



Influence of dental filling material type on the concentration of interleukin 9 in the samples of gingival crevicular fluid

Uticaj tipa materijala za plombiranje zuba na koncentraciju interleukina 9 u uzorcima gingivalne sulkusne tečnosti

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Abstract

Background/Aim. Several cytokines and lymphokines (IL1 β , ENA78, IL6, TNF α , IL8 and S100A8) are expressed during dental pulp inflammation. Analysis of gingival crevicular fluid (GCF) offers a non-invasive means of studying general host response in oral cavity. Although GCF levels of various mediators could reflect the state of inflammation both in dental pulp and gingiva adjacent to a tooth, GCF samples of those without significant gingivitis could be interpreted as reflection of pulpal process. The aim of this study was to investigate IL9 GCF values in patients with dental caries and to assess possible influence of various dental fillings materials on local IL9 production. **Methods.** The study group included 90 patients, aged 18–70, with inclusion and exclusion criteria in the prospective clinical study. Of the 6 types of material used for the restoration of prepared cavities, 3 were intended for temporary and 3 for definitive restoration. According to dental fillings weight, all the participants were divided into 3 groups: those with fillings lighter than 0.50 g, those with 0.50–1.00 g, and those with fillings heavier than 1.00 g. Samples were taken from gingival sulcus using the filter paper technique. Clinical parameters were determined by bleeding index, plaque index (Silness-Lou, 0–3),

gingival index (0–3), and gingival sulcus depth. Cytokine concentrations were assessed using commercially available cytotoxicity. **Results.** According to the weight of dental fillings, there was a clear decreasement trend of IL9 values meaning that dental defects greater than 1.00 g of dental filling were associated with lower GCF IL9 concentration. The IL9 values correlated with the degree of gingival index and depth of gingival sulcus, being higher with more advanced gingivitis and more pronounced anatomical changes in the tooth edge. Different filling materials exerted various local IL9 responses. Zink polycarbonate cement and amalgam fillings induced a significant and long-lasting local IL9 decreasement, while the use of Tetric EvoCeram and GMA-BISK significantly increased IL9 levels. **Conclusion.** The obtained results indicate that IL9 GCF could be regarded as a measure of odontoblasts' response to the extensivity of dental caries. The type of material used for dental fillings could profoundly alter biological function of gingival and pulpal cells. Also, the results obtained in this study suggest that some materials could even enhance wound repair by modulating macrophage activation.

Key words: dental caries; dental materials; gingival crevicular fluid; cytokines; interleukin-9.

Apstrakt

Uvod/Cilj. Jedan broj citokina i limfokina (IL1 β , ENA78, IL6, TNF α , IL8 i S100A8) izlučuje se tokom upale zubne pulpe. Analiza gingivalne sulkusne tečnosti (*gingival crevicular fluid* – GCF) omogućava neinvazivno proučavanje opšteg

odgovora pacijenta na lokalne promene u usnoj duplji. Iako nivoi GCF mogu da odražavaju stanje upale kako u zubnoj pulpi, tako i u gingivi susednog zuba, uzorci GCF zuba bez značajnijeg gingivitisa mogli bi se tumačiti kao pokazatelji procesa u pulpi. Cilj ovog istraživanja bio je da se ispituju nivoi IL9 u GCF kod pacijenata sa zubnim karijesom i da se

utvrdi eventualni uticaj različitih materijala za zubne ispune na lokalnu proizvodnju IL9. **Metode.** U studiju je bilo uključeno 90 pacijenata, starih od 18 do 70 godina, uz primenu kriterijuma za uključivanje/isključivanje za prospektivne kliničke studije. Od ukupno šest materijala za punjenje pripremljenih kaviteta, tri je korišćeno za privremenu i tri za trajnu ispunu. Prema težini zubnog ispuna, pacijenti su bili podeljeni na tri grupe: oni sa ispunama lakšim od 0,50 g, sa ispunama od 0,50 do 1,00 g i sa ispunama težim od 1,00 g. Uzorci su uzimani iz gingivalnog sulkusa primenom tehnike filter papira. Korišćeni klinički parametri bili su indeks krvarenja, plak indeks (Silness-Lou, 0–3), gingivalni indeks (0–3) i dubina gingivalnog sulkusa. Koncentracije citokina određene su komercijalnim citomiksom. **Rezultati.** Težina zubne ispune ukazivala je na tendenciju opadanja vrednosti IL9, što je značilo da je veće oštećenje zuba, sa zubnom ispunom težom od 1,00 g, praćeno nižom koncentracijom IL9 u GCF. Vrednosti IL9 bile su u korelaciji sa stepenom gingi-

