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Research Article

The Effect of E-cigarette on Periodontal Health: Microbiology Aspect, Immunology Aspect and Cell's Consequences

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Abstract

Introduction: The E-cigarette has enjoyed a great popularity but no standard is yet known for the liquid's composition and combustion system. Actually, the effect of conventional tobacco on oral health is well studied; however, the effect of E-cigarette on oral health is not studied enough

The aim of this literature review is to determine the effect of E-cigarette on periodontal.

Material and methods: 23 articles are selected on PubMed, Cochrane and Google scholar data base. This literature review use *in-vitro* and *vivo* study with globally the same protocol of smoke exposition. Article older than ten years are excluded.

Result: The E-cigarette is able to decrease the bacterial diversity and realize a selection pressure favorable for perio-agressive bacteria's. In addition, the E-cigarette is able to increase the invasion of perio-agressive bacteria's.

In another way, the E-cigarette is able to place the periodontal in a pro-inflammatory condition with different way activation.

The E-cigarette generates a decrease of fibroblast activity, growth and mobility. The same result is finding on bone cells and epithelial cells.

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Conclusion: Even if the E-liquid is exempt of nicotine, it is no harmless on periodontal cells. However the negative impact of nicotine's E-cig. Is less bad than nicotine E-cig. Globally, the negative impact is less than conventional cigarette, but some effect are very specific to E-cig., like bacterium selection pressure.

Keywords

Electronic Cigarettes; Health Professionals; Periodontal Cells; Nicotine

Introduction

The electronic cigarette has enjoyed a great popularity in recent years in our societies. For the general population, and more particularly among the young population, it is accepted as an alternative to tobacco, and often proposed for smoking cessation [1].

It consists of an electronic combustion device which receives a vaporizable liquid. The vapors are directed towards the oral cavity thus ensuring a phenomenon of “vaping”.

No standard is yet known for the composition of the combustion liquid [2]. Depending on the brand, the presence or absence of nicotine is mentioned with a concentration varying from 0 to 18 mg/ml. A correspondence, variable according to the brands, is given by the cigarette manufacturers to help the conventional smoker to choose his nicotine concentration according to the number of cigarettes smoked per day.

The effect of nicotine and conventional tobacco on the periodontium is well studied today.

However, the specificity of electronic cigarettes on the periodontium does not yet have sufficient studies or sufficient clinical experience in health professionals.

Even though it may be nicotine-free (thus suggesting that it is harmless), it has a multitude of constituents specific to its operation (not present in conventional cigarettes):

- Toxic metals: aluminum, chromium, nickel, etc.
- Carbon compound: glycerol, glycerin, propylene glycol
- Silicate pearl, pewter pearl [3]
- Aromas

The large variation in composition of the liquid between manufacturers, associated with variations in vapor quality, depending on combustion operation and the environment, makes it difficult to carry out comparative studies [4].

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In this literature review, we propose to study in a first part, the effects of electronic cigarettes on the periodontal microbiota. Then, in the second part, we will discuss the consequences of electronic cigarettes on the periodontal inflammatory response.

Then, the third part will be focus on the effects of electronic cigarettes on periodontal ligament. The fourth and fifth parts will deal respectively with the effects of electronic cigarettes on bone and epithelial tissue.

Material and Methods

The articles are taken from the PubMed, Cochrane and Google-scholar databases. The search used the following keywords

- Electronic cigarette
- Oral health
- Oral infection
- Periodontitis
- Oral microbiome
- Human periodontal cell
- Fibroblast
- Osteoblast
- Epithelial cell

Are excluded the Articles dealing with:

- Liquid burn / explosion / ingestion-inhalation accident
- Carcinology disease
- Articles older than 10 years except historic studies

Are included the articles with *in-vivo* protocol and *in-vitro* studies articles with similarity in smoke exposure protocol.

A total of 23 references were used for writing this review.

Results 1: Effect on oral microbiota Bacterial diversity and ecological pressure

In 2020, Ganesan's team published *in-vivo* research on the effect of electronic cigarettes on the human microbiota, in comparison with the non-smoker or conventional smoker [5].

She selected 123 patients without history of periodontal disease: less than 3 sites greater than 4 mm pocket depth, bleeding less than 20% on probing, loss of attachment less than 1 mm.

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She divides them into 5 groups: conventional smoker, non-smoker, electronic cigarette from 6 to 18 mg/ml of nicotine, regular or occasional double consumer (conventional + electronic); in which she collects subgingival plaque and gingival fluid from 15 sites located in the anterior maxilla and mandible.

The microbiota is analyzed by DNA sequencing: 37 million sequences have appeared, identifying 9730 bacterial species (by classification of the seed subsystem [6]. In order to facilitate the study, it eliminates any genome less than 0.01% of abundance, so in the end 7035 selected species (Fig. 1).

A comparison is given concerning the expression of genes, the diversity of bacterial taxonomic groups and bacterial profiles encountered.

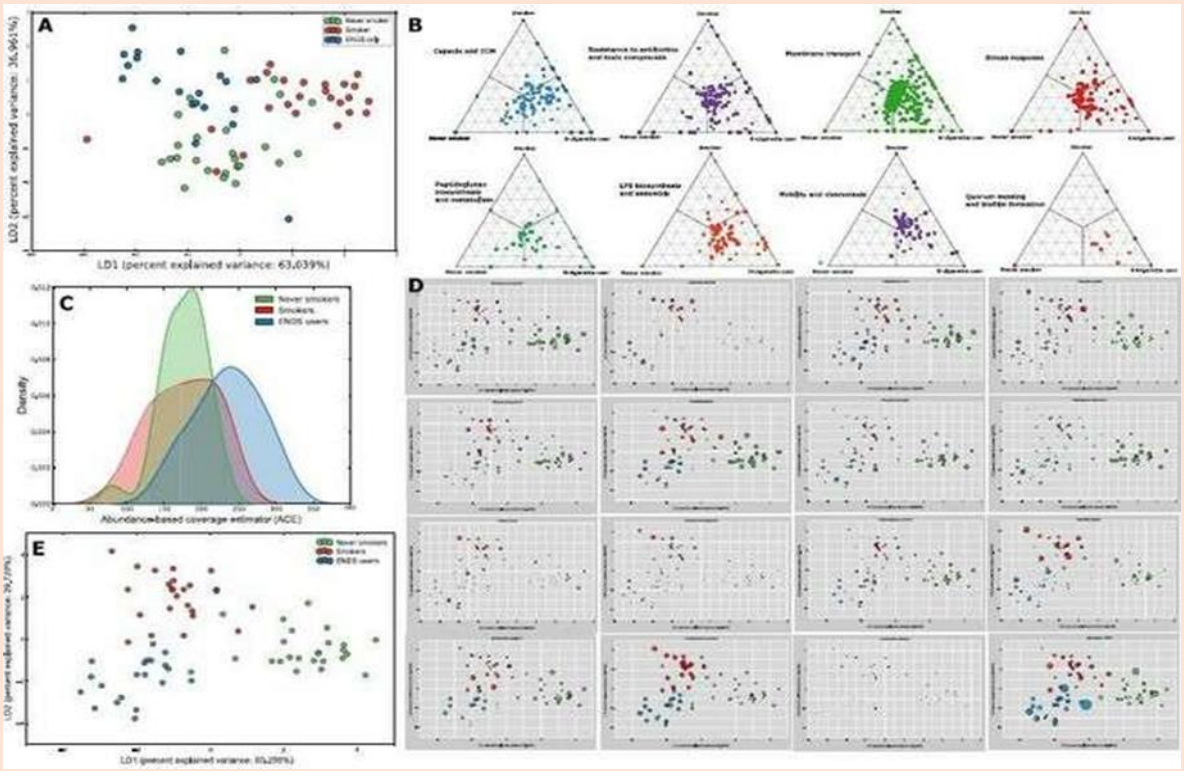

Figure 1: Structural and functional differences between the bacteria's communities regarding the different test group [5].

Concerning the E-cigarette group, the authors note a 4 to 7% increase in the expression of genes involved in the formation of the biofilm and the expression of virulence factors (synthesis of lipopolysaccharide, quorum sensing, flagella mobility, etc.) and a decrease of bacterial diversity: 59 species represent 75% of the bacterial abundance and 136 species represent 96%

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of the abundance. In addition, there is a main bacterial hub of 203 interconnected species, composed mainly of actinomyces, fusobacterium, treponeme, tanerrela and prevotella. The E-cigarette group as a whole shows remarkable homogeneity in these results, which suggests that the electronic cigarette assure a real selective pressure subgingival in favor of pathogenic periodontal bacteria, as described by Sockransky [7].

This study also indicates that the bacterial diversity is different between the conventional smoker and the electronic smoker. Analysis of bacterial diversity and its characteristics in mixed users (conventional + electronic) indicate similar results to the electronic cigarette user alone. This being an additional argument in favor of the selective pressure linked to the electronic cigarette. The authors discuss the potential implication of the combustion mechanism (Table 1).

This is also suggested by the study of Pushalkar, et al., published in 2020 [8]. This team is studying *in-vivo* the effect of electronic cigarette aerosols on the periodontal microbiome.

They collect the saliva of 119 patients, including 39 non-smokers, 40 conventional smokers and 40 electronic cigarette smokers, and then they analyze various clinical data followed by RNA sequencing.

	Control	**E-cigarette**	**Tabaco**	**Results**
BOP %	53	64,5	57,2	X
Depth Probing mm	2.7	3.3	3	X
Salivary Flow g/mn	2.4	2.9	2.2	X
Carbon monoxide ppm	1.8	18.8*	5.1*	* Significant increase compared to control
Cotinine mg/ml	11.1	535,4*	103,7*	* Significant increase compared to control

Table 1: Clinical data and saliva dosages are shown.

The level of carbon monoxide and salivary cotinine is higher in the electronic cigarette user compared to the conventional smoker and control.

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Regarding bacterial diversity, 8,254,494 RNA sequences are identified, corresponding to: 11 phylum's / 22 classes / 33 orders / 55 families / 99 genre / 162 species / 911 operational taxonomic unit OUT (Fig. 2) (Table 2).

A calculate of alpha diversity, Shannon diversity and OTU diversity is performed via a Mann Whitney test.

97% Taxon	Spirochete	Proteo bacteria	Fusobacterium	Actino bacteria	Firmicute
Control	0.2%	17.5%	7.6%	8%	45%
E cigarette	0.4%	15%	7%	10%	48%
Tabaco	0.45%	7.5%	5%	12.5%	50%
Results	Significant increase	Significant decrease	Significant decrease	Significant increase	Significant increase

Table 2: The difference between the main taxa is shown.

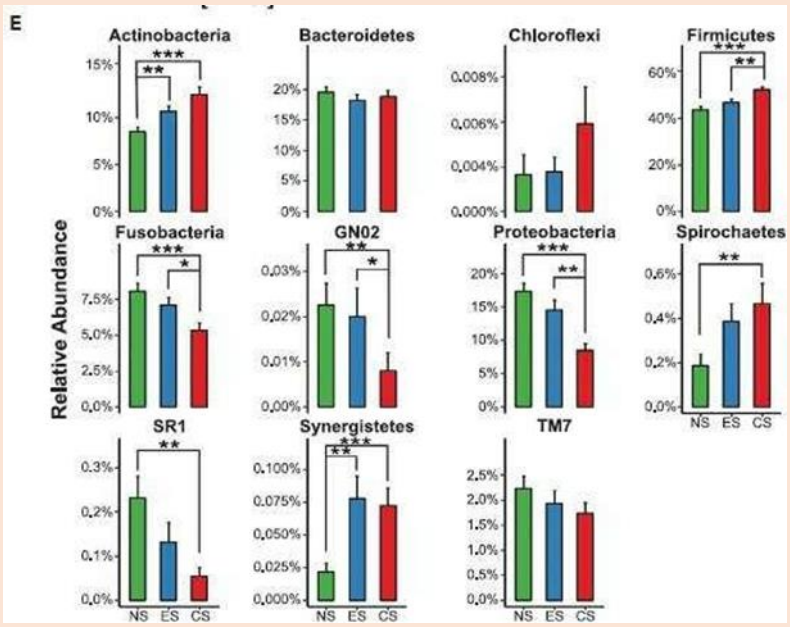

Figure 2: Relative abundance of bacterium species [8].

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As in the previous study, a relative increase in pathogenic periodontal bacteria is described. In this case, the effects are higher in the conventional smoker.

Bacterial Virulence and Cell Invasion

The previous study by Pushalkar, et al., also investigated the *in-vitro* effect of electronic cigarettes on the cell invasion capacities of pathogenic periodontal bacteria such as Porphyromonas gingivalis (Pg) and Fusobacterium nucleatum (Fn) [8].

In vitro, they produce a cell culture of Fadu cancer cells (hypopharyngeal carcinoma, subjected to 40 minutes of exposure to electronic cigarette vapor, then cultured for 2 hours in the presence of Pg or Fn [9].

Flow cytometer followed by relative quantification is performed to distinguish between healthy cells and infected cells. The results are shown below (Fig. 3) (Table 3).

Relative Percentage	Pg	Fn
Control	8	25
E cigarette	14	28
Results	Significant increase	Significant increase

Table 3: Relative percentage.

