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Cytokine release from human leukocytes exposed to silorane- and methacrylate-based dental materials

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ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form
8 November 2011
Accepted 9 March 2012

Keywords:
Silorane
Methacrylate
Cytokine
Allergic reaction
Immune response
In vitro testing
Biocompatibility
Cytokine release assay
Sensitization
Immune toxicology

ABSTRACT

Objectives. Silorane-based dental monomers contain an epoxy functional group. Less is known about the toxicological and inflammatory potential of silorane-based composites. Therefore we compared the release of 24 cytokines from human leukocytes after incubation with silorane-based Filtek™ Silorane (Silo) and methacrylate-based TetricEvo Flow® (TC).

Methods. Leukocytes from nine healthy test persons (P) were incubated with Silo or TC for up to 72 h. All 24 h cytokines were quantified with a magnetic bead assay.

Results. Silo stimulates the leukocytes to higher release of cytokines when compared to TC. 72 h after beginning the experiment, leukocytes from P6 incubated with Silo secreted more than an 18-fold amount of interleukin (IL)-6 when compared with leukocytes incubated with TC (771.8 vs 42.1 pg/ml).

Only leukocytes from P8 incubated with Silo release up to 14.4 pg/ml IL-2 after 72 h.

Significance. The significantly higher induction of cytokines with Silo in comparison to TC is test person independent. This indicates a higher sensitization potential for Silo. Because of the cytokine release pattern (especially the release of T-cell dependent IL-2) from leukocytes from P8 after incubation with Silo it is likely that P8 can develop an allergic Type IV sensitization to Silo. Therefore the cytokine release assay is a helpful tool for providing information about possible immunological reactions to dental resins in individual cases as well as for a general risk assessment and comparison between different dental materials.

1. Introduction

The inflammatory mediators are a large family of molecules which play an important role in numerous physiological and pathological processes in our organism [1]. Within this cross-serial regulatory network they can have additive/synergistic as well as antagonistic effects. Inflammatory mediators can be released from different type of cells, e.g. from peripheral (blood) leukocytes after their stimulation. To start an immune response, human leukocytes can produce different inflammatory mediators like polypeptide hormones, complement factors, coagulation factors, enzymes like proteases and lipases [2]. Cytokines, a subgroup of the polypeptide

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doi:10.1016/j.dental.2012.03.009

hormones, interact with specific receptors and can trigger autocrine, paracrine and endocrine reactions by the release of proinflammatory substances, e.g. interleukin (IL)-1, IL-6, IL-10 and tumor necrosis factor (TNF)-α [2].

The release of inflammatory mediators from cells like human leukocytes can be caused for different reasons, e.g. defence against microorganisms and parasites. In our modern times this release can also be stimulated by xenobiotic substances like 2-Hydroxyethyl methacrylate (HEMA) or Triethylene glycol dimethacrylate (TEGDMA) [3] or erosion particles from methacrylate based polymer networks [4]. In relation to methacrylate based materials it is known that they can cause allergic reactions in patient and dental personal as well as cytotoxic and genotoxic effects in vitro [5–7]. One reason for these effects is the release of dental monomers and additives after polymerization of the dental material because of the low conversion rate of about 55–65% [8,9].

A new class of monomers, named silorane, an acronym for siloaxane and oxirane, was presented a few years ago [10]. Filtek™ Silorane has lower polymerization shrinkage and a higher depth of cure compared with a lot of methacrylate based dental composites [11]. However less data is available regarding possible cytotoxic effects and the release of substances from the polymerized silorane.

The release of components from dental materials has a marked influence on the structural stability and especially on the biocompatibility of the material. The induction or alterations in the regulation of inflammatory markers can be helpful in the assessment of the biocompatibility of the dental composite [12]. Particularly in the early stages, after exposition to dental composites as well as by exposure to sublethal concentrations the secretion and expression of inflammatory markers is an important indicator for latent cellular damage.

Therefore the aim of our study was to compare the amount and the releasing kinetic of 24 inflammatory markers after exposure of human leukocytes to the dental methacrylate based restorative material TetricEvo Flow® and the silorane based restorative material Filtek™ Silorane to the 72 h after beginning the experiment.