valnog indeksa i dubinom gingivalnog sulkusa, pogotovu u poodmaklom gingivitisu i izraženijim anatomskim promena ivica zuba. Različite ispune izazivale su različite lokalne sekrecije IL9. Cink-polikarbonatni cement i amalgamska ispuna izazvale su značajan i dugotrajan pad nivoa lokalnog IL9, dok je primena tetric-evocerama i GMA-BISK znatno povisila nivo IL9. **Zaključak.** Rezultati dobijeni u ovoj studiji ukazuju da se IL9 u GCF može koristiti kao mera za reakciju odontoblasta na veličinu karijesa. Tip zubne ispune može da promeni biološku funkciju ćelija gingive i pulpe. Rezultati ove studije, takođe, ukazuju i na to da neke vrste ispuna mogu čak da ubrzaju zarastanje rane modulacijom aktivnosti makrofaga.

Ključne reči:
zub, karijes; zub, materijali za punjenje korenskog kanala; gingivalna sulkusna tečnost; citokini; interleukin-9.

Introduction

Intensive inflammatory and immune processes are mediated with numerous cytokines and lymphokines, which were detected in dental tissue and the pulp of the affected teeth, at gene and/or protein level. Dental tissue of carious teeth contains much more IL1b, ENA78, IL6, TNFa, IL8 and S100A8 than samples of healthy teeth¹. Dental pulp of caries affected tooth is associated with high levels of IL-6, IL-8, IL-10, TNF- α and IFN- γ , CXCL10, VEGF, TNFa, and IL2²⁻⁶. Finally, gingival crevicular fluid (GCF) around tooth with caries also shows elevated levels of IL8⁷. Pulpal odontoblasts have several physiological roles. Their basic role of production extracellular dentin structure is supplemented in a carious tooth, because they are crucial in making the reparative dentin. Odontoblasts mediate local inflammatory response both directly and indirectly, with numerous cytokines, lymphokines and antimicrobial peptides^{8,9}. Studying the inflammatory gene expression profile of 96 different mediators in pulp and odontoblasts culture of carious and normal teeth, Horst et al.⁸ reported that there were distinct profiles, and different dominant profiles of cytokines and lymphokines. Both micro-environments had rich expression of chemokines and IL1, but only odontoblasts expressed IL9, IL9R and IL13.

Interleukin 9 is one among mediators which exerts influence on numerous functions of different cell types. Produced by T cells, IL9 induces significant biological response on mast cells, hematopoietic progenitor cells, epithelial cells, smooth muscle cells, antigen presenting cells, B-lymphocytes and T-lymphocytes themselves¹⁰⁻²⁰. IL9 was recognized as a growth factor of mast cells that enhances their survival, production of IL6 and proteases, and induce expression of IgE receptor²¹. Together with IL5, IL9 induces maturation and activation of eosinophils, making it crucial in allergic inflammation^{22,23}. It is assumed that IL9 together with IL5 and IL13 coordinately controls epithelial barrier functions²⁴.

The aim of this study was to investigate the concentration of IL9 in GCF of caries affected teeth and to correlate it with clinical parameters, as well as to follow IL9 dynamics

after dental filling procedures and to evaluate IL9 response to different dental filling materials.

Methods

A total of 90 patients, aged 18–70, were included in this prospective clinical study. The inclusion criteria were the diagnosed approximal caries on frontal and side teeth, the existence of the same type of antagonists or natural teeth for the test or the control group, no fresh post-extraction or traumatic wounds in the restoration area or the area of restored surfaces, no signs of infection in the area of restored surfaces. Also, each patient had to meet the conditions for the duration of one restoration, and with satisfactory level of oral hygiene. The exclusion criteria were the presence of infection of endodontal or periodontal origin in the area of approximal or cervical filling, the presence of prominent periodontal pockets, the presence of fillings that were prominent outside the cavity, the patients who were on immunosuppressive therapy or those with heavy chronic bone metabolic or treated malignant diseases, the patients whose medical history included alcohol and drug abuse problems or mental diseases, those who smoked more than 20 cigarettes a day, those with bad oral hygiene, and unreliable for cooperation.

The monitored clinical parameters included bleeding index on probing (BOP), plaque index (PI, 0–3), gingival index (GI, 0–3), and depth of gingival sulcus (DGS). The second sample was taken from gingival sulcus fifteen days after setting the approximal filling.

All potential participants in the study filled out a form within a dental record in order to get information on their general health and oral condition. The patients were familiarized with the aim of the research, as well as with all of its procedures and duration, and gave written consent to participate in the research.