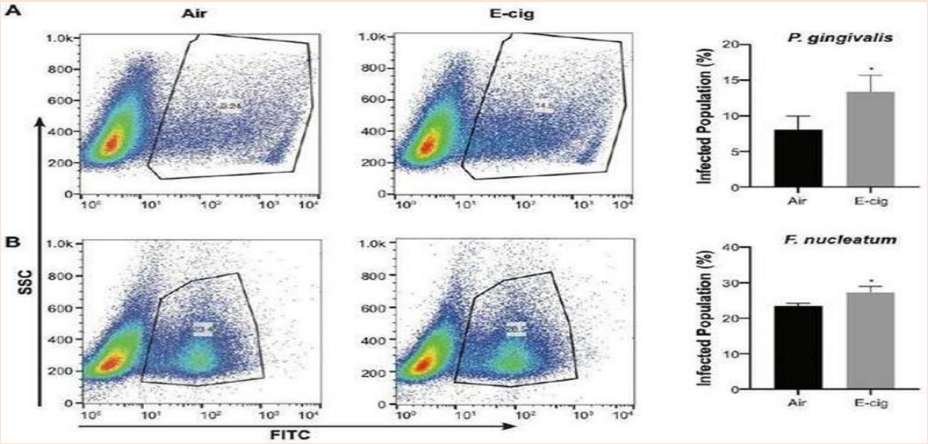

Figure 3: Flow cytometer and quantification after co-culture Fadu/Pg or Fadu/Fn exposed [8].

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Mycotic Growth

In 2019, Alanazi, et al., published their research on the effect of E-cigarettes on Candida albicans. After culture of Candida, they expose it 2 times 15 minutes per day to the vapor of the following collected cigarettes: conventional tobacco CCS, electronic cigarette "nicotine rich NR" 18 mg/ml, electronic cigarette "nicotine free NF" 0 mg/ml, air (control) [10].

The growth is analyzed over 2 days and 3 days of culture + exposure. Mtt staining is performed, followed by relative absorbance measurement by spectrophotometry. The results are shown below (Fig. 4) (Table 4).

Relative absorbance	2 days	3 days
Ctrl	0.35	0.5
NF	0.6	0.9
NR	0.8	1.1
CCS	1	1.5

Table 4: Relative absorbance.

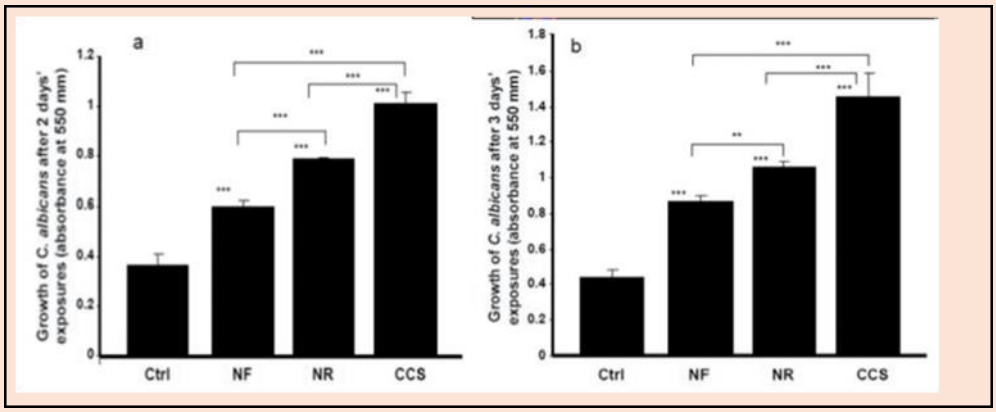

Figure 4: Candida growth regarding smoke exposition [10].

Thus we notice a significant increase in the growth of Candida on Day 2 and Day 3 for the electronic cigarette with or without nicotine and according to the following pattern: CCS> NR> NF> control.

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We understand that even without nicotine, the combustion system and the intrinsic components of the liquid have an impact on the growth of Candida.

Mycotic Virulence

The study by Alanazi, et al., continues with the analysis of virulence factors of the fungus [10]. Thus, they are exploring the impact of the E-cigarette on the production of membrane proteins including the protein Chitin. After cell culture, according to the previous protocol, they perform a white calcofluor staining, accompanied by microscopic epifluorescence observation and dosage.

The results are shown below (Fig. 5) (Table 5).

Relative Dosage	Control	NF	NR	CCS
	100	200	250	350

Table 5: Relative dosage.

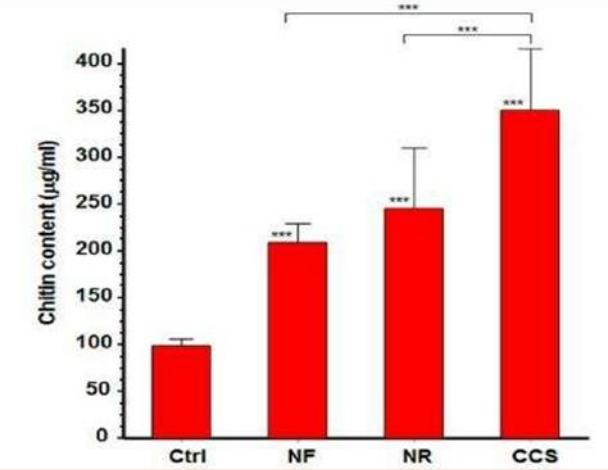

Figure 5: Chitin's production regarding smoke exposition [10].

The significant increase in chitin's production occurs according to the following pattern: CCS> NR> NF> ctrl.

Remember that the protein will produce an immune system avoidance effect.

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The team is also investigating whether exposure to vapors has an impact on the Mycotic phenotype. They are looking for a change from the commensal phenotype (blastopore) to the pathological phenotype (hyphal), in particular via the expression of Hyphae / pseudo-hyphae and their length (implicated in cell invasion)

Thus, after 15 minutes of exposure to the vapors, they analyze the relative length of the Hyphae's in NIH imaging at H3 and H6. The results are mentioned below (Fig. 6) (Table 6).

Relative Length	3 Hours	6 Hours
CTRL	1	1
NF	1	1.5
NR	1.2	1.7
CS	1.5	1.8

Table 6: Relative length.

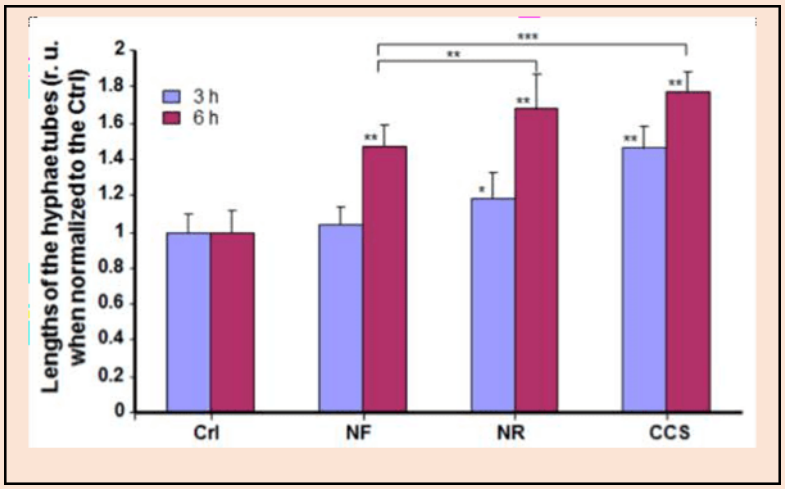

Figure 6: Hyphae's relative length regarding smoke exposition [10].

The electronic cigarette with and without nicotine, significantly promotes the growth of hyphae's and therefore cell invasion, and according to the following scheme: NF <NR <CCS.

The team then turns to the expression of the SAP genes. As a reminder, SAP 2 is involved in the change in phenotype, SAP 3 codes for the enzyme aspartase and SAP 9 codes for the

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synthesis of membrane proteins and the modulation of host immunity. Thus, the cell culture is subjected to 2 exposures 15 minutes to 6 hours apart. An Rt PCR is performed for the corresponding RNA. A relative quantification is given below (Fig. 7) (Table 7).

Relative Quantification	SAP2	SAP 3	SAP 9
CTRL	0.7	0.8	0.7
NF	1 .1	1	1
NR	1.1	1.1	1.2
CS	1.2	1.3	1.3

Table 7: Relative quantification.

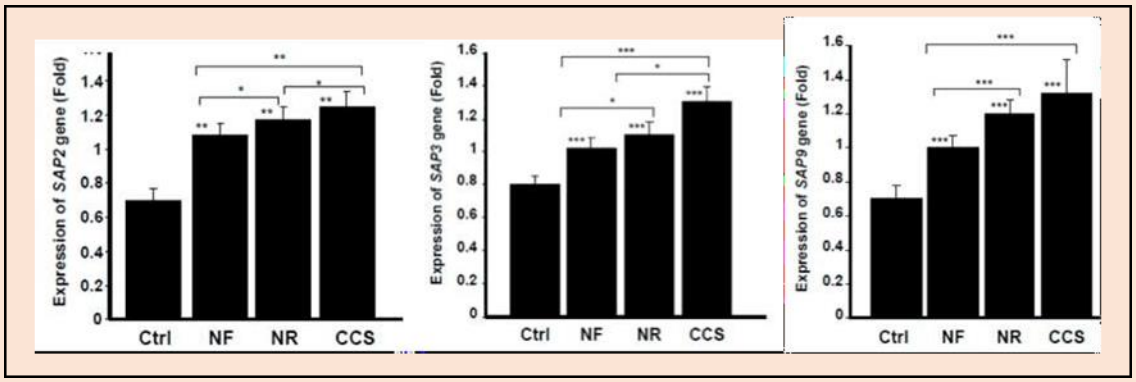

Figure 7: SAP expression regarding smoke exposition [10].

A significant increase in the expression of genes responsible for virulence factors is demonstrated, always with the following diagram: NF <NR <CCS.

Finally, the team is analyzing the ability of Candida to adhere to epithelial cells. The fungus previously exposed to the vapors is cultured with cancerous epithelial cells for 24 hours. A microscopic analysis after staining with Crystal violet is carried out at 6.00 am and midnight.

The team reveals an increase in growth and phenotypic transformation for NR and CCS, as well as an increase in cell adhesion by increase in Hyphae's according to the following pattern: NF.

<NR <CCS. Correlates with previous results (Fig. 8).

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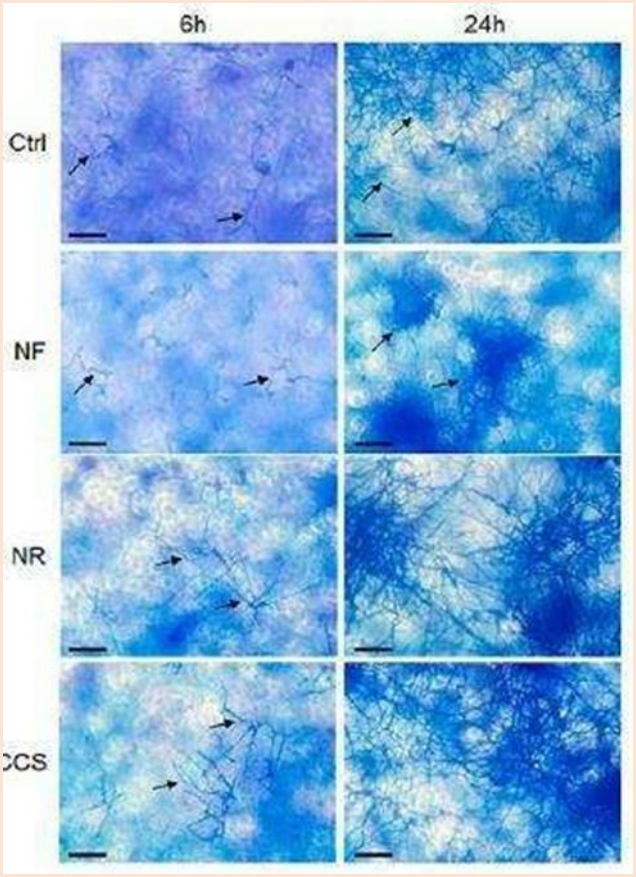

Figure 8: Candida's capacity to cell adhesion regarding smoke exposition [10].

Resume of this part

Previous studies tell us that electronic cigarettes lead to a decrease in periodontal bacterial diversity *in-vivo*, and exert selection pressure in favor of pathogenic periodontal bacteria. Some authors consider the potential role of carbon monoxide as a potential responsible for the ecological switch.

The consumption of electronic cigarettes also generates *in-vitro* an acceleration of the growth of Candida.

Some bacteria, such as Porphyromonas gingivalis and Fusobacterium nucleatum, see their virulence increased and benefit from an increased capacity for cell invasion.

Regarding Candida, the electronic cigarette is capable of transforming its phenotype (towards the pathological phenotype) and of increasing the expression of virulence factors.

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We can therefore conclude that electronic cigarettes, with or without nicotine, have a negative effect promoting the proliferation and aggressiveness of pathogenic periodontics.

Results 2: Effect on periodontal inflammatory response oxidative stress, mechanism of initiation of inflammation and DNA degradation

In 2016, Sancilio and his team, studied the mechanisms of cellular cytotoxicity linked to electronic cigarettes [11]. During wisdom tooth surgery, he performed a retro-molar gingival sample for fibroblast cell culture. These samples are exposed to different electronic cigarette vapor: Nicotine Rich NR (24 mg/ml nicotine), nicotine free NF (0 mg/ml). These vapors are themselves diluted as follows: 0.5/1/2/5 mg/ml

An analysis of the production of free radicals is carried out at 6, 24 and 48 hours. This analysis is performed by specific oxygen reagent immunofluorescence. Microscopic analysis is performed along with a relative immunofluorescence density measurement. The results are shown below (Fig. 9).

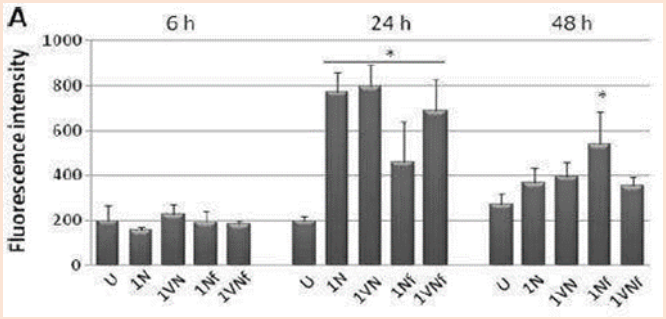

Figure 9: Relative fluorescence after exposition [11].

Here is the summary of the values at 24h (Table 8).