2. Materials and methods

2.1. Preparation of specimen

From the light-curable tooth restorative materials TetricEvo Flow® (TC) (Ivoclar Vivadent, Ellwangen, Germany; LOT M61775) and Filtek™ Silorane (Silo) (3M ESPE, Seefeld, Germany; LOT 0FM) specimens of approximately 100 mg (thickness of 1.8 mm, diameter of 6 mm; color A2, with a resulting surface of the cylinder of 90.4 mm^2) were prepared under photolaboratory conditions. The specimens were covered with plastic matrix strips (Frasaco, Tettnang, Germany) and polymerized according to the instructions of the manufacturer by using an Astralis 10® light source (Ivoclar Vivadent).

2.2. Cell culture

9 ml blood was taken from 9 healthy test persons (P) (8 female, 1 male) in a Li-heparin tube (Becton Dickinson Vacutainer®, Heidelberg, Germany). 500 μl Roswell Park Memorial Institute medium (RPMI)-1640 medium (Sigma–Aldrich, Steinheim, Germany) were added to 500 μl whole-blood sample in 5 ml tubes (Sarstedt, Nuermbracht, Germany; LOT 1071001) under cell culture conditions. From the blood from each test person 4 tubes were prepared. 100 μl lectin from *Phytolacca americana* (Sigma–Aldrich; LOT 110M77102V) were added to tube 1 (positive control). Silo or TC were added to tubes 2 and 3, respectively. No further substances were added to tube 4 (negative control). The cells were stored at 37 °C in 5% (v/v) CO_2 atmosphere. 24, 48 and 72 h after the beginning of the experiment 50 μl from the cell free supernatant were taken and stored at −20 °C.

2.3. Quantification of cytokines

Inflammatory mediators were quantified with the magnetic bead assay Bio-Plex Pro™ (Bio Rad Laboratories, Munich, Germany; LOT 310009465) test kit using the Bio-Plex® detection system and software (Bio Rad Laboratories). Following mediators were quantified: IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL15, IL-17, granulocyte colony-stimulating factor (G-CSF), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1); major Cdk9-interacting elongation factor (MCAF)), macrophage inflammatory proteins (MIP)-1β, MIP-1α, TNF-α, vascular endothelial growth factor (VGEF), Eotaxin, fibroblast growth factor (FGF) basic, granulocyte-macrophage colony-stimulating factor (GM-CSF). The treatment of the samples as well as standard dilution and calibration followed the manufacturer's instructions. Briefly, all standards and samples were equilibrated to 25 °C. The test was performed on 96-well filter plates. After wetting the filter plates with assay buffer and removing it, 50 μl of the coupled bead solution was added to each well. Then 50 μl of the standard or sample were added to the wells, respectively, and incubated for 30 min at 25 °C with constant shaking (300 rpm). Afterwards, each well was washed three times with wash buffer and 25 μl of the solution with detection antibodies was added to each well and incubated for 30 min at 25 °C with constant shaking (300 rpm). Next, each well was washed three times with wash buffer and 50 μl streptavidin–phycoerythrin (streptavidin–pe) solution was added to each well and incubated for 10 min at 25 °C with constant shaking (300 rpm). Hereafter, each well was washed three times with wash buffer and 125 μl assay buffer were added to each well and shaken for 30 s at 1100 rpm. Then the 96-well filter plates were analyzed in the Bio-Plex® detection system.

2.4. Calculations and statistics

The data were analyzed with the Bio-Plex Manager™ software. From the measured amount of inflammatory mediators in the leukocyte cultures incubated with Silo or TC, the amount of inflammatory mediators measured in the native culture was subtracted. P8 was excluded from the statistics and separately discussed. The data are presented as mean ± CV. The statistical significance ($p < 0.05$) of the differences between the release of inflammatory mediators caused