Before sampling GCF, the DGS was measured out against the approximal caries lesion graded with periodontal probe, and after that the procedure was repeated on the oppo-

site side with the gingival sulcus depth measured out against the surface of a healthy tooth.

Six types of material were used for the restoration of prepared cavities, 3 for temporary and 3 for definitive restoration. The materials used for temporary restoration of cavities were glass ionomer cement – filling group F (GC Fuji PLUS®, Green Circle, USA), zinc phosphate cement – filling group E (Cegal NV, Galenika), and zinc polycarboxylate cement – filling group A (Harvard). The materials used for definitive restoration were amalgam – filling group B (Extracap D caps, Galenika), Beautiful (Shofu, Japan), and Tetric EvoCeram – filling group C (Ivoclar Vivadent). Tetric EvoCeram and Beautiful – filling group D are nanohybrid composite materials that require UV light for binding in the cavity. The other materials in the cavity tend to bind, *ie* harden by themselves.

Upon the removal of caries lesions and rinsing the cavity, a matrix with the appropriate holder was placed into the interdental space, whose role was to prevent sub-gingival impression of the material and, on the other hand, enable construction of the contact point and bringing back the morphology to the restored tooth.

Results

Influence of dental fillings weight on IL9 GCF concentration

According to dental fillings weight, all the participants were divided into 3 groups (those with fillings lighter than 0.50 g, those with 0.50–1.00 g, and those with fillings heavier than 1.00 g). The highest average IL9 concentration was estimated in the samples of the group with the smallest dental defects, and consequently the smallest used dental filling weight (Table 1). The average IL9 concentration before the procedure was significantly decreased in GCF of teeth with biggest defects, that consequently needed more dental filling material. There was a clear decreament trend of IL9 values according to dental filling weight, meaning that larger dental defects were associated with the lower GCF IL9 concentration. The only significant difference in the average IL9 concentration was between the smallest weight filling group (< 0.50 g) and those that needed more than 1.00 g fillings (Mann Whitney $p = 0.0337$), with IL9 higher in those with smaller dental defects. Analysis of individual, serial samples

Table 1

Average interleukin 9 (IL9) gingival cervicular fluid (GCF) concentration according to the investigated parameters

Parameters	IL9 (pg), $\bar{x} \pm SD$		
	before (0)	control I	control II
Dental filling weight (g)			
< 0.50	50 ± 72	74 ± 84	47 ± 84
0.50–1.00	61 ± 74	43 ± 85	21 ± 33
> 1.00	12 ± 17	10 ± 14	17 ± 30
Filling material type			
A – zinc polycarboxilate cement	33 ± 23	13 ± 28	3 ± 5
B – amalgam	31 ± 40	14 ± 29	18 ± 30
C – tetric evoceram	53 ± 26	153 ± 102	117 ± 124
D – beautiful	44 ± 54	110 ± 103	78 ± 125
E – zinc phospate cement	37 ± 35	52 ± 44	30 ± 25
F – glass ionomer cement	42 ± 46	64 ± 52	40 ± 31
GI			
0	32 ± 47	31 ± 39	17 ± 27
1	51 ± 79	76 ± 102	50 ± 92
2	49 ± 69	64 ± 71	32 ± 55
3	29 ± 23	44 ± 32	135 ± 180
PI			
0	31 ± 36	43 ± 41	29 ± 35
1	50 ± 71	71 ± 90	43 ± 82
2	45 ± 71	59 ± 71	42 ± 74
3	42 ± 1	36 ± 40	5 ± 1
BI			
0	48 ± 75	69 ± 96	45 ± 85
1	45 ± 62	62 ± 70	34 ± 53
2	51 ± 75	57 ± 69	49 ± 92
3	21 ± 29	117 ± 75	11 ± 16
DGS			
0	27 ± 32	75 ± 99	74 ± 120
1	39 ± 62	52 ± 76	28 ± 57
2	73 ± 94	84 ± 88	55 ± 90
3	49 ± 51	66 ± 47	38 ± 25

GI – gingival index; PI – plaque index; BI – bleeding index; DGS – depth of gingival values.

showed significant concentration changes in the smallest (Wilcoxon test, O/I $p = 0.0073$, I/II $p = 0.0453$) and the intermediate (I/II $p = 0.0313$) dental fillings groups. There were no significant changes of IL9 in the group treated with dental filling heavier than 1.00 g.

IL9 GCF concentration and different dental filling materials

The highest average IL9 concentration was estimated in the C dental filling group, while the smallest detected was in the A dental filling group, at both check points. After the first 15 days, in the first control interval, the average IL9 values in samples treated with A and B fillings were significantly lower comparing to those estimated in the groups C and D (Table 2). Furthermore, the group with the highest average IL9 level showed a significantly higher concentration than the group with E and F type of dental fillings.