Control	200
NR vapor	800
NF vapor	700

Table 8: Summary of the values.

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A significant increase in the production of free radicals is observed compared to the controls. This increase takes place with or without nicotine, indicating that the intrinsic composition of the liquid generates the production of free radicals.

In 2016, Sundar, investigated the molecular pathways responsible for inflammation and free radical production after exposure to electronic cigarettes [12].

It measures the expression of the COX 2, RAGE, HDAC2, and S100A8 pathways, in human fibroblast and human epithelial cell cultures exposed to air (control), to “nicotine rich” vapors NR (16 mg/ml) and “Nicotine free” NF (0 mg/ml), in puffs of 5 seconds, every 30 seconds for 15 minutes.

The measurements are carried out via quantitative Western blot, accompanied by comet and a nova test to analyze DNA damage (Fig. 8,9) (Table 9.10).

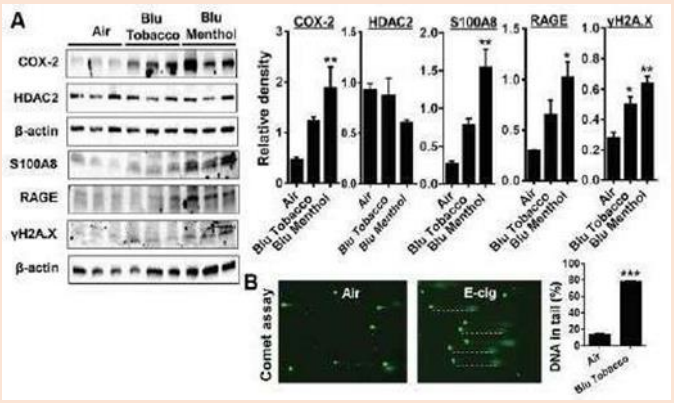

Figure 10: Molecular expression in fibroblast culture regarding the vapor exposition [12].

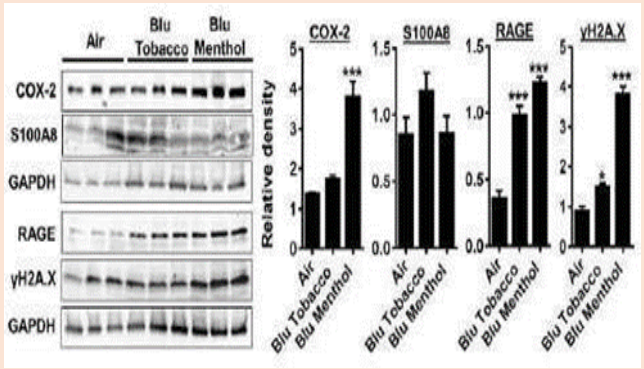

Figure 11: Molecular expression in fibroblast culture regarding the exposition [12].

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Relative Density Epithelial Cells	Cox 2	S100A8	RAGE	Gamma H2Ax
Control	1.5	0.9	0.4	1
NR	1.9	1.2	1*	1.7*
NF	3.8*	0.9	1.25*	3.8*

Table 9: Relative density epithelial cells.

Relative Density Fibroblast	Cox 2	HDAC2	S100A8	RAGE	Gamma H2AX
Control	0.5	0.9	0.25	0.3	0.3
NR	1.2	0.85	0.75	0.6	0.5*
NF	1.8*	0.6	1.5*	1*	0.6*

Table 10: Relative density fibroblast.

Thus, the expression of cox 2, RAGE and H2AX is significantly increased by epithelial and fibroblast cells in the presence of NR and / or NF. Likewise, we understand that the aromas and intrinsic components cause an inflammatory reaction and the production of free radicals, even in the absence of nicotine.

Remember that cox 2 (cyclooxido reductase) is responsible for the inflammatory activity in the event of overexpression by synthesis of prostaglandin E2. Receptors for Advanced Glycation Products (RAGEs) are a functional element of inflammation known in periodontal disease and autoimmune diseases, by pro-inflammatory and pro-oxidant effects. Finally, the intracellular protein A8 fixed to calcium S100 plays a role of mediator of intracellular inflammation.

The enzyme HDAC2 histone deacetylase is involved in cell degeneration by DNA damage [13]. Little is known about the activation mechanisms. Likewise, the H2AX gamma marker indicates DNA damage in localization of the H2AX gene, intervening in the stability of DNA [14,15].

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Cytokine Production

In his study on the impact of electronic cigarettes on the human microbiota, Ganesan explores the impact of the latter on cytokine synthesis [5]. This measurement is made *in-vivo* from subgingival dental plaques and gingival fluids. The results are shown below.

Remember that in this study, 123 patients are classified into 5 groups: strict conventional smoker, non-smoker, and strict E-cigarette smoker, regular or occasional mixed user. For the comparison of cytokine synthesis, only the first three groups are compared (Fig. 12).

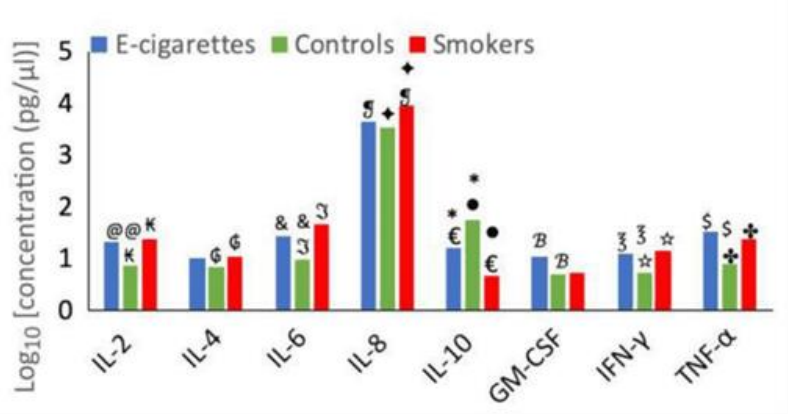

Figure 12: Cytokine production regarding the exposition [5].

Concentration n pg./micro log10	IL 2	IL 4	IL 6	IL 8	IL 10	GMCSF	IFN	TNF
E-cigarette	1.5	1.3	1.5	3.6	1.5	1	1	1.5
Control	1	1	1	3.5	1.9	0.5	0.8	1
Tobacco	1.5	1.3	1.6	4	0.5	0.8	1.1	1.5

Table 11: Concentration of E-cigarette.

Thus, there is a significant increase in the amount of pro-inflammatory cytokines and a decrease in the amount of anti-inflammatory cytokines (IL10). The significant variation in the IL8 level suggests an alteration in chemotaxis.

In 2018, Mokeem studied the periodontal impact of different tobacco consumption (cigarettes, E-cigarettes, and hookah) [16]. In 147 patients classified into 4 groups (non-smoker, cigarette,

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e-cigarette, hookah) it passively collects the salivary flow for 5 minutes. The concentration of interleukin 6 and interleukin 1beta is mentioned below (Fig. 13) (Table 12).

Concentration ng./ml	IL6	IL1 b
Tobacco	100	120
E-cigarette	18	20
hookah	100	120
Control	15	10

Table 12: Concentration different tobacco consumption (cigarettes, E-cigarettes, and hookah).

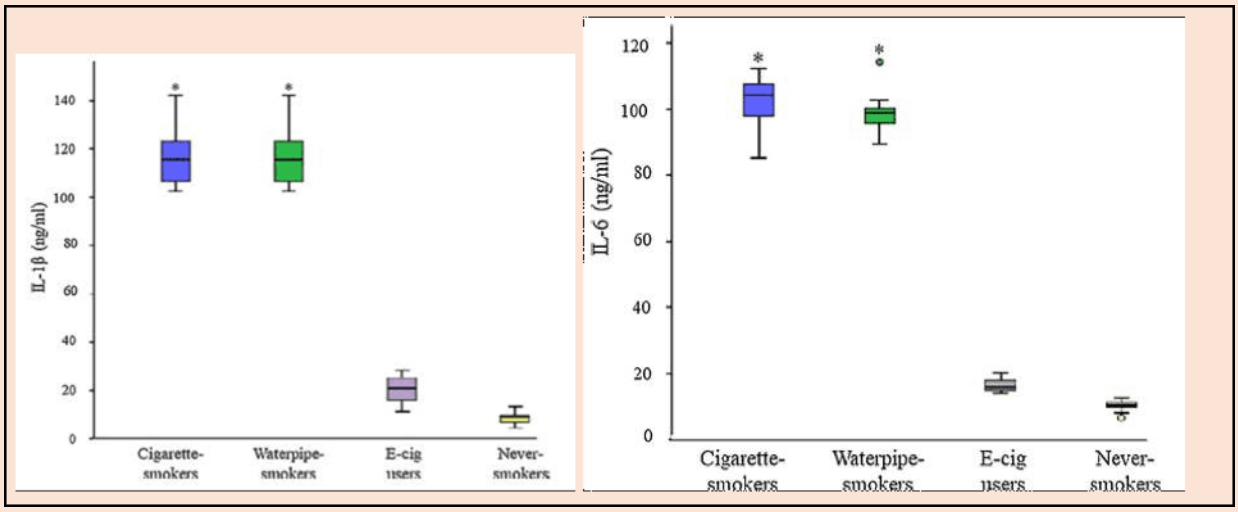

Figure 13: IL6 and IL1b concentrations regarding exposition [16].

In this study, no significance for the increase in IL1b and IL6 concentration in e-cigarette consumers, unlike consumers of hookah and conventional tobacco. But an increasing trend is recorded. Let us recall the implication of IL1b in chronic inflammatory pathologies such as periodontal disease or atherosclerosis [17]. This isomer is associated with the hyperinflammatory monocytes phenotype.

The study by Sundar in 2016 on the metabolic pathways of inflammation and the production of free radicals made it possible *in-vitro* to measure the production of interleukin 8 and

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prostaglandin E2 from the supernatant of fibroblast cell cultures [12]. Human and human epithelial cells exposed to vapors from electronic cigarettes with (NR 16 mg/ml) and without nicotine (NF), 2x5seconds per minute for 15 minutes. Here are the results (Fig. 14) (Table 13,14).

Concentration pg./ml epithelial	IL8	PGE2
Control	550	600
NR	1100*	1000*
NF	1000*	900*

Table 13: Concentration pg/ml epithelial.

Pg /ml Fibroblast	IL8	PGE2
Control	250	500
NR	400*	1000*
NF	400*	1500*

Table 14: Pg /ml fibroblast.

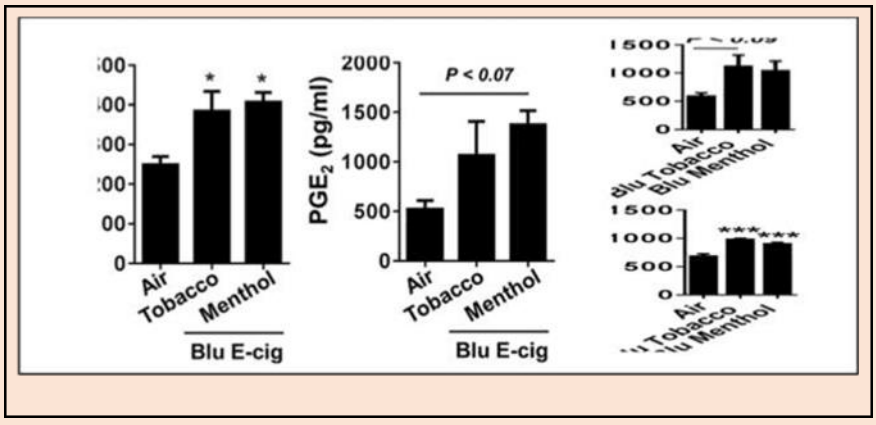

Figure 14: Cytokine's concentrations in fibroblast (left) and epithelial (right) culture [12].

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Thus a significant increase in the production of IL8 and PGE2 is noted *in-vitro* for epithelial and fibroblast cells.

Increased inflammatory response to periodontal pathogenic bacteria

In 2020, Pushalkar is using a cell culture of Fadu cancer cells (hypopharynx carcinoma exposed to vapors from electronic cigarettes for 40 minutes [9,18]. These cells are then exposed for 2 hours to a pathogen: Porphyromonas gingivalis or Fusobacterium nucleatum.

The mRNAs of the major inflammatory cytokines are relatively quantified via qPCR. Here are the results (Fig. 15,16) (Table 15,16).

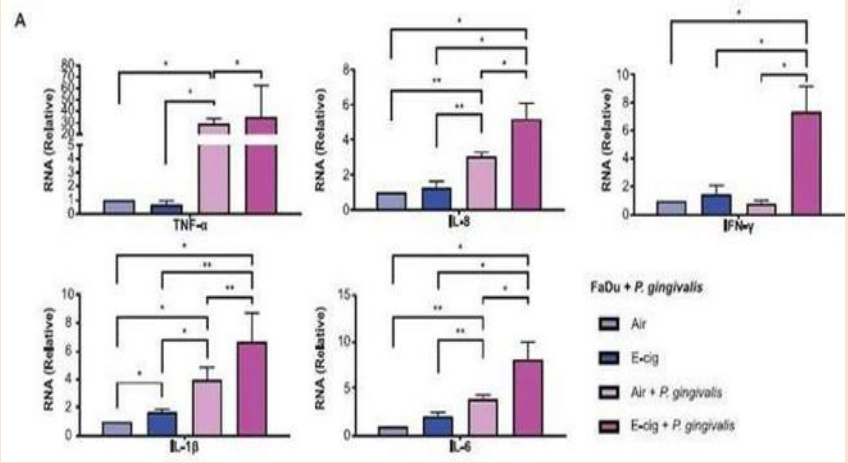

Figure 15: RNA expression with Pg. contact [18].