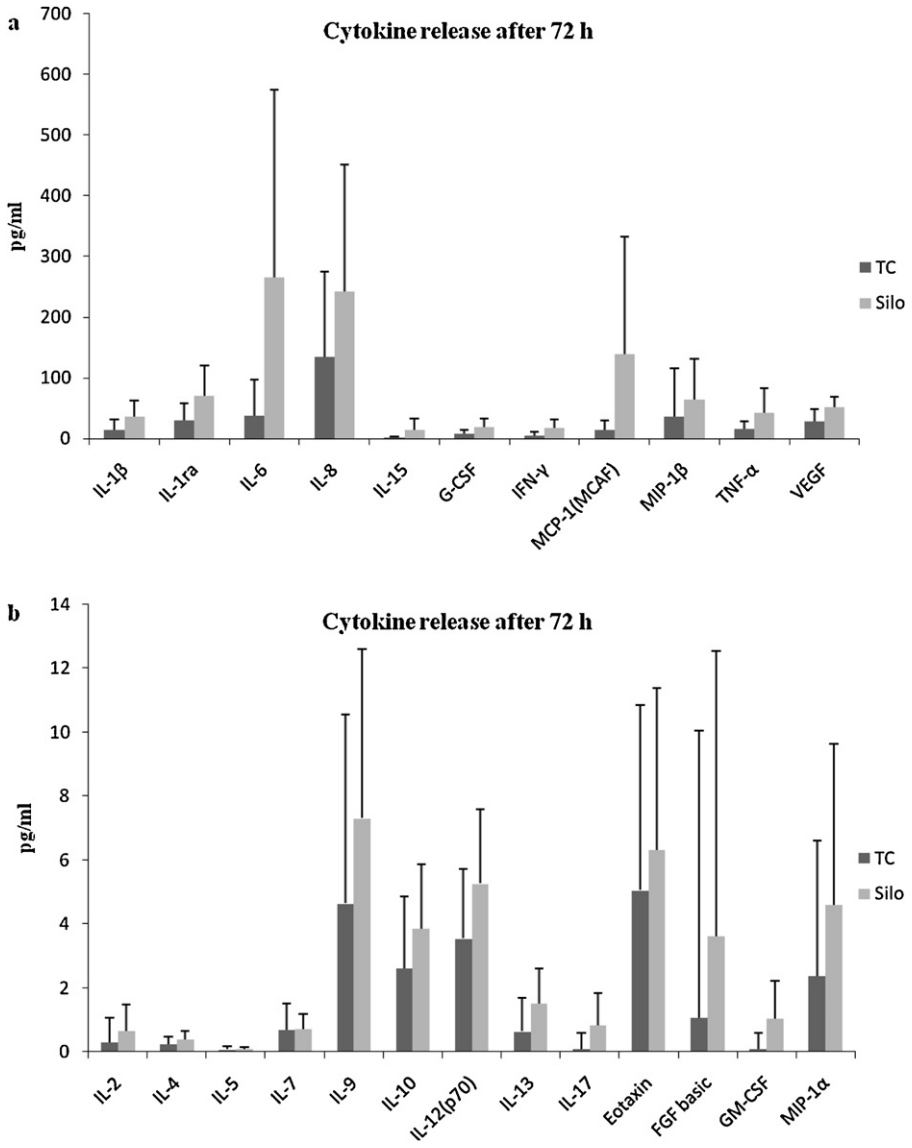

Fig. 1 – (a and b) Amount of cytokines (pg/ml ± SD) released from human leukocytes after 72 h after incubation with specimen from Filtek™ Silorane (Silo) or TetricEvo Flow® (TC). Abbreviations used: IL, interleukine; G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon-gamma; MCP-1, monocyte chemoattractant protein-1 (=major Cdk9-interacting elongation factor (MCAF)); MIP, macrophage inflammatory proteins; TNF, tumor necrosis factor; VGEF, vascular endothelial growth factor; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

by the dental restoratives was tested using the t-test, corrected according to Bonferroni–Holm [13].

2.5. *Declaration*

These experiments comply with the current laws of Germany.

3. Results

The amounts of the inflammatory mediators in the standards was in the range defined by the manufacturer.