In the second control interval, the only significant difference was IL9 increment detected in the group C comparing with the group A. There were no significant differences between IL9 concentration in the first or the second control interval among the samples of the same group, neither between the average values, nor individual ones in serial samples.

Gingival index and IL9 GCF concentration

Before filling treatment, the smallest average IL9 concentrations were detected in the group with the smallest and the highest value of GI. At the first and the second control, the smallest IL9 concentrations were in the group with GI = 0. There were no significant differences in average IL9 levels between the groups with different GI. Analysis of individual serial samples showed a significant concentration change in the GI = 2 group (I/II $p = 0.0063$).

Plaque index and IL9 GCF concentration

The highest average IL9 level was detected in the samples of the group that had PI = 1, within the first control.

There were no significant differences in the average IL9 levels between the groups with different PI. Analysis of individual, serial samples showed a significant concentration change in the PI = 1 group (I/II $p = 0.0256$).

BOP index and IL9 GCF concentration

The highest average IL9 level was detected in samples of the group that had BOP = 3 within the first control. There were no significant differences in the average IL9 levels among the groups with different BOP. Analysis of individual serial samples showed a significant concentration change in BOP = 1 group (I/O $p = 0.0129$, I/II $p = 0.0124$).

Depth of gingival sulcus and IL9 GCF

The lowest IL9 concentration was detected before the filling treatment in the group without bleeding, contrary to other groups with different BOP values. On the other hand, the highest average IL9 value was detected in the BOP = 0 group within the second control. All these differences were not statistically significant. Analysis of individual, serial samples showed a significant IL9 concentration change in the DGS = 0 group (I/O $p = 0.0481$).

Frequency of IL9 increment in response to type of dental filling used

In the groups treated with various dental filling materials the percent of patients that had IL9 increment in samples comparing with the previous time interval was estimated (Table 3). So, the frequency of IL9 stimulation was estimated within the first control point relative to the basal level, before the treatment (I/O), the second comparing to basal (II/O) and the second comparing with the first control point (II/I).

The most frequent IL9 increase was detected in GCF samples of the patients treated with D, F and than E and C types of dental materials, in more than a half within the first time interval, after 15 days. At the second check point, IL9

Table 2

Statistical analysis of differences in the interleukin 9 (IL9) concentration in the investigated groups treated with various dental fillings[#]

Check points	A/B	A/C	A/D	A/E	A/F	B/C	B/D	B/E	B/F	C/D	C/E	C/F	D/E	D/F	E/F
I	ns	***	**	ns	ns	***	**	ns	ns	ns	**	*	ns	ns	ns
II	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

(* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$); ns – no significant difference; I – control I; II – control II.

[#]A – zinc polycarboxilate cement; B – amalgam; C – Tetric EvoCeram; D – Beautifill; E – zinc phosphate cement; F – glass ionomer cement.

Table 3

Frequency of interleukin 9 (IL9) increment (%) in response to the type of dental filling used[#]

Check points	A	B	C	D	E	F
I / O	29	29	50	67	53	57
II / O	12	27	44	33	31	38
II / I	12	27	33	27	36	27

[#]A – zinc polycarboxilate cement; B – amalgam; C – Tetric EvoCeram;

D – Beautifill; E – zinc phosphate cement; F – glass ionomer cement.

O – before dental filling; I – control I; II – control II.

was frequently increased in C and F types material treated teeth, but the percentage was notably lower comparing to those after the first 15 days. The pattern of IL9 elevation was the same after we compared the second to the first time interval, around one third of those teeth filled with E and C types material had the increase in IL9.

Based on this observation it could be concluded that the materials C, E and F induce local production of IL9 in most GCF samples, and that the A and B materials are IL9 inducers of low potency.

Discussion

The predominant cause of local inflammation in dental pulp is the presence and activity of bacteria originating from oral flora. Interaction of bacterial products with dental pulp immune cells realized through dental tubules results in the induction of local immune reaction by dental carious lesion²⁵. The consequent inflammation is the product of mediators produced and released by various immune and non-immune cells in dental pulp, such as TNF α , IFN γ , IL1b, IL6, IL10 and NO²⁶⁻²⁸. Deep invasion of bacteria into tooth structures induces a significant mononuclear infiltration of dental pulp. Microbial products, probably through toll receptors, stimulate dental pulp fibroblasts to produce chemoattractants needed for inflammatory cell accumulation. Takahashi et al.²⁹ report that CCL20 mRNA is highly expressed in inflamed comparing to healthy pulp, on fibroblasts, endothelial cells and macrophages. Generally, it could be expected that the size and the duration of dental lesion correspond to the level of local dental pulp inflammation. Karapanou et al.⁷ show that GCF samples of caries affected teeth could be valuable in the assessment of staging acute pulpitis. The level of CXCL8 in GCF was sensitive biological inflammatory marker, which highly correlates with pulpitis and subjective feel of pain that had a high value even in adjacent teeth to the affected one, and had decreased GCF values in those patients who received local anesthesia before sampling.