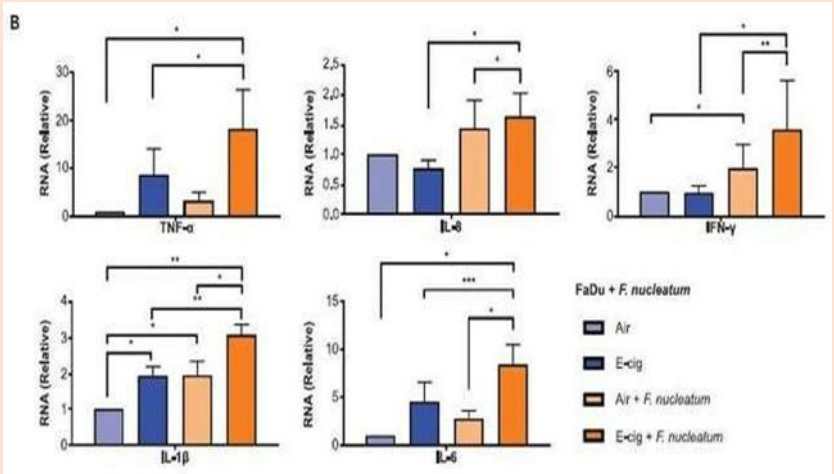

Figure 16: RNA expression with Fn contact [18].

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qPCR Pg.	TNFx	IL 8	IFN gamma	IL 1B	IL 6
Control + pg.	30	3	1	4	4
E cig+ pg.	40	5	9	7	9
	Significative augmentation	Significative augmentation	Significative augmentation	Significative augmentation	Significative augmentation

Table 15: Significative augmentation.

qPCR Fn	TNFx	IL 8	IFN gamma	IL 1B	IL 6
Control + Fn	5	1.4	2	2	2.5
E cig+ Fn	18	1.6	3.5	3	7.5
	Significative augmentation	Significative augmentation	Significative augmentation	Significative augmentation	Significative augmentation

Table 16: qPCR Fn augmentation.

An Elisa test is undertaken to quantify the expression of IL8 and TNFx in the two previous cases (Fig. 17) (Table 17,18).

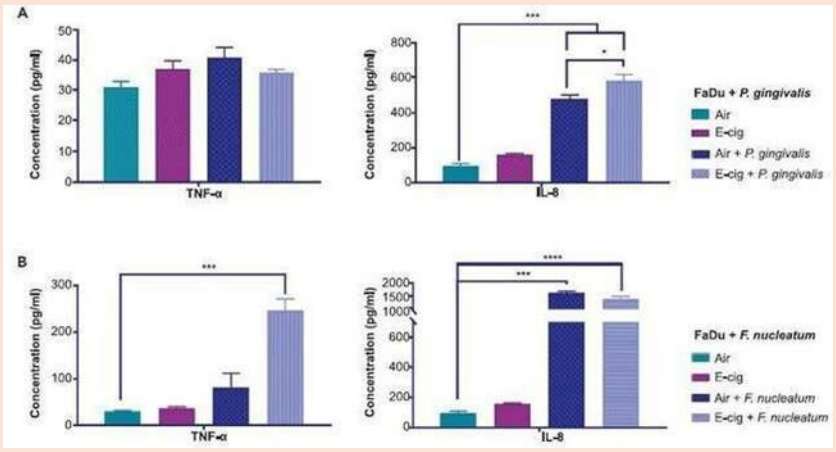

Figure 17: Elisa's test for IL8 and TNFx expression [18].

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Pg./ml pg.	IL8	TNFx
Control + pg.	500	35
E-cig + pg.	600	40
	Significative augmentation	No Significative augmentation

Table 17: Pg./ml pg. augmentation.

Pg./ml Fn	IL8	TNFx
Control + pg.	1600	90
E-cig + pg.	1400	250
	No Significative augmentation	No Significative augmentation

Table 18: Pg./ml Fn. augmentation.

Thus, all of this data allows us to confirm the following data:

- Electronic cigarettes in the absence of pathogenic bacteria increase the production of pro-inflammatory cytokine mRNA and chemotaxis (*in-vivo* and *in-vitro*)
- In the presence of periodontal pathogens such as Fn and Pg., the expression of pro-inflammatory cytokine appears to be increased in comparison with cells not exposed to electronic cigarette vapor (*in-vitro*)

Clinical Periodontal Measurement

In 2018, the study by Mokeem provides a comparative clinical approach between consumers of tobacco, hookah, and E-cigarettes [16].

It measures *in-vivo*, salivary flow, salivary cotinine's, and periodontal clinical data. Here are the results and characteristics of the groups tested (Table 19).

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Group	Tobacco	Hookah	E cigarette	Control
Inclusion	At least 5 cig per day since more than 1 year	At least once a day since more than 1 year	Never tobacco smoke At least once a day since more than 1 year	O
Sexes + middle age	Man 39	Man 40	Man 28	Man 40
Consummation	16 cig	17 min/day	8mn/day	0
Percentage of site with plaque	50	52	28	23
Percentage of BOP site	20	17,5	17,5	35
Average depth pocket mm	4,5	4,5	1,8	1,5
Average clinical attachment loss mm	3,2	2,8	0,5	0,3
Radiologic crestal bone loss mm	4,2	4,5	2	1
Salvia flow ml/mn	0,53	0,52	0,53	0,46
Salivar Cotinine mg/ml	247	252	221	2,3

Table 19: Results and characteristics of the groups tested.

The level of salivary cotinine in E-cigarette consumers appears to be similar to that of conventional cigarette consumers. Thus, vaping of 8 min per day is equivalent here to 16 cigarettes per day, depending on the initial concentration of nicotine in the combustion liquid, not specified here. No difference in the variation in salivary flow was noted. The average bone loss, pocket depth, and clinical loss of attachment of e cigarette users appear to be similar to non-smokers, and lower than conventional cigarette users. For an equivalent amount of plaque, the electronic cigarette consumer presents less gingival bleeding compared to the non-smoker group (like the conventional smoker).

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Conclusion of the parts

In this part, we understand that the consumption of electronic cigarettes, with or without nicotine, generates the *in-vitro* and *in-vivo* production of free radicals by periodontal cells, resulting in intracellular and intranuclear lesions (DNA damage). This reaction is due to the cytotoxic components of the combustible liquid and the aromas.

In addition, several metabolic pathways are involved, in particular via the COX and RAGE pathways.

This leads to overexpression of pro-inflammatory cytokines.

This chronic pro-inflammatory context seems to be further exacerbated in the presence of periodontal pathogenic bacteria.

The presence of nicotine in the combustion fluid makes the level of salivary cotinine similar to conventional consumption.

In the presence of a plaque index similar to non-smokers, gum bleeding is reduced by the action of the vasoconstrictor nicotine, just like conventional consumption, and therefore, masks the clinical signs of periodontal disease.

Results 3: Effect on periodontal ligament effect on cell activity, growth and morphology

In 2014, Willershausen studied the impact of intrinsic constituents of the combustion fluid of electronic cigarettes on the fibroblast of the human periodontal ligament [19].

Thus, he uses an Hpdlf (Human periodontal ligament fibroblast) culture distributed in 6 culture conditions:

- Control group
- 10 µg diluted in Nicotine culture medium
- 10 µg diluted in propylene glycol culture medium
- 10 µg diluted in the culture medium of Hazelnut flavoring liquid (nicotine 20 mg/ml + propylene glycol)
- 10 µg diluted in the culture medium of lemon flavored eliquid (nicotine 20 mg/ml + propylene glycol)
- 10 µg diluted in the culture medium of Eliquide aroma mint (nicotine 22 mg/ml + propylene glycol)

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After 24 h of culture, the cell activity is recorded with an Apoglow bio test, using immunofluorescence targeted on the atp. Here are the results (Fig. 18) (Table 20).

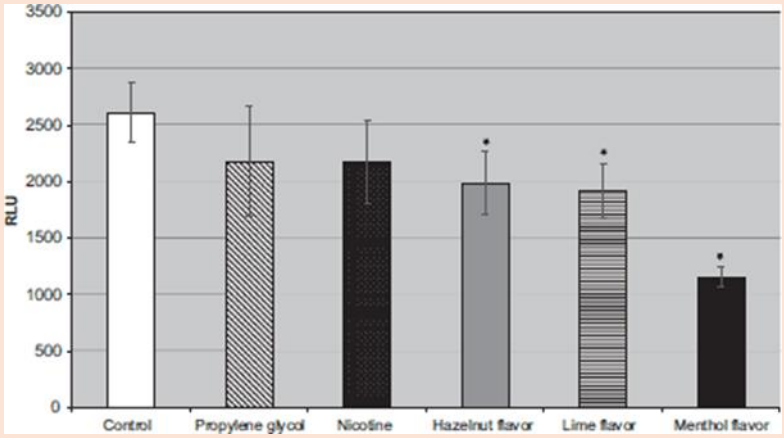

Figure 18: Relative fluorescence measuring the cellular activity [19].

Growing Condition	Relative Fluorescence (rfu)
Control	2600
Propylene	2100
Nicotine	2100
Hazelnut	2000
Lemon	1900
Menthol	1100

Table 20: Relative fluorescence measuring the cellular activity [19].

Thus, there is a significant decrease in fibroblast cell activity, as part of the Aroma / nicotine / propylene glycol combination. Propylene glycol or nicotine alone indicates an insignificant decrease. Under these same conditions, a microscopy study is carried out after calcein / ethidium cell staining (Fig. 19).

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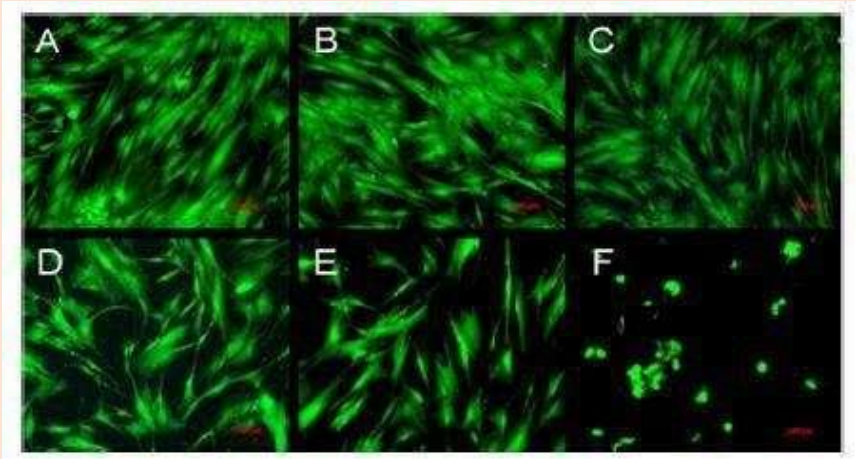

Figure 19: Epifluorescence microscope results at 24 hours.

The control (A) / propylene (B) / nicotine (C) group have elongated, cross-linked cells and an equivalent cell density. The Hazelnut (D) and Lemon (E) group shows fewer, more elongated cells. The Mint group (F) shows a virtual cellular disappearance, with cells of small sizes, round and not digitized.

On the other hand in 2018, Alanazi carried out a comparative study on the effects of tobacco and electronic cigarettes on the human periodontal fibroblast [20].

After collecting smoke from conventional CSC cigarettes, from electronic cigarettes with nicotine NR 12 mg/ml and from electronic cigarettes without nicotine NF 0 mg/ml, the fibroblasts are exposed to these smoke by dilution of 1, 5 and 10%.

The cells are observed by microscope and epifluorescence at 24 hours.

For all exposures, there is a cellular change ranging from cell elongation to cytoplasm depletion, but also a reduction in the overall population. The effects are more marked with tobacco (from 1%) than with electronic cigarettes (from 10% for NF, against 5% for NR) (Fig. 20) (Table 20).

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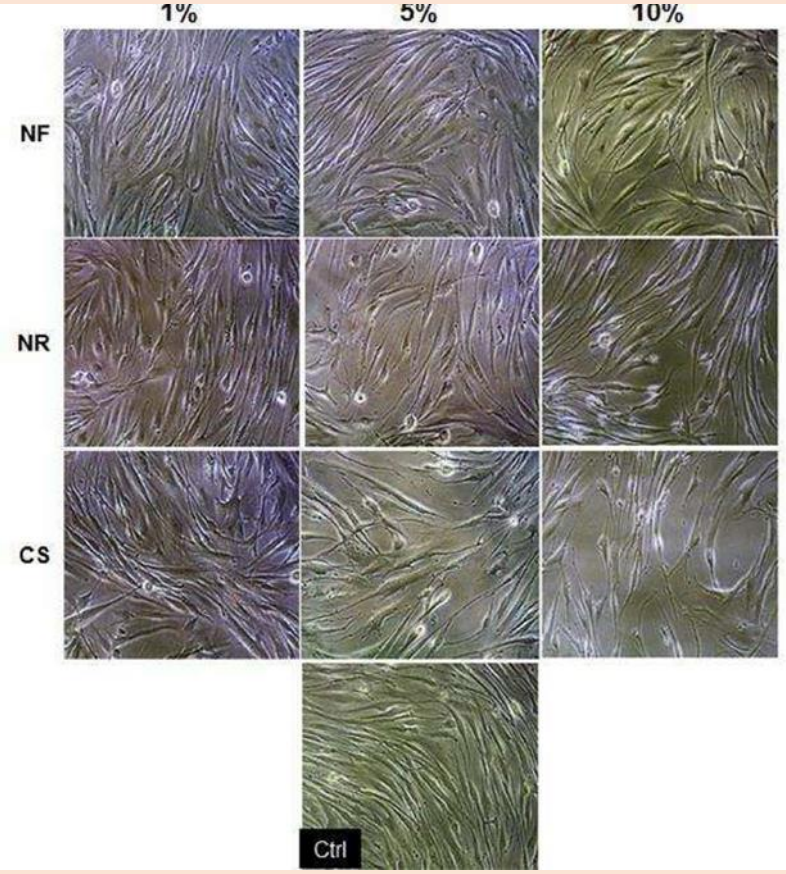

Figure 20: Cellular analyses using microscope at 24 hours [20].