Higher amounts of all measured inflammatory mediators were found after incubation of human leukocytes with Silo as compared to TC after 24, 48 and 72 h (Fig. 1a and b). In detail the differences of the IL-6, TNF-α and IL-1β release kinetic from leukocytes after incubation with Silo or TC from P6, P8 and P9 are shown in Figs. 2–4. 72 h after the beginning of the experiment leukocytes from P6 incubated with Silo secreted more than an 18-fold amount of IL-6 as compared to the leukocytes incubated with TC (771.8 vs 42.1 pg/ml) (Fig. 2a), the 2-fold amount of IL-1β (99.3 vs 35.9 pg/ml) (Fig. 3a) and the 3-fold amount of TNF-α (87.2 vs 24.4 pg/ml) (Fig. 4a). Leukocytes from P9 incubated with Silo secreted more than the 9-fold amount of IL-6 compared with leukocytes incubated with TC (50.5 vs 5.6 pg/ml) (Fig. 2b), the 6-fold amount of IL-1β (20.0 vs 3.1 pg/ml) (Fig. 3b) and the 4-fold amount of TNF-α (24.4 vs 5.7 pg/ml) (Fig. 4b).

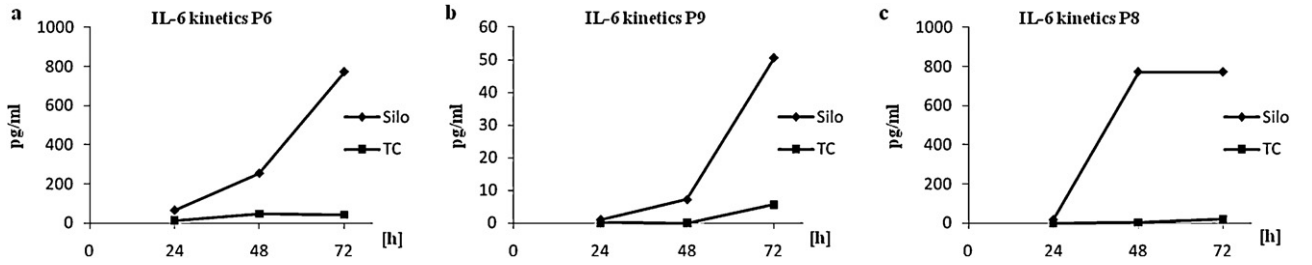

Fig. 2 – (a–c) Amount of interleukin (IL)-6 (pg/ml) released from human leukocytes from the test persons P6, P8 and P9 24, 48 and 72 h after incubation with specimen from Filtek™ Silorane (Silo) or TetricEvo Flow® (TC). The releasing kinetic showed that leukocytes from P6, P8 and P9 released more IL-6 after incubation with Silo compared to TC.

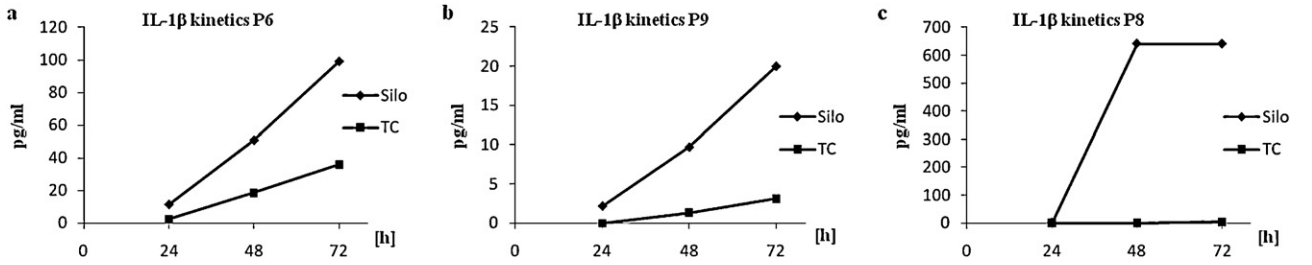

Fig. 3 – (a–c) Amount of interleukin (IL)-1β (pg/ml) released from human leukocytes from the test persons P6, P8 and P9 24, 48 and 72 h after incubation with specimen from Filtek™ Silorane (Silo) or TetricEvo Flow® (TC). The releasing kinetic showed that leukocytes from P6, P8 and P9 released more IL-1β after incubation with Silo compared to TC.