To our knowledge there has been no published data concerning IL9 in GCF till now. Innate lymphoid cells (ILC2 type) together with Th2 and Th9 lymphocytes are considered as main producers of IL9, along with the so-called type 2 cytokines IL4, IL5 and IL13^{30,31}. In physiological conditions these cytokines coordinately control epithelial barrier functions, inducing goblet cells proliferation and production of mucus. They also induce polarization and activation of certain functional macrophage types, important for tissue repair. Since data on IL9 in pulp or gingival tissue are seldom, all the results of our study could be interpreted with speculation. It is reasonable to suppose that dental caries that lasts long and which is extensive enough induce a significant pulp inflammation^{7,29}. In our study, larger dental lesions that needed more than 1.00 g of dental filling were accompanied with low concentrations of IL9 GCF. This finding indicates that more extensive dental caries is mediated with cytokines that down-regulate local IL9 production, or *vice versa*, that the presence of IL9 in GCF could implicate the capability to confine destruction of dental tissue.

According to the accepted view, physiological role of IL9 is represented in local response of innate lymphoid cells type 2 (ILC2) to IL33 and IL25 produced after damage of epithelial tissue with viruses, helminthes, and allergens²⁴. Locally produced IL9 induce other ILC and T to produce IL5 and IL13 and mediate in restoration of epithelial integrity. Since there is no correlation of IL9 and IL13 values in GCF of our patients (unpublished data), it is hard to assume that the same mechanism of IL9 is operative in tooth microenvironment.

The importance of IL9 is now recognized in allergic and chronic inflammation, so the other speculation would be that IL9 GCF levels correspond to the degree of inflammatory process, both in the pulp and gingiva. In the samples of our patients, IL9 values correlated with the degree of gingival index and the depth of gingival sulcus, being higher with more advanced gingivitis and more pronounced anatomical changes in tooth ledge^{32,33}. The patients with most intensive gingivitis had the highest average IL9 GCF values 30 days after filling procedures, contrary to those without any signs of gingival inflammation, where the average IL9 level was significantly reduced. Clearly, inflammation that takes place in gingiva and those happening only in dental pulp could have completely different mechanisms. While intensive inflammation in gingiva would be mediated with T lymphocytes, macrophages and neutrophils, mild dental pulp inflammation would include odontoblasts, dental pulp fibroblasts and rare immune cells, which will generate different mediator profile. There also could be a possibility that a certain type of oral microorganism could selectively induce local IL9 production in susceptible person, like *Lactobacillus casei* that induce a strong IL10 response, *P. alactolyticis* that induces predominantly type 2 cytokine local production and *S. mutans* that induce IFN γ mediated response in early pulpitis³⁴⁻³⁶.

Horst et al.⁸ show that caries induces strong response in pulp and odontoblast layer, represented by the expression of various cytokine and chemokine genes. It is important that each microenvironment, pulp and odontoblast layers, has a different dominant profile of mediators. While the pulp of carious teeth was rich with expression of various chemokines and IL1, cytokine production in odontoblast layer was dominated by IL8, IL1a, IFN α , IL9, IL9R, IL13 and chemokines. This was in concordance with the authors' hypothesis that primary role in local tooth immune response is carried by odontoblasts, which use and are governed by these cytokines to produce antibacterial proteins. In line with this attitude, our results indicate that IL9 GCF could be accepted as a measure of odontoblasts response to the extensity of dental caries.

The results of our study show that different filling materials exert various local IL9 responses. Zink polycarbonate cement and amalgam fillings induced a significant and long-lasting local IL9 decrement, while the use of tetric evoceram and GMA-BISK significantly increased IL9 levels at both check points. The frequency of patients who responded with IL9 GCF increase was highest in the Tetro EvoCeram group, but even the average IL9 level was insignificantly elevated, almost 40% of zink polycarboxilate cement treated patients showed IL9 elevation.