Effect on cell migration: the healing potential. The healing ability of periodontal tissue can be assessed with the ability of fibroblasts to migrate and adhere.

The study by Willershausen carried out an attic test for the 6 growing conditions described above [19]. Thus, 2 culture dishes are separated by a porous PET membrane. Migration analysis is carried out by fluorescent labeling at 72 hours (Fig.19) (Table 21).

Growing Condition	Relative Fluorescence
Control	2500
Propylene	2400
Nicotine	2000

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Hazelnut	2000
Lemon	2100
Menthol	600

Table 21: Relative fluorescence measuring the cellular activity.

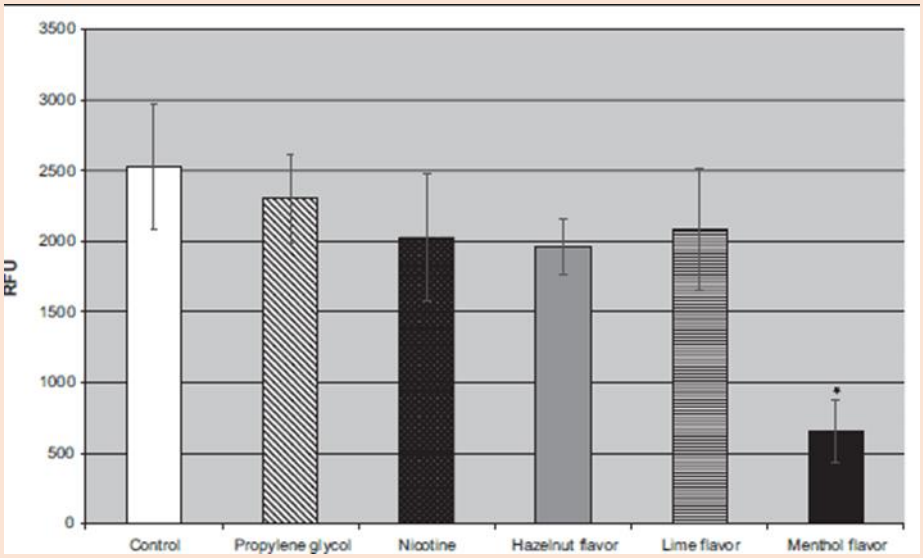

Figure 21: Relative fluorescence concerning migration [19].

Although all growing conditions indicate a decrease in migration capacity, only the Mint aroma indicates a significant decrease.

In Alanazi's study, the migration capacity is assessed by measuring the volume unoccupied by cells, every hour until the 2nd day [20].

The concentration of 1% of the different fumes (tobacco, NR, NF) does not show any significant difference. However, a concentration of 5 and 10% indicates a significant decrease for all CSC, NR, NF conditions and a more marked effect for tobacco. The effects are almost similar for the electronic cigarette with or without nicotine (Fig. 22) (Table 22).

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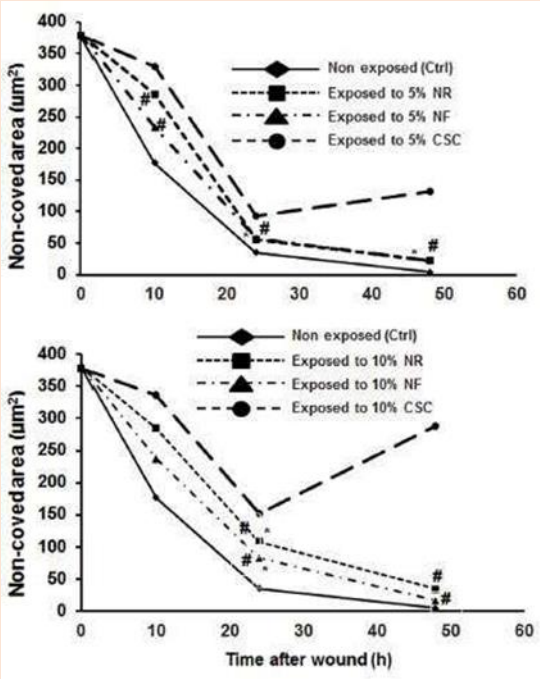

Figure 22: Box covering by fibroblast [20].

Growing Condition	No Covered Area µm² 5% 48h	No Covered Area µm² 10% 48h
Ctrl	0	0
NR	25	10
NF	25	40
CSC	180	300

Table 22: Concentration of 1% of the different fumes (tobacco, NR, NF).

Effect on Cell Vitality and Apoptosis

In 2016, Sancilio measured the cellular toxicity of electronic cigarette liquids on human fibroblast [11]. After collection of the NR (24 mg/ml) and NF (0 mg/ml) and air (control) fumes, these are diluted by injection of 0.5, 1, 2 mg or 5 mg in the culture medium. After 24 hours and 48 hours, an Mtt test quantifies cell activity and mitosis (Fig. 23) (Table 23).

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Percentage of Viable Cells	24h	48h
Témoin	100	150
VNR 2 mg	30*	25*
VNR 5 mg	25*	25*
VNF 2 mg	50*	50*
VNF 5 mg	15*	0*

Table 23: Cell vitality.

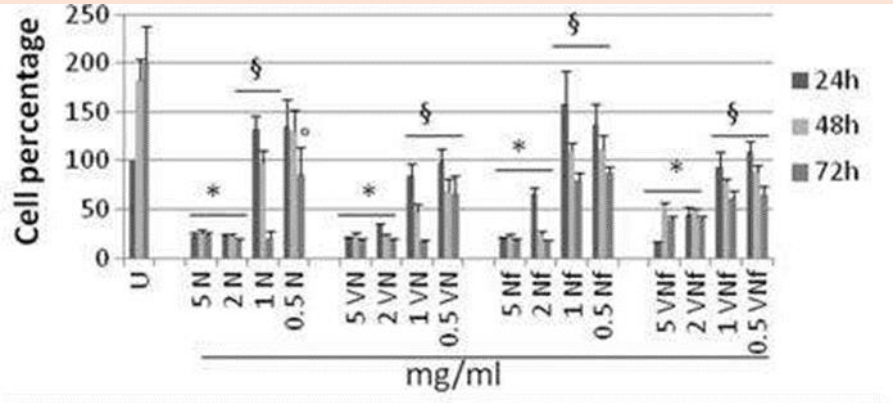

Figure 23: Percentage of viable cells regarding the exposition time [11].

Thus, a significant reduction in mitotic activity is demonstrated, with a dose dependent effect for liquids with or without nicotine. Annexin V / PI detection flow cytometer separates viable cells from apoptotic cells (Viable: V-/ p-; pre-apoptosis: V + / P-; advanced apoptosis V- / pi +; necrotic V + / Pi +).The quantification is carried out by percentage of cells in each stage. The table shows the results at 48 hours for the 1 mg dilution of vaporized collected smoke (Fig. 24) (Table 24).

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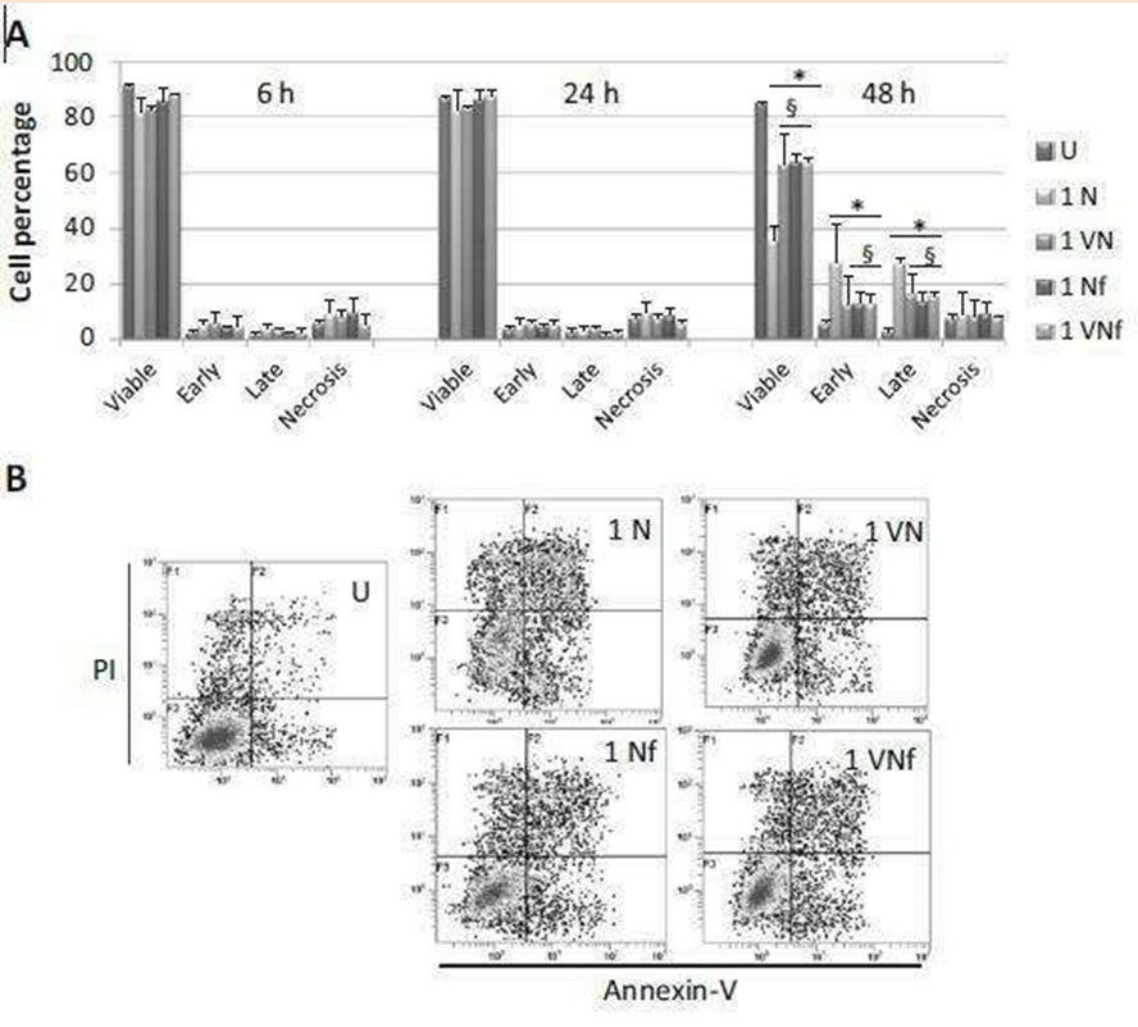

Figure 24: Flow cytometer and quantification of viable cells [11].

Percentage at 48h	Viable cells	Pre apoptosis	Advanced Apoptosis	Necrosis
Control	85	5	0	10
Vaporized NR	60*	15*	15*	10
Vaporized NF	60*	15*	15*	10

Table 24: Percentage of cell activity at 48h.

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In parallel, the expression of a pro-apoptotic gene such as Bax is demonstrated by fluorescence at 24 hours, under the conditions of 1 mg/ml vaporized with NR and NF. Relative fluorescence is expressed as follows (Fig. 23) (Table 25).

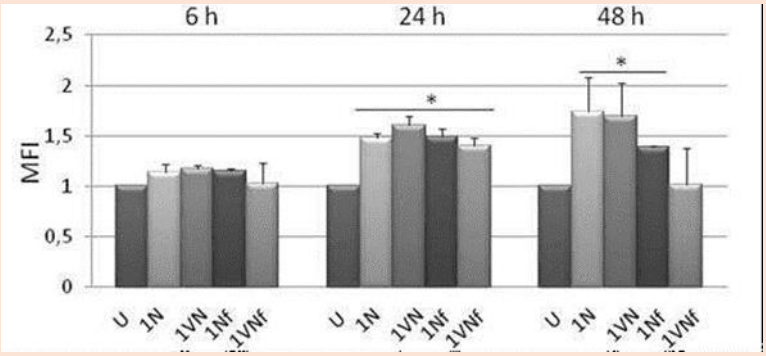

Figure 25: Bax expression [11].

Control	1
VNR	1.6*
VNF	1.4*

Table 25: Relative fluorescence.

Above, the summary of the values for 24 hours, with 1 mg/ml of NR or NF vaporized. A significant increase in Bax transcription confirms the increase in cell apoptosis for NF and NR cultures. In the article by Alanazi (20), fibroblastic mitosis is demonstrated by an Mtt test at 3, 5 and 7 days, after fibroblast culture under the following conditions: conventional tobacco CSC, E- cigarette NR 12 mg/ml nicotine, E-cigarette NF without nicotine, and this up to 60 minutes of exposure per day. The vapors are diluted to 1, 5 and 10% (Fig. 26).

