cells and Kupffer cells.[52,53] Interestingly, in the study by Lerner *et al.*,[53] levels of IL-8 from lung epithelial cells were higher when exposed to e-cigarettes in comparison to the air control.

In conclusion, this study found that substitution of smoking with vaping was associated with a statistically significant increase in gingival bleeding on probing, but these results

are preliminary and must be interpreted with extreme caution since this is only a pilot study. However, this finding merits further investigation and the study provides data about the gingival condition and local cytokine levels on which to base future sample size calculations.

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