There are no data on direct influence of Tetric EvoCeram on cytokine production, while there are reports on fluoride release from tetric evoceram dental fillings, which was significantly associated with local mediator micro environment. Naoum et al.³⁷ show that the 4 different materials tested *in vitro* (Beautiful II, Tetric EvoCeram, Gradia Direct X, and Fuji IX Extra) differ in their mechanical stability, and ability to release or recharge fluoride. There are several lines of evidence based on *in vitro* experiments on ameloblast cell lines that showed profound influence of fluoride on cytokine production and cell functions. Riksen et al.³⁸ showed that exposing of cells to sodium fluoride for various time induced reduced cell proliferation, decreased production of VEGF, MCP1 and IP10 and decreased mRNA expression of structural enamel proteins (amelogenin, ameloblastin, enamelin, enamel protease MMP-20). Kubota et al.³⁹ show that fluoride concentration higher than those in drinking water caused endoplasmic reticulum stress in cultivated ameloblasts. Few animal experiments pointed out that even a 7-day fluoride supplementation given through drinking water resulted in systemic effects on the whole organism, reflected in increased levels of serum cytokines IL2, IL6 and TNF α .⁴⁰ The influences of mineral trioxide aggregate (MTA), calcium hydroxide (Life) and zinc oxide eugenol based materials were assessed on human osteosarcoma cell line (U2OS) through evaluation of cell attachment and cytokine production. The best degree of osteosarcoma cells attachment was to MTA, together with the higher levels of IL4 and IL10 produced⁴¹. The TEC group in our study showed the highest average IL9 GCF value, both at 7 day and 30 day controls.

Restoration of amalgam dental filling with other materials could even have systemic effects. Bjorkman et al.⁴² reports that the patients with amalgam fillings have increased serum values of IL12, IL7, IFN α , IL6, GM-CSF and IL2R comparing to the controls. The use of different dental filling materials instead of amalgam in these patients induced reduction of serum cytokine levels. Immunocytochemical

analysis of cultivated fibroblasts from periodontal ligament after exposure to various dental materials showed the highest collagen expression after 24 h incubation with MTA, but the group exposed to Portland cement demonstrated the highest late (7 days) production of collagen, fibronectin and TGF β .⁴³ In this system, amalgam showed the weakest influence on connective structures. Modified Portland cement and MTA did not exhibit any cytotoxic activity on mouse fibroblast cell line L929⁴⁴. Both materials induced IL1 β cytokine production after a short-term culture (24 h), without differences between them.

The type of material used for dental fillings could profoundly alter biological function of gingival and pulpal cells. Materials with more rough surfaces induced, at least *in vitro* on macrophage cell line, transformation of M2 like phenotype, with increased MCP1 and MIP1 α production and without arginase 1 and NOS expression⁴⁵. These data indicate that some materials could even enhance wound repair by modulating macrophage activation.

Conclusion

The obtained results indicate that IL9 in GCF could be regarded as a measure of odontoblasts' response to extensity of dental caries. The type of material used for dental fillings could profoundly alter biological function of gingival and pulpal cells. Also, the results obtained in this study suggest that some materials could even enhance wound repair by modulating macrophage activation.

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R E F E R E N C E S

1. McLachlan JL, Sloan AJ, Smith AJ, Landini G, Cooper PR. S100 and Cytokine Expression in Caries. *Infect Immun* 2004; 72(7): 4102–8.
2. Elsalhy M, Azizi F, Ragbupathy R. Cytokines as diagnostic markers of pulpal inflammation. *Int Endod J* 2013; 46(6): 573–80.
3. Adachi T, Nakanishi T, Yumoto H, Hirao K, Takahashi K, Mukai K, et al. Caries-related bacteria and cytokines induce CXCL10 in dental pulp. *J Dent Res* 2007; 86(12): 1217–22.
4. Artese L, Rubini C, Ferrero G, Fioroni M, Santinelli A, Piattelli A. Vascular endothelial growth factor (VEGF) expression in healthy and inflamed human dental pulps. *J Endod* 2002; 28(1): 20–3.
5. Kokkas AB, Goulas A, Varsamidis K, Mirtsoy V, Tzioufas D. Irreversible but not reversible pulpitis is associated with up-regulation of tumour necrosis factor- α gene expression in human pulp. *Int Endod J* 2007; 40(3): 198–203.
6. Rauschenberger CR, Bailey JC, Cootanco CJ. Detection of human IL-2 in normal and inflamed dental pulps. *J Endod* 1997; 23(6): 366–70.
7. Karapanou V, Kempuraj D, Theoharides TC. Interleukin-8 Is Increased in Gingival Crevicular Fluid from Patients with Acute Pulpitis. *J Endod* 2008; 34(2): 148–51.
8. Horst OV, Horst JA, Samudrala R, Dale BA. Caries induced cytokine network in the odontoblast layer of human teeth. *BMC Immunol* 2011; 12(1): 9.
9. Farges J, Keller J, Carron F, Durand SH, Romeas A, Bleicher F, et al. Odontoblasts in the dental pulp immune response. *J Exp Zool B Mol Dev Evol B* 2009; 312B(5): 425–36.
10. Beriou G, Bradshaw EM, Lozano E, Costantino CM, Hastings WD, Orban T, et al. TGF- β induces IL-9 production from human Th17 cells. *J Immunol* 2010; 185(1): 46–54.
11. Matsuzawa S, Sakashita K, Kinoshita T, Ito S, Yamashita T, Koike K. IL-9 enhances the growth of human mast cell progenitors under stimulation with stem cell factor. *J Immunol* 2003; 170(7): 3461–7.
12. Wiener Z, Falus A, Toth S. IL-9 increases the expression of several cytokines in activated mast cells, while the IL-9-induced IL-9 production is inhibited in mast cells of histamine-free transgenic mice. *Cytokine* 2004; 26(3): 122–30.