The amounts of measured inflammatory mediators vary significantly between the test persons, shown for IL-6 (Fig. 2a and b), IL-1β (Fig. 3a and b) and TNF-α (Fig. 4a and b). 72 h after the beginning of the experiment leukocytes incubated with Silo from P6 secreted more than the 15-fold amount of IL-6 as compared with leukocytes from P9 (771.8 vs 50.5 pg/ml) (Fig. 2a and b), the 4-fold amount of IL-1β (99.3 vs 20.0 pg/ml) (Fig. 3a and b) and the 4-fold amount of TNF-α (87.2 vs 20.4 pg/ml) (Fig. 4a and b).

The increase of the inflammatory mediators over time was in almost linear in some cases while in other cases more exponential, e.g. IL-1β release from leukocytes from P2 after incubation with Silo showed a linear release kinetics ($y = 0.977x - 22.74$; $R^2 = 0.999$; Fig. 5) and from P3 showed an exponential release kinetics ($y = 0.353e^{0.057x}$; $R^2 = 0.998$; Fig. 5).

The release of IL-2 from leukocytes from all test persons with exception of P8 was not detectable independent of incubation with Silo or TC 24 and 48 h after the beginning of the experiment. 72 h after the beginning of the experiments the leukocytes from P1 release 2.6 pg/ml IL-2 after incubation with Silo and 2.0 pg/ml after incubation with TC. The leukocytes from P8 released no measurable amounts of IL-2 up to 72 h after incubation with TC. After incubation with Silo the released amounts of IL-2 were 5.4 pg/ml 48 h and 14.4 pg/ml 72 h after the beginning of the experiment.

72 h after the beginning of the experiments leukocytes incubated with Silo or TC from P6 released 3.1 vs <0.1 pg/ml IL-10, from P9 7.2 vs 1.5 pg/ml and from P8 59.8 vs 1.0 pg/ml.

4. Discussion

Attention has been directed to studies addressing the cytotoxic, estrogenic and genotoxic potential of dental composites, because of the degree of exposure to eluted components from the dental materials like residual (co)monomers, additives

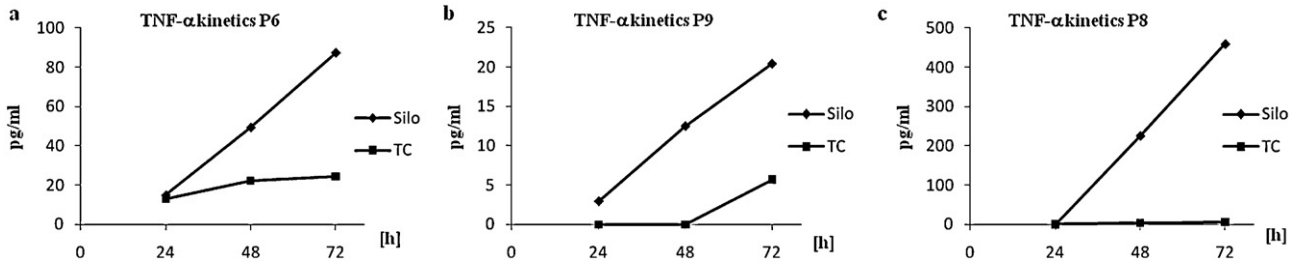

Fig. 4 – (a–c) Amount of tumor necrosis factor (TNF)-α (pg/ml) released from human leukocytes from the test persons P6, P8 and P9 24, 48 and 72 h after incubation with specimen from Filtek™ Silorane (Silo) or TetricEvo Flow® (TC). The releasing kinetic showed that leukocytes from P6, P8 and P9 released more TNF-α after incubation with Silo compared to TC.