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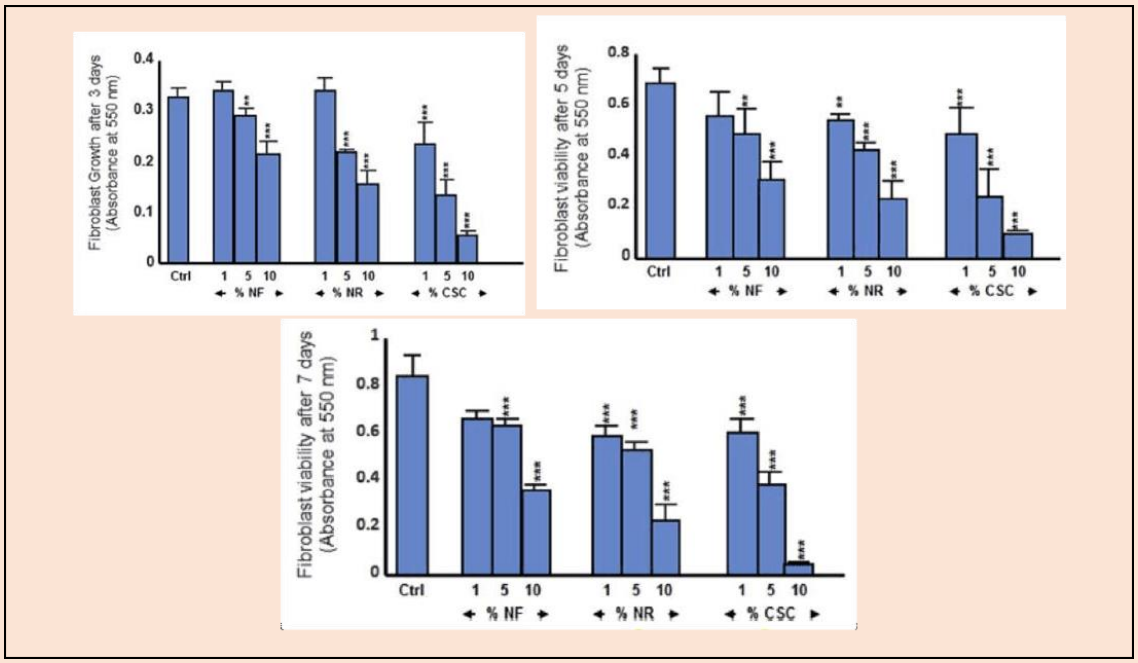

Figure 26: Test Mtt to appreciate the cellular vitality [20].

Thus, from 3 days, a significant decrease in cell growth is observed with a dose-dependent effect compared to the control. NF appears to be less deleterious than NR, itself less harmful than CSC. In parallel, a tunnel test (characteristic of DNA lesions) with proteinase K and TACS Blue staining (marking DNA lesions) is carried out on the cell pull on the 3rd day. The percentage of apoptotic cells is shown below (Fig. 27).

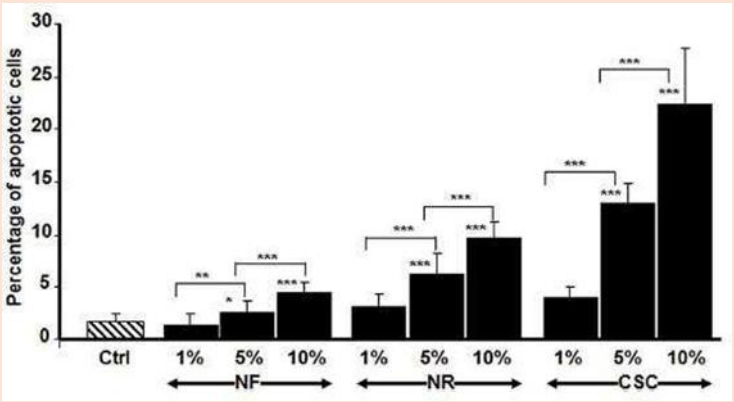

Figure 27: Apoptosis cells percentage at 3 days [20].

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Likewise, a significant increase in apoptosis is demonstrated, in a dose-dependent manner, with the same diagram: CSC> NR> NF.

Conclusion of the part

This section highlights the negative effect of electronic cigarettes on periodontal tissue. Thus, the electronic cigarette is able to decrease *in-vitro* cell activity and fibroblast growth.

In addition, the fibroblast morphology is modified by depleting, for example, the intracellular cytoplasm.

This decline in fibroblast growth and mitosis is coupled with an increase in apoptosis, in particular through the increased expression of pro-apoptotic genes, leading to increased DNA damage.

Fibroblast function is also impaired, reducing the ability of the fibroblast to migrate and adhere, which may limit the capacity for periodontal healing.

It is important to note that *in-vitro* studies prove a negative effect of electric cigarette liquids, even in the absence of nicotine, thus incriminating constituents such as propylene glycol or flavors.

Results 4: Effect on bone

Effect on osteoplastic growth and vitality

In 2020, Florence measures the impact of electronic cigarette flavors on the human bone cell using a similar cell line: osteosarcoma MG63 [21]. After collection of nicotine-free cigarette smoke, with different aromas, the bone cells are exposed to 2 puffs per minute, with different concentrations of vapor. At 48 hours, an Mtt test associated with an absorbance measurement by spectrophotometry (Fig. 28) (Table 26).

% alive cells/ concentration	0%	0.0025	0.025	0.25	2.5
No flavor	100	100	100	90	40
Flavor cinnamon candy brand	100	110	100	90	Non measurable
Flavor cinnamon napalm brand	100	110	105	70	Non measurable

Table 26: Effect on osteoplastic growth and vitality.

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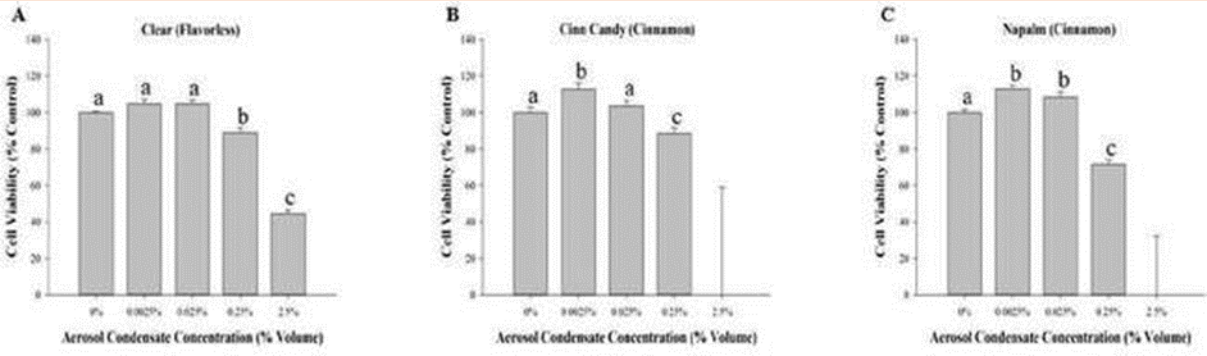

Figure 28: Bone cells 'viability regarding concentration and flavour [21].

Thus, a significant drop in cell viability is observed with a dose-dependent effect. The impact of aromas seems greater than that of a liquid without aroma. The intrinsic constitution of the liquid (without nicotine and without aroma) seems to be deleterious for cellular vitality.

In addition to the viability test, the analysis of the production of free radicals is carried out using a fluorescent marker (485/528 nm) reactive to oxidized DNA: Rox green. The fluorescence is then related to the number of cells present (Fig. 29) (Table 27).

Condition/ concentration	0%	0.0025	0.025	0.25	1
No flavor	145	260	230	180	280
Flavor cinnamon candy brand	300	400	350	500	2000
Flavor cinnamon napalm brand	125	125	125	900	1750

Table 27: Cellular vitality.

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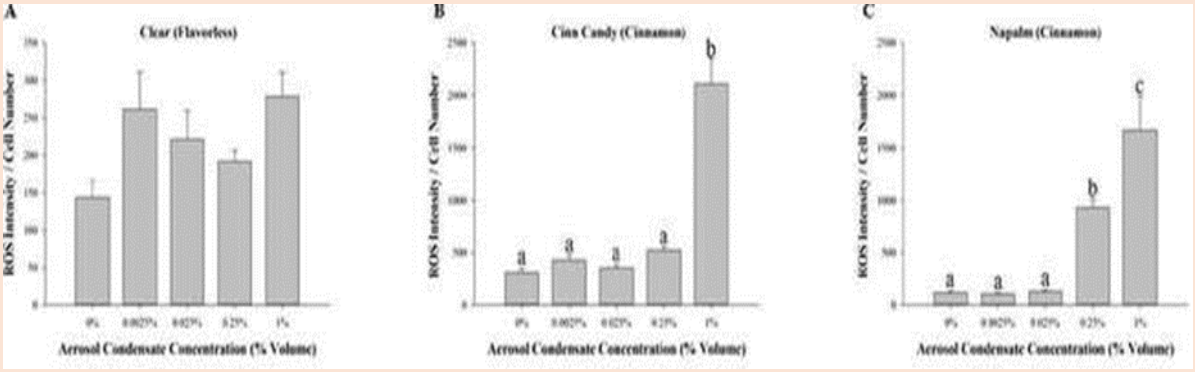

Figure 29: ROs production depending flavor used [21].

The increase in the production of free radicals is significant with the presence of aromas. Oxidative stress caused by the presence of electric cigarette smoke could be a pathway explaining osteoplastic cell death *in-vitro.*

Similarly, in 2019 Rouabhia measured the effect of conventional cigarettes and electronic cigarettes (with 18mg/ml or without nicotine) on the SAOS 2 cell (human osteosarcoma) [22]. He exposes them to the vapors collected for 15 or 30 minutes a day for 3 days. An Mtt test quantifies cell growth by measuring absorbance by spectrophotometry (Fig. 30) (Table 28).

Hour 1	**15 min**	**30mn**
Control	0,35	0,35
CS	0,2	0,1
NR	0,25	0,15
NF	0,3	0,25
Hour 2	**15 min**	**30mn**
Control	0,4	0,4
CS	0,1	0,5
NR	0,12	0,8
NF	0,25	0,15
Hour 3	**15 min**	**30mn**
Control	0,45	0,45

Table 28: Measured absorbance by spectrophotometry.

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DOI: http://dx.doi.org/10.46889/JCIM.2022.3102

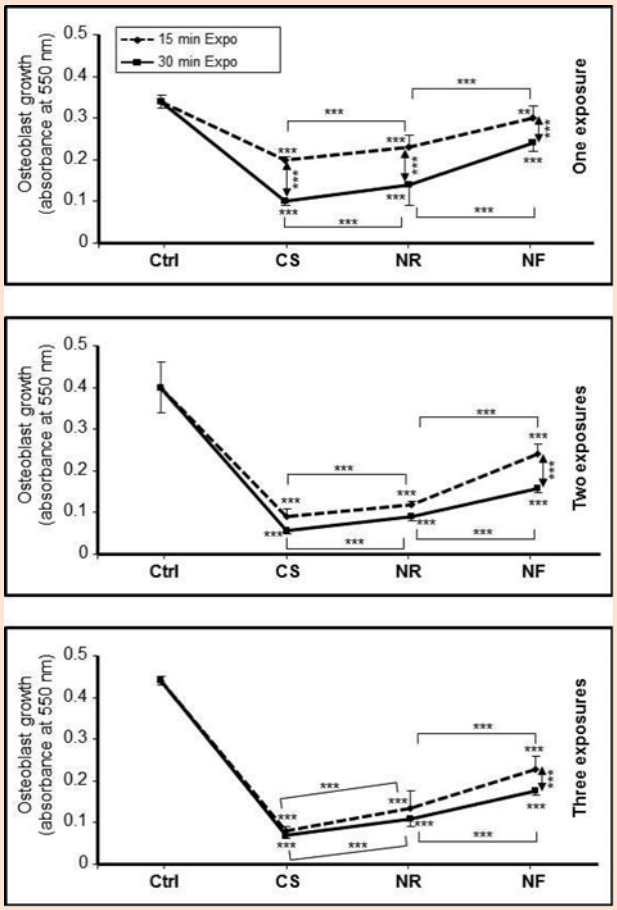

Figure 30: Cellular growing regarding time and exposition [22].

The conventional CS cigarette causes a significant drop in cell growth and cell vitality from the first exposure. The same is true for the electronic cigarette with NR nicotine. Regarding the NF nicotine-free liquid, the drop is lighter but significant and occurs after 2 days. In parallel, the expression of pro-apoptotic genes (caspase 3) is sought by western blot, associated with a measurement of fluorescence density (immunofluorescence) (Fig. 31) (Table 29).

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DOI: http://dx.doi.org/10.46889/JCIM.2022.3102

	1 Exposition	2 Expositions
Control	0	0
NF	0	1
NR	0	9
CS	1	Non measurable

Table 29: Fluorescence density.

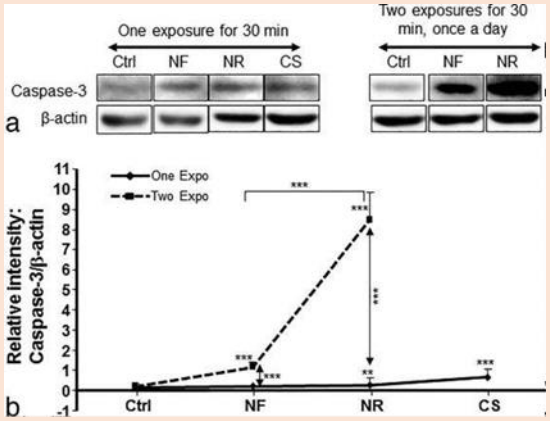

Figure 31: Proapoptosis DNA expression regarding exposition [22].

The significant increase in the expression of caspase 3, depending on the number of exposures, is one way of explaining the drop in osteoblastic cell vitality in the presence of electronic cigarette vapor.

Effect on protein synthesis and mineralization

Let us return to the study by Florence testing the impact of aromas on cells close to the osteoblast [21]. For a concentration of 0.25% of the smoke vapors collected, the expression of the gene coding for type I collagen at 48 hours is demonstrated by immunofluorescence and quantification of the relative fluorescence.

No significant difference is noted on intracytoplasmic detection and type I collagen synthesis (Fig. 32).