13. Lemoli RM, Fortuna A, Fogli M, Motta MR, Rizzi S, Benini C, et al. Stem cell factor (c-kit ligand) enhances the interleukin-9-dependent proliferation of human CD34+ and CD34+CD33-DR- cells. *Exp Hematol* 1994; 22(9): 919–23.
14. Fujiki H, Kimura T, Minamiguchi H, Harada S, Wang J, Nakao M, et al. Role of human interleukin-9 as a megakaryocyte potentiator in culture. *Exp Hematol* 2002; 30(12): 1373–80.
15. Steenwinckel V, Louahed J, Lemaire MM, Sommereyns C, Warnier G, McKenzie A, et al. IL-9 promotes IL-13-dependent paneth cell hyperplasia and up-regulation of innate immunity mediators in intestinal mucosa. *J Immunol* 2009; 182(8): 4737–43.
16. Yamasaki A, Saleh A, Koussib L, Muro S, Halayko AJ, Gounni AS. IL-9 induces CCL11 expression via STAT3 signalling in human airway smooth muscle cells. *PLoS One* 2010; 5(2): e9178.
17. Pilette C, Ouadrhiri Y, van Snick J, Renauld JC, Staquet P, Vaerman JP, et al. IL9 inhibits oxidative burst and TNF- α release in lipopolysaccharide-stimulated human monocytes through TGF- β . *J Immunol* 2002; 168(8): 4103–11.
18. Fawaz LM, Sharif-Askari E, Hajoui O, Soussi-Gounni A, Hamid Q, Mazer BD. Expression of IL-9 receptor alpha chain on human germinal center B cells modulates IgE secretion. *J Allergy Clin Immunol* 2007; 120(5): 1208–15.
19. Nowak EC, Weaver CT, Turner H, Begum-Haque S, Becher B, Schreiner B, et al. IL-9 as a mediator of Th17-driven inflammatory disease. *J Exp Med* 2009; 206(8): 1653–60.
20. Ehyaman W, Bradshaw EM, Uyttenhove C, Dardalbon V, Awasthi A, Imitola J, et al. IL-9 induces differentiation of TH17 cells and enhances function of FoxP3+ natural regulatory T cells. *Proc Natl Acad Sci USA* 2009; 106(31): 12885–90.
21. Hültner L, Druetz C, Moeller J, Uyttenhove C, Schmitt E, Rüde E, et al. Mast cell growth-enhancing activity (MEA) is structurally related and functionally identical to the novel mouse T cell growth factor P40/TCGFIII (interleukin 9). *Eur J Immunol* 1990; 20(6): 1413–6.
22. Louahed J, Zhou Y, Maloy WL, Rani PU, Weiss C, Tomer Y, et al. Interleukin 9 promotes influx and local maturation of eosinophils. *Blood* 2001; 97(4): 1035–42.
23. Gounni AS, Gregory B, Nutku E, Aris F, Latifa K, Minshall E, et al. Interleukin-9 enhances interleukin-5 receptor expression, differentiation, and survival of human eosinophils. *Blood* 2000; 96(6): 2163–71.
24. Wilhelm C, Stockinger B. Innate Lymphoid Cells and Type 2 (Th2) Mediated Immune Responses - Pathogenic or Beneficial. *Front Immunol* 2011; 2: 68.
25. Love RM, Jenkinson HF. Invasion of dental tubules by oral bacteria. *Crit Rev Oral Biol Med* 2002; 13(2): 171–83.
26. Farges J, Keller J, Carrouel F, Durand SH, Romeas A, Bleicher F, et al. Odontoblasts in the dental pulp immune response. *J Exp Zool B Mol Dev Evol* 2009; 312B(5): 425–36.
27. Connelly L, Palacios-Callender M, Ameixa C, Moncada S, Hobbs AJ. Biphasic Regulation of NF- κ B Activity Underlies the Pro- and Anti-Inflammatory Actions of Nitric Oxide. *J Immunol* 2001; 166(6): 3873–81.
28. di Maio FD, Lohinai Z, d'Arcangelo C, de Fazio PE, Speranza L, de Lutiis MA, et al. Nitric Oxide Synthase in Healthy and Inflamed Human Dental Pulp. *J Dent Res* 2004; 83(4): 312–6.