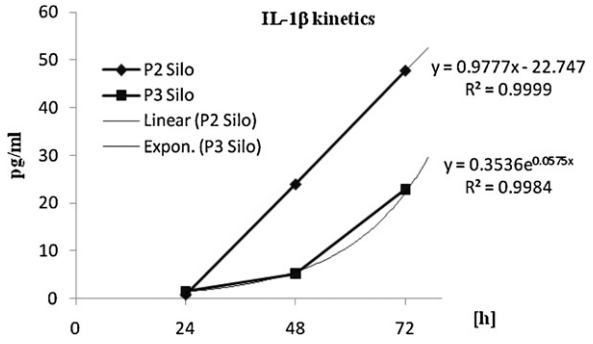

Fig. 5 – Amount of interleukin (IL)-1β (pg/ml) released from human leukocytes from the test persons P2 and P3 24, 48 and 72 h after incubation with specimen from Filtek™ Silorane (Silo). The releasing kinetic of IL-1β from leukocytes from P2 is nearly linear and from P3 nearly exponential.

and degradation products into the oral cavity and into the saliva [7,8]. The release of inflammatory mediators is also important because some cytotoxic processes are mediated by inflammatory mediators and immunotoxic processes can be initiated. Moreover some authors postulate that "inflammatory mediators produced in periodontal tissues, might enter the bloodstream, causing systemic effects and/or contributing to systemic diseases" [14].

TNF-α and IL-1β were matched to the primary cytokines, that means that they can initiate the inflammatory reaction [15]. Our results showed that more TNF-α and IL-1β were released after incubation of human leukocytes with Silo as compared to TC in all test persons. The amount of cytokine release varied with the human leukocytes from different test persons. The release of TNF-α (Fig. 3a and b) and of IL-1β (Fig. 4a and b) from leukocytes from P6 is higher compared to leukocytes from P9. The release kinetics of TNF-α (Fig. 3a and b) and of IL-1β (Fig. 4a and b) however clearly showed that in both cases Silo induced higher amounts when compared to TC. The individual variability in the inflammatory response is well known [16]. The variability relates not only to the amount of cytokines, but also the releasing kinetic (linear vs exponential release; Fig. 5).

The same results as those for TNF-α and IL-1β were found for IL-6 and the other measured cytokines. IL-6 can be released from various cells, e.g. monocytes, T- und B-lymphocytes after stimulation with lipopolysaccharides (LPS), TNF-α, IL-1β and IFN-γ [17]. IL-6 has influence on cell proliferation and differentiation of cytotoxic T-cells.

After the induction of the inflammatory cascade the anti-inflammatory cascade is activated. IL-10 is one of the most important cytokines with anti-inflammatory attributes [18]. IL-10 inhibits the release of inflammatory mediators from monocytes and macrophages, it up-regulates the soluble IL-1ra and inhibits the formation of the induceable NO-syntheses (iNOS) and therefore the formation of NO [19]. Comparable anti-inflammatory mechanisms are found for IL-13 [20]. By analyzing the measured cytokine panel it could be clearly demonstrated that the leukocytes from test persons releasing high amounts of inflammatory cytokines also released high amounts of anti-inflammatory cytokines stimulated by the feedback inhibition of the inflammatory cytokines [21].

One reason why it is important to know if materials can have an inflammatory potential is that the release of one cytokine can stimulate the release of others. TNF-α activates the synthesis of other cytokines like IL-1, IL-6, IL-8 as well as the expression of surface and adhesion molecules [22]. The effect of cytokines like TNF-α is not limited to the immune system. TNF-α also has a procoagulatoric effect on endothelial cells [23]. Moreover the release of other inflammatory mediators which can perpetuate the inflammatory response like reactive oxygen species or NO can be stimulated [24]. IL-1β has a comparable range of efficacy with TNF-α. In experiments it could be shown that TNF-α can potentiate the effect of IL-1β [25].

To get a representative statement from a material comparison of different (dental) materials based on the releasing pattern of cytokines from leukocytes it is important to measure a panel of cytokines and not only a few because of the different possible inflammatory reactions but also the pleiotropy of the cytokines and their interactivity. Only in this case a different interpretation is possible [26].

One possible explanation for the differences in the cytokine release after stimulation of leukocytes incubated with Silo or TC could be the chemical reactivity of the elutable monomers used in these composites. The organic matrix of methacrylate based composites consists of different methacrylic acid based monomers. Methacrylic acid has a double bounding and carboxyl group as functional groups. Normally, the carboxyl group was esterified. The double bounding must be activated for chemical reactions.