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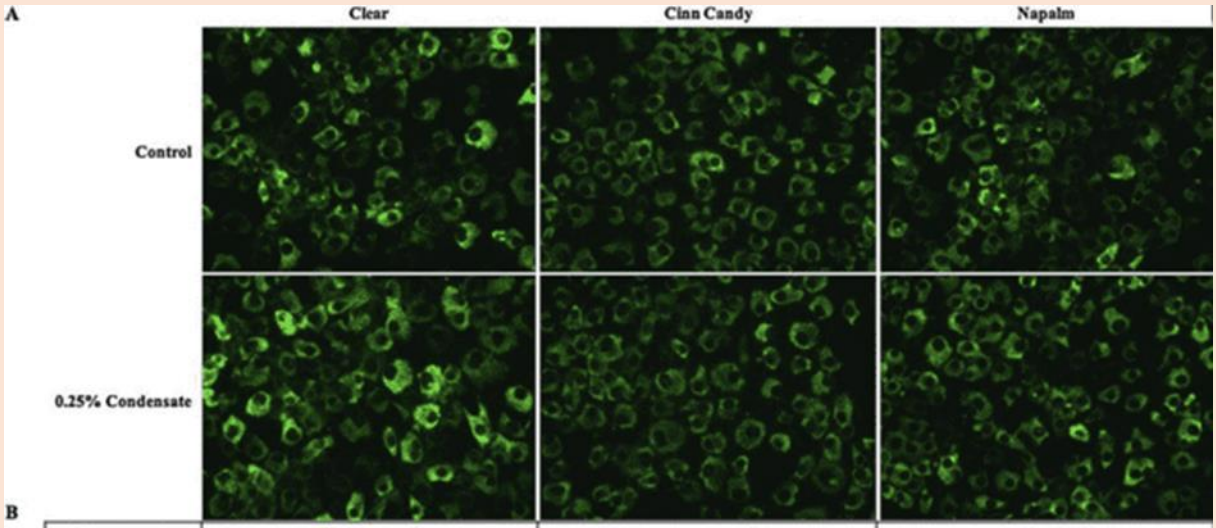

E-liquid	Clear	Cinn Candy	Napalm
Control	15.687±2.223	10.409±0.492	12.801±2.104
Treatment	19.442±1.007	10.237±0.308	14.757±1.018

Figure 32: Detection and type I collagen synthesis.

The mineralization capacities of the extra cellular matrix by osteoblasts in contact with electric cigarette vapor have been explored by Rouabhia [22].

The study is carried out on the activity of alkaline phosphatase at 24 hours, after exposure for 2 and 3 days (for 15 or 30 minutes per day). The cellular supernatant is harvested, and then receives the pNPP substrate of the enzyme tested (Fig. 33) (Table 30).

15 mn Exposition	**Control**	**CS**	**NR**	**NF**
2 days	3.5	2.8	3	3.4
3 days	3.9	2.5	2.9	3.4
30 mn Exposition	**Control**	**CS**	**NR**	**NF**
2 days	3.55	2.6	2.9	3.4
3 days	3.8	2	2.75	3.35

Table 30: Activity of alkaline phosphatase.

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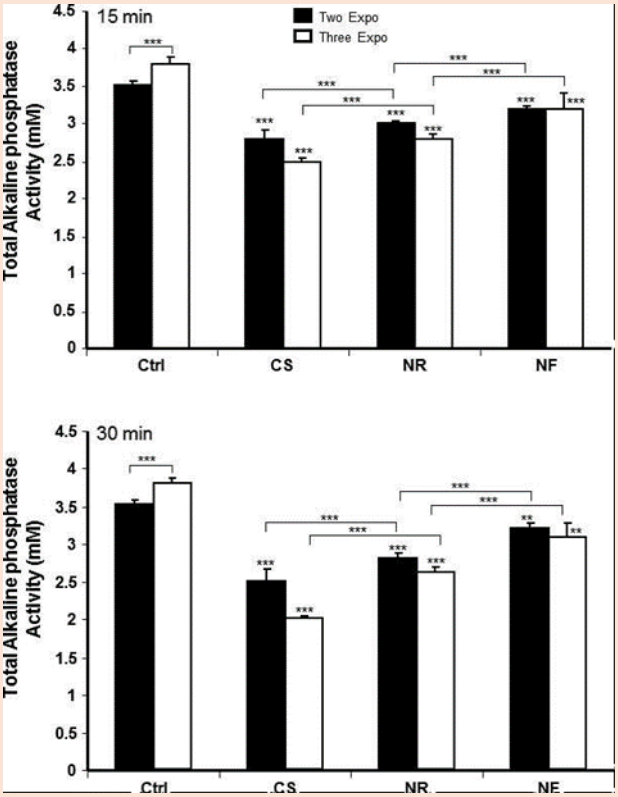

Figure 33: Phosphatase alkaline activities regarding exposition [22].

A significant decrease in the activity of alkaline phosphatase is noted concerning exposure to conventional tobacco CS, to the electric cigarette with nicotine 18 mg/ml NR and without nicotine NF. The drop was significantly greater for CS compared to NR which itself was significantly greater than NF. In the control group, the activity of alkaline phosphatase increased significantly over time, unlike the test groups in which the activity decreased over time.

The study continues with 3 weeks of cell culture without exposure, and then these cells are exposed to 30 minutes of steam per day for 1.2 and 3 days. The nodules of mineralization are marked by staining with alizarin red ARS. The amount of ARS absorption by spectrophotometry is shown above (Fig. 34) (Table 31).

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Relative absorption regarding number of exposition	1	2	3
Control	1,5	1,7	2,3
CS	1,25	1,4	1,45
NR	1,4	1,4	1,6
NF	1,45	1,6	2

Table 31: Relative absorption.

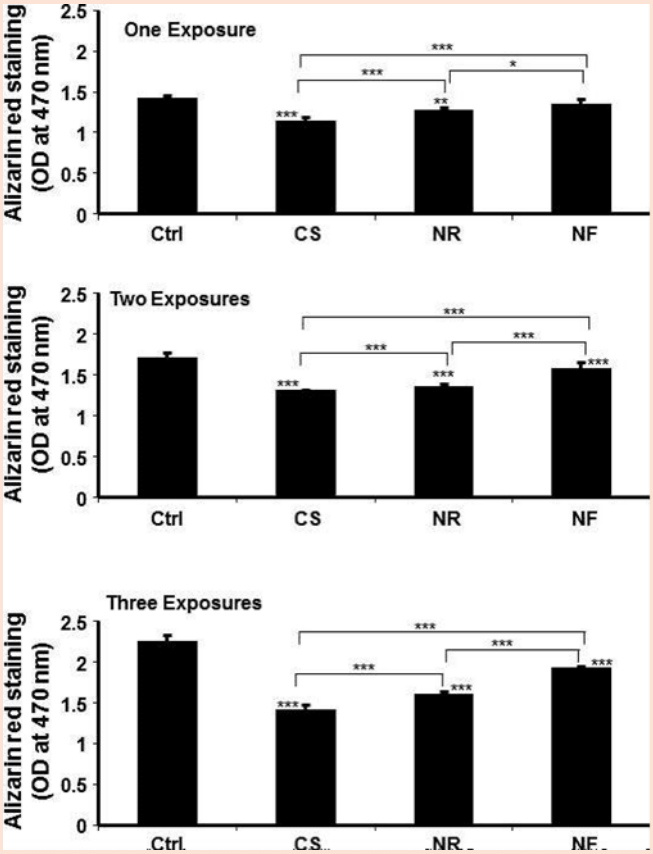

Figure 34: Relative absorption of the mineralization's nodules using spectrophotometry [22].

A significant drop in the quantity of mineralized tissue is observed compared to the control, from the 1st exposure for CS and NR, and from the 2nd exposure for NF. It should be noted that the magnitude of the drop is significantly different according to the different test conditions and is carried out according to the following diagram: CS>NR>NF

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Effect on the adhesion and integrity of bone cells on the implant surface.

Rouabhia carried out the cell culture of cells close to osteoblasts (osteosarcoma) on a sterile Ti6Al4v titanium disc [22]. As described previously, it exposes the cells to the vapors of conventional tobacco CS, electronic cigarette with nicotine NR (18 mg/ml) and without nicotine NF. After 3 days of culture at the rate of 1 exposure per day of 15 or 30 min, the cells adherent to titanium are highlighted by Hoechst staining and fixation with methanol. The comparison is carried out under an epifluorescence microscope (Fig. 35).

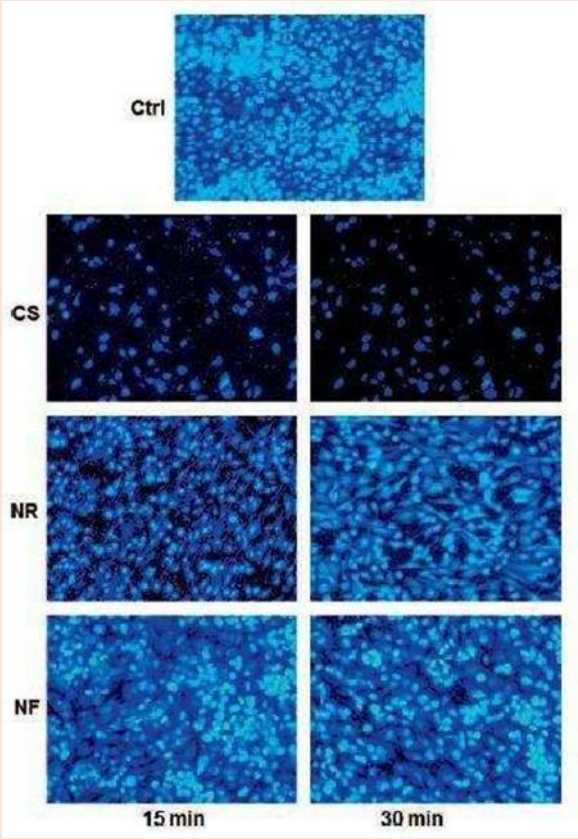

Figure 35: Adherent's cells on titan regarding exposition [22].

Concerning the conventional cigarette, a very strong decrease in cell density is noticed. It is also noticed less strongly for the electronic cigarette with nicotine. Few differences are objectifiable concerning the cell density on the titanium surface for NF compared to the control.

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The cytoskeleton is studied for titanium adhesive cells, in particular F-actin filaments, by fixation with fluorescent phalloidin. Fluorescence density and microscopic analysis are available below (Fig. 36) (Table 32).

Relative Fluorescence	15 min	30 min
Control	40	40
CS	15	10
NR	35	20
NF	40	30

Table 32: Relative fluorescence.

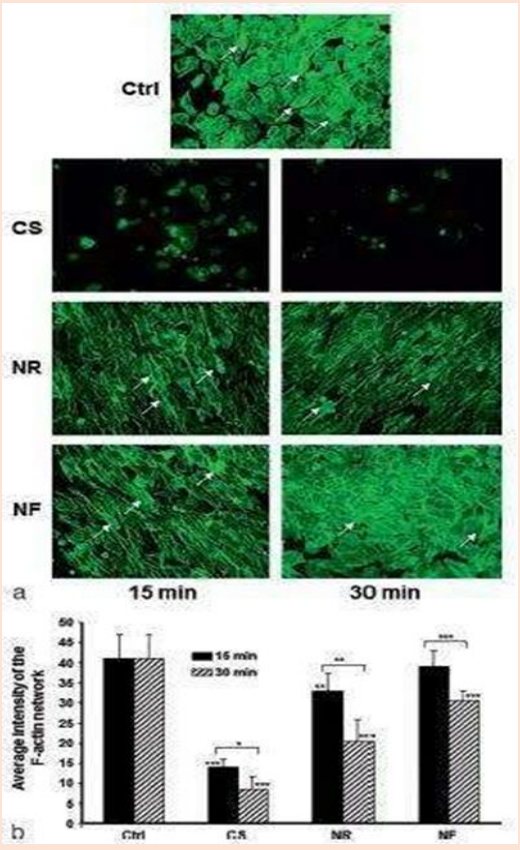

Figure 36: Actin coloration and relative density regarding exposition [22].

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After 30 min of exposure, the density of the action network is significantly lower between the control and NF, then between NF and NR, then between NR and CS. The longer the exposure time, the greater the effect.

Conclusion of the parts

Previous studies tell us the impact of E-cigarettes on bone tissue *in-vivo*, using cancer cells. The electronic cigarette seems to cause an increase in the production of free radicals and activate the expression of pro-apoptotic gene *in-vivo*.

These elements have the consequence of reducing bone cell growth, but also of damaging the cytoplasmic integrity of bone cells; In particular by damaging the cytoskeleton of bone cells in contact with an implant surface.

We can specify that nicotine is not the only deleterious agent involved, the aromas and the intrinsic elements constituting the liquid have a negative impact on cell growth and integrity.

These effects could affect the cellular ability to adhere to implant surfaces and therefore impact osteointegration.

The capacities of collagen synthesis and mineralization of the extra cellular organic framework seem to be altered *in-vitro* in repeated contact with electronic cigarette vapors.

Results 5: Effect on epithelium

Effect on cell vitality and morphology

In 2017, Rouabhia measured the effect of electronic cigarettes on the epithelial cell [23]. He collects human epithelial cells from young patients (18-25) with no periodontal history or smoking. The cell culture is then exposed to 15 min per day to electronic cigarette vapors with 12 mg/ml of nicotine, at the rate of 2 puffs per minute, for 3 days. The cells are analyzed by optical microscope.

The test group presents cubic cells with a small nucleus accompanied by a high cell density. Concerning the test group, we find from the 2nd day large, long cells, with a decrease in cell density (Fig. 37).

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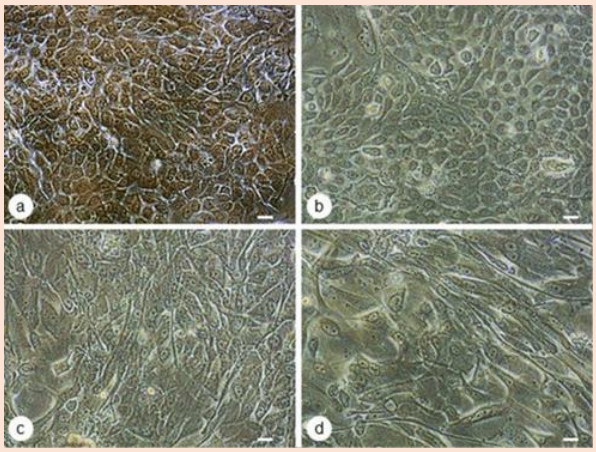

Figure 37: Cell's aspect on optic microscope, A control, B 24hours exposition, C 48h, D 72h [23].