29. Takahashi K, Nakanishi T, Yumoto H, Adachi T, Matsuo T. CCL20 production is induced in human dental pulp upon stimulation by *Streptococcus mutans* and proinflammatory cytokines. *Oral Microbiol Immunol* 2008; 23(4): 320–7.
30. Wilhelm C, Turner J, van Snick J, Stockinger B. The many lives of IL-9: a question of survival. *Nat Immunol* 2012; 13(7): 637–41.
31. Palm NW, Rosenstein RK, Medzhitov R. Allergic host defences. *Nature* 2012; 484(7395): 465–72.
32. Yao W, Zhang Y, Jabeen R, Nguyen ET, Wilkes DS, Tepper RS, et al. Interleukin-9 is required for allergic airway inflammation mediated by the cytokine TSLP. *Immunity* 2013; 38(2): 360–72.
33. Gregersen I, Skjelland M, Holm S, Holven KB, Krogh-Sørensen K, Russell D, et al. Increased Systemic and Local Interleukin 9 Levels in Patients with Carotid and Coronary Atherosclerosis. *PLoS One* 2013; 8(8): e72769.
34. Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* 2005; 115(6): 1260–7.
35. Habn C, Liewehr F. Relationships between Caries Bacteria, Host Responses, and Clinical Signs and Symptoms of Pulpitis. *J Endod* 2007; 33(3): 213–9.
36. Habn CL, Best AM, Tew JG. Cytokine induction by *Streptococcus mutans* and pulpal pathogenesis. *Infect Immun* 2000; 68(12): 6785–9.
37. Naoum S, Martin E, Ellakwa A. Long-Term Fluoride Exchanges at Restoration Surfaces and Effects on Surface Mechanical Properties. *ISRN Dent* 2013; 2013: 579039.
38. Riksen EA, Kalvik A, Brookes S, Hynne A, Snead ML, Lyngstadaa SP, et al. Fluoride reduces the expression of enamel proteins and cytokines in an ameloblast-derived cell line. *Arch Oral Biol* 2011; 56(4): 324–30.
39. Kubota K, Lee DH, Tsuchiya M, Young CS, Everett ET, Martinez-Mier EA, et al. Fluoride Induces Endoplasmic Reticulum Stress in Ameloblasts Responsible for Dental Enamel Formation. *J Biol Chem* 2005; 280(24): 23194–202.
40. Afolabi OK, Oyevo EB, Adekunle AS, Adedosu OT, Adediji AL. Oxidative indices correlate with dyslipidemia and pro-inflammatory cytokine levels in fluoride-exposed rats. *Arch Hig Rada Toksikol* 2013; 64(4): 521–9.
41. Huang TH, Yang CC, Ding SJ, Yeng M, Kao CT, Chou MY. Inflammatory cytokines reaction elicited by root-end filling materials. *J Biomed Mater Res B Appl Biomater* 2005; 73(1): 123–8.
42. Björkman L, Brokstad KA, Moen K, Jonsson R. Minor changes in serum levels of cytokines after removal of amalgam restorations. *Toxicol Lett* 2012; 211(2): 120–5.
43. Fayazi S, Ostad SN, Razmi H. Effect of ProRoot MTA, Portland cement, and amalgam on the expression of fibronectin, collagen I, and TGF β by human periodontal ligament fibroblasts in vitro. *Indian J Dent Res* 2011; 22(2): 190–4.
44. Filho GJ, Cintra LT, Junior DE, Watanabe S, Faria MD, Gomes AC, et al. Effects of modified Portland cement and MTA on fibroblast viability and cytokine production. *Dental Press Endod* 2012; 2(2): 20–24.
45. Barth KA, Waterfield JD, Brunette DM. The effect of surface roughness on RAW 264. macrophage phenotype. *J Biomed Mater Res A* 2013; 101(9): 2679–88.

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