The functional group of the siloranes is an oxirane ring [10]. Oxirane (=ethylene oxide, a member of the large group of epoxides) is a reactive heterocyclic three-membered ring consisting of two carbon and one oxygen atom. Kopperud et al. [27] identified with the help of liquid chromatography in ethanol/water (75%/25%) eluates from Filtek™ Silorane specimen the three siloranes bis-3,4-epoxycyclohexylethyl-phenylmethylsilane, 1,3,5,7-tetra(3,4-epoxycyclohexylethyl)-1,3,5,7-tetramethyl-cyclotetrasiloxane and 1,3,5,7,9-penta(3,4-epoxycyclohexylethyl)-1,3,5,7,9-pentamethylcyclopentasiloxane as well as isopropyl-methyl-diphenyliodonium which initiate the cationic polymerization process of siloranes. It cannot be excluded that this elutable and chemical reactive epoxides are also released into cell free supernatant and can stimulate the release of inflammatory mediators from leukocytes.

Besides the monomers other substances from the organic matrix (e.g. photoinitiators, photostabilisators) and inorganic matrix (e.g. fillers, particles for opacity) as well as coupling agents and reaction products may be also released and can contribute to the differences in the cytokine release after incubated of leukocytes with Silo or TC.

All test persons deny known allergic reaction to methacrylate- or silorane-based materials. Leukocytes from P8 showed a high and a fast decrease in the release of inflammatory mediators (Figs. 2c, 3c and 4c) compared with the release from the leukocytes from other test persons (Figs. 2a, b, 3a, b and 4a, b). Moreover a release of IL-2 was noted (14.4 pg/ml 72 h after incubation of leukocytes with Silo),

whereas nearly no release of IL-2 was measured 72 h after incubation with TC. IL-2 also called T-cell growth factor (TCGF) stimulates T-cells. One of the major biological functions of IL-2 is the autocrine activation of T-cells. If T-cells are exposed to an antigen which was identified by prior contact, then they will secrete IL-2 and stimulate themselves [28]. The highest amounts of IL-2 were found in the cell free supernatant from leukocytes from P8 72 h after incubation with Silo (TC did not stimulate the release of IL-2 in measurable amounts from leukocytes from P8). In the case of P8 it was very unlikely that P8 had had no contact with silorane/epoxides. Further anamnesis has shown that P8 designed resin-based costume jewelry years ago without any problems relating to the wide field of allergic response like contact dermatitis. In this context it is likely that P8 had contact with epoxy resin. In this case it is advisable to do further allergic testing against materials based on silorane/epoxides.

5. Conclusions

Our data show that Silo leads to the excretion of increased amounts of inflammatory markers from leukocytes when compared to TC. As in our case, it could be possible that a material comparison experiment based on the cytokine release assay can show significant differences in the immunological potential of the materials and provide information relating to their biocompatibility. Moreover our results show that the in vitro measurement of the released cytokines also gives hints about individual sensibilization reactions. In this case it is advisable to do further allergic examinations or use another dental restorative.

Elution experiments are a good and necessary addendum to identify and quantify elutable substances from Silo.

Acknowledgments

The authors acknowledge Susanne Zapf and Bernd Niedereiner for their excellent technical assistance.