The impact of electric cigarette vapors on epithelial cells can be studied indirectly. Thus Alanazi (10) proposes to carry out a cell co-culture between epithelial cells and candida Albicans exposed or not to different vapors (air control, conventional tobacco CS, electric cigarette without nicotine NF, and electric cigarette with 18mg/ml of nicotine NR).

Test groups show epithelial cell transformation, with increased cell size, larger cell nuclei, and looser cytoplasm, compared to cell co-culture with unexposed *Candida Albicans* (Fig. 38).

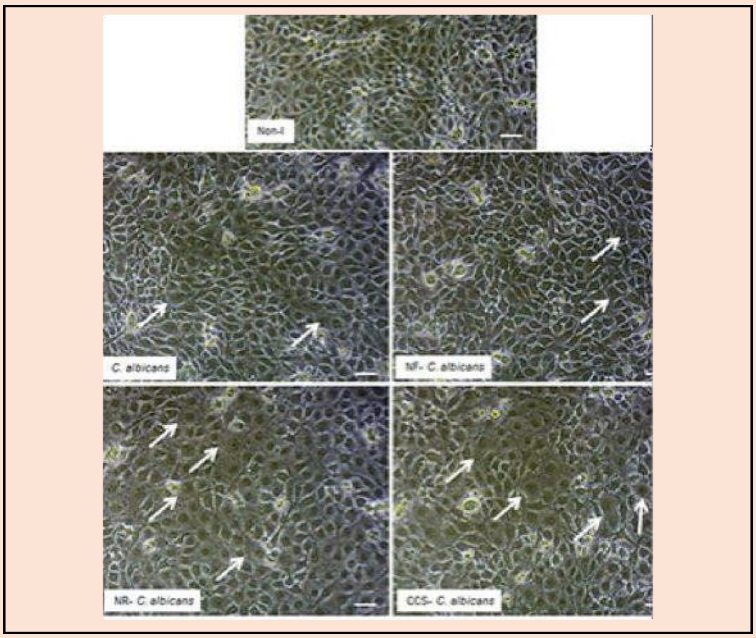

Figure 38: Microscopic analysis of culture with Candida regarding exposition [10].

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Cells were then stained with tryptan blue to measure viable cells: l (Fig. 39) (Table 33).

	Control	NF	NR	CS
Number of cells x10 ^4	130	130	105	80

Table 33: Viable cells measured by tryptan blue.

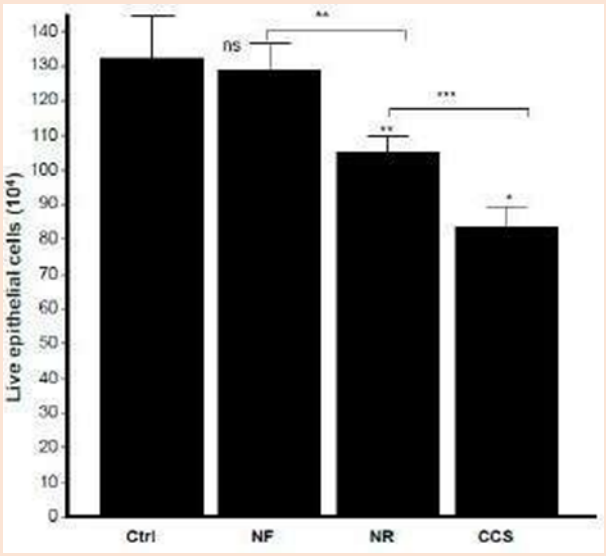

Figure 39: Alive epithelial cell's number regarding exposition [10].

Exposure of Candida Albicans to vapors from an electric cigarette or conventional tobacco acts negatively on epithelial cell viability in co-culture compared to no exposure.

Effect on DNA integrity and apoptosis

Rouabhia highlights the cellular damage caused by the vapors of electronic cigarettes on epithelial cells [23].

He focuses his research on membrane damage, in particular via the activity of Lactate Dehydrogenase (LDH). This is demonstrated by the Promega LDH test, within the supernatant.

At 24h, an absorbance spectrophotometry at 490nm is carried out (Fig. 40) (Table 34).

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Relative absorption à 490 nm	Control	E-cig.
D1	1.5	3
D2	3	5
D3	4	8

Table 34: Spectrophotometry absorbance.

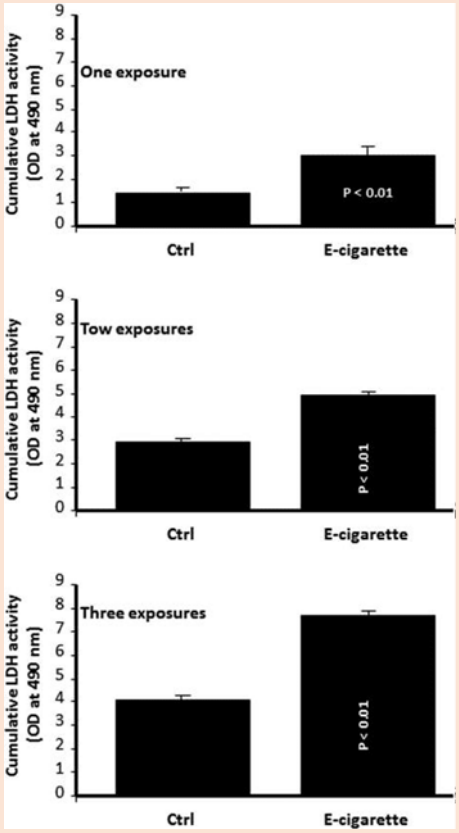

Figure 40: LDH activity regarding time and exposition [23].

The electric cigarette with nicotine *in-vitro* is able to increase the cellular lesions of the epithelium in a significant way and this from the first exposure. Then the DNA lesions are noted by tunnel test (tacs blue label) with counting of the marked cells, considered as apoptotic (Fig. 41) (Table 35).

Control	1 Exposition	2 Expositions	3 Expositions
2	18*	40*	52*

Table 35: Apoptotic exposition.

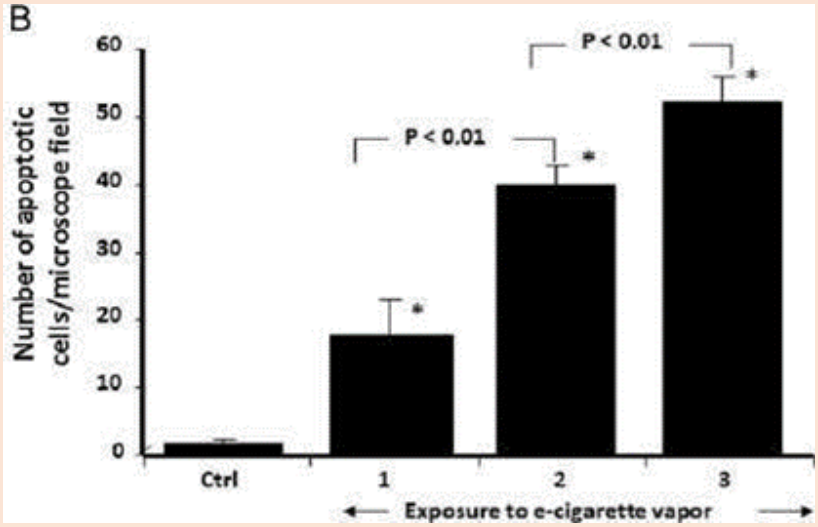

Figure 41: Apoptotic cells regarding exposition [23].

Exposure to the vapors of electric cigarettes induces *in-vitro* a significant increase in the number of apoptotic cells, with a significant dose-dependent effect. These first measurements are confirmed by flow cytometer after labeling Annexin V/pi (fluorescein isothiocyanate) (Fig. 42) (Table 36).

Percentage of Cell	Control	D1	D2	D3
Apoptotic cell V+	1%	1%	2.5%*	3.5% *
Necrotic cell PI +	12%	15%	20%*	25%*

Table 36: Flow cytometer measurement.

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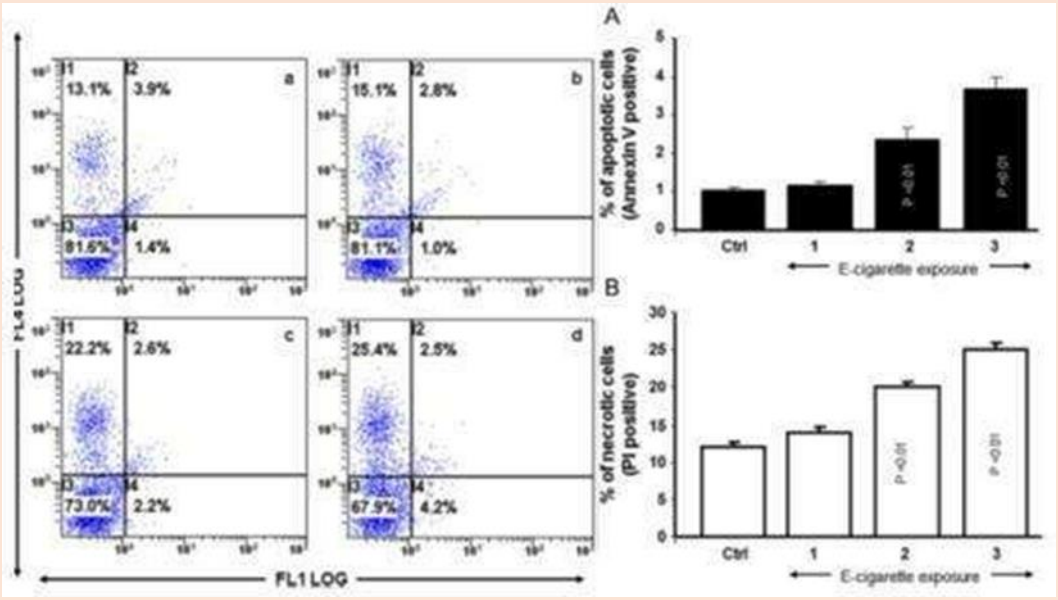

Figure 42: Flow cytometer and analyses of apoptotic and necrotic cells [23].

From 2 exposures to electronic cigarette vapors, a significant increase in the number of apoptotic and necrotic epithelial cells is highlighted. A western blot test accompanied by NIH imaging, allowing a band ratio calculation, is carried out in order to highlight the expression of a pro-apoptotic gene such as caspase 3 (Fig. 43) (Table 37).

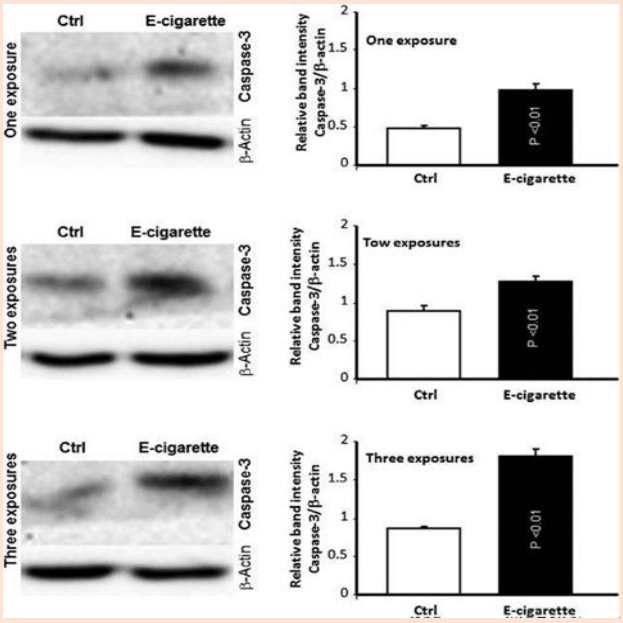

Figure 43: Caspase 3's expression regarding exposition [23].

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Relative Intensity	Control	E cig
D1	0.5	1*
D2	0.8	1.4*
D3	0.8	1.8*

Table 37: Relative intensity.

From the first exposure in vitro, a significant increase in the expression of caspase 3 is noticed, which may explain the elements described above.

Conclusion of the part

Electric cigarette vapors with nicotine are able to act *in-vitro* on epithelial cells directly:

- Decreased cell vitality
- Morphological modification
- Cytoplasmic and membrane damage by activation of LDH
- Apoptosis and cellular necrosis by activation of pro-apoptotic genes. But also indirectly, through contact with exposed pathogens
- Morphological modification
- Decreased cell vitality

Conclusion

This literature review is based on recent reviews, mostly *in-vitro*, on human cells, collected after *in-vivo* sampling or from cancer cell banks.

In-vivo, electric cigarettes appear to cause a decrease in bacterial diversity, ecological pressure for the growth of periodontal pathogens and increased growth of Candida albicans. In addition, the latter, as well as bacteria such as Pg. and Fn, see their virulence increased.

In-vitro and *in-vivo*, the electric cigarette causes the production of free radicals, in turn ensuring cellular and genomic damage, and this via several inflammatory pathways (including COX). The production of pro-inflammatory cytokines is exacerbated.

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The tissue reaction to pathogens is unsuitable and exacerbated, ensuring a local context favoring periodontal disease, the clinical signs of which will be partly masked, as with conventional cigarettes.

In vitro, bone tissue indicates an increase in free radicals after exposure to vapors from electronic cigarettes. The growth of bone cells is reduced, cellular damage is noted. The mineralization of the organic fabric is weakened, and the adhesion of bone cells to implant surfaces is impacted.

Periodontium and epithelial tissue are *in-vitro* subject to decreased activity and fibroblast growth in favor of apoptosis and cell necrosis. Fibroblasts and epithelial cells have an abnormal morphology and appear to be less functional.

It is important to note that all these elements intervene with vapors with or without nicotine. The combustion liquid thus contains intrinsic cytotoxic substances (propylene glycol, aromas, etc.). This is why even without nicotine; we cannot offer the electric cigarette as a long-term substitute for conventional cigarettes.

Conflicts of Interest

The authors declare that have no competing interest and not any conflict of interest.

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