REFERENCES

[1] Rankin JA. Biological mediators of acute inflammation. AACN Clin Issues 2004;15:3–17.
[2] Vilcek J. The cytokines: an overview. In: Thomson A, editor. The cytokine handbook. 3rd ed. London: Academic Press; 1998. p. 1–20.
[3] Andersson J, Dahlgren U. Effects on mouse immunity of long-term exposure in vivo to minute amounts of HEMA. Eur J Oral Sci 2011;119:109–14.
[4] Huang Z, Ma T, Ren PG, Smith RL, Goodman SB. Effects of orthopedic polymer particles on chemotaxis of macrophages and mesenchymal stem cells. J Biomed Mater Res A 2010;94:1264–9.
[5] Henriks-Eckerman ML, Suuronen K, Jolanki R, Alanko K. Methacrylates in dental restorative materials. Contact Dermatitis 2004;50:233–7.
[6] Reichl FX, Durner J, Muckter H, Elsenhans B, Forth W, Kunzelmann KH, et al. Effect of dental materials on gluconeogenesis in rat kidney tubules. Arch Toxicol 1999;73:381–6.
[7] Durner J, Debiak M, Burkle A, Hickel R, Reichl FX. Induction of DNA strand breaks by dental composite components compared to X-ray exposure in human gingival fibroblasts. Arch Toxicol 2011;85:143–8.
[8] Durner J, Spahl W, Zaspel J, Schweikl H, Hickel R, Reichl FX. Eluted substances from unpolymerized and polymerized dental restorative materials and their Nernst partition coefficient. Dent Mater 2010;26:91–9.
[9] Miletic VJ, Santini A. Remaining unreacted methacrylate groups in resin-based composite with respect to sample preparation and storing conditions using micro-Raman spectroscopy. J Biomed Mater Res B Appl Biomater 2008;87:468–74.
[10] Weinmann W, Thalacker C, Guggenberger R. Siloranes in dental composites. Dent Mater 2005;21:68–74.
[11] Ilie N, Hickel R. Silorane-based dental composite: behavior and abilities. Dent Mater J 2006;25:445–54.
[12] Schmalz G, Schuster U, Schweikl H. Influence of metals on IL-6 release in vitro. Biomaterials 1998;19:1689–94.
[13] Forst HT. Problems of multiple tests and evaluations in drug research. Arzneim Forsch 1985;35:563–9.
[14] Pizzo G, Guiglia R, Lo Russo L, Campisi G. Dentistry and internal medicine: from the focal infection theory to the periodontal medicine concept. Eur J Intern Med 2010;21:496–502.
[15] Hack CE, Aarden LA, Thijs LG. Role of cytokines in sepsis. Adv Immunol 1997;66:101–95.
[16] Shapira L, Wilensky A, Kinane DF. Effect of genetic variability on the inflammatory response to periodontal infection. J Clin Periodontol 2005;32(Suppl. 6):72–86.
[17] Loppnow H, Libby P. Adult human vascular endothelial cells express the IL6 gene differentially in response to LPS or IL1. Cell Immunol 1989;122:493–503.
[18] Sabat R, Grutz G, Warszawska K, Kirsch S, Witte E, Wolk K, et al. Biology of interleukin-10. Cytokine Growth Factor Rev 2010;21:331–44.
[19] Chernoff AE, Granowitz EV, Shapiro L, Vannier E, Lonnemann G, Angel JB, et al. A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. J Immunol 1995;154:5492–9.
[20] de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991;174:1209–20.
[21] Watzl C, Long EO. Signal transduction during activation and inhibition of natural killer cells. Curr Protoc Immunol 2010:19B [chapter 11: unit 11].
[22] Moldawer LL. Biology of proinflammatory cytokines and their antagonists. Crit Care Med 1994;22:S3–7.
[23] Simpson SQ, Casey LC. Role of tumor necrosis factor in sepsis and acute lung injury. Crit Care Clin 1989;5:27–47.
[24] Nathan C, Sporn M. Cytokines in context. J Cell Biol 1991;113:981–6.
[25] Schalkwijk CG, Vervoordeldonk M, Pfeilschifter J, van den Bosch H. Interleukin-1 beta-induced cytosolic phospholipase A2 activity and protein synthesis is blocked by dexamethasone in rat mesangial cells. FEBS Lett 1993;333:339–43.
[26] Durner J. Clinical chemistry: challenges for analytical chemistry and the nanosciences from medicine. Angew Chem Int Ed Engl 2010;49:1026–51.
[27] Kopperud HM, Schmidt M, Kleven IS. Elution of substances from a silorane-based dental composite. Eur J Oral Sci 2010;118:100–2.
[28] Cox MA, Harrington LE, Zajac AJ. Cytokines and the inception of CD8T cell responses. Trends Immunol 2011;32:180–6.