Biomonitoring of DNA damage in peripheral blood lymphocytes of subjects with dental restorative fillings

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Abstract

Dental fillings provide a major iatrogenic exposure to xenobiotic compounds due to the high prevalence of surface restorations in developed countries. Experimental data suggest that both amalgams, which contain mercury, and resin-based dental materials cause an impairment of the cellular pro- and anti-oxidant redox balance. The aim of this study was to assess the potential genotoxicity of dental restorative compounds in peripheral blood lymphocytes of young exposed subjects compared with controls. The study examined, by use of the comet assay, 68 carefully selected subjects taking into account the major known confounding factors. In the 44 exposed subjects, the mean numbers of restored surfaces was 3.0 and 3.8 in males and females, respectively. Tail length, percentage of DNA in the tail, tail moment or Olive tail moment were twofold higher in the exposed group than in unexposed controls, with significant differences. No significant difference was observed between amalgam and composite fillings. Furthermore, as shown by multivariate analysis, the association between dental fillings and DNA damage was enhanced by the number of fillings and by the exposure time. Among the lifestyle variables, a moderate physical activity showed a protective effect, being inversely correlated to the DNA damage parameters evaluated. On the whole, the use of DNA-migration allowed us to detect for the first time the potential adverse impact on human health of both kinds of dental filling constituents, the amalgams and the methacrylates. The main mechanism underlying the genotoxicity of dental restorative materials of various nature may be ascribed to the ability of both amalgams and methacrylates to trigger the generation of cellular reactive oxygen species, able to cause oxidative DNA lesions.

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1. Introduction

Dental fillings provide a major iatrogenic exposure to xenobiotic compounds, because the prevalence of restored surfaces is very high in developed countries, where millions of restorative procedures are performed annually [1,2]. Biocompatibility is the principal requisite for a successful dental restorative treatment, but potential adverse effects on human health present a serious problem.

Amalgam, which was introduced more than 150 years ago, is the most frequently used material as a tooth filling restoration. In spite of the availability of new materials, today amalgam is still a popular restorative owing to its wide potential applications, easiness of manipulation, adequate mechanical properties and relatively low cost [3]. Conventional dental amalgam is a mercury (Hg) and silver-based alloy that consists of ~50% mercury, 35% silver, 15% tin, plus copper and zinc in various amounts. Amalgam may also contain traces of cadmium, platinum and palladium. It is widely accepted that amalgams are the main source of human exposure to mercury and they contribute substantially to the load of this metal in human body tissues [4]. Several investigations have shown that elemental mercury vapour can be released from hardened dental amalgam, and the
urine metal concentration in non-occupationally exposed individuals is directly related to the number of filling surfaces [5,6].

Mercury release in the oral cavity is a function of several factors, including temperature, chewing, brushing, biological corrosion due to bacteria, electrochemical corrosion, and saliva pH [7]. The released Hg vapour is inhaled, absorbed by lung and gastrointestinal tissue and retained mainly in the kidney, brain and liver [6]. Moreover, it has been observed that there is a direct absorption across the oral mucosa and by migration through teeth into tissues [8]. The estimates of total daily intake of Hg vapour from amalgams range from 5 to 9 μg [9–11].

The toxicological consequences of long-term exposure to Hg from amalgam fillings are still a matter of debate in several countries. Despite the well-known neurotoxicity of Hg vapour [2], it is estimated that there are 190 million people in the US with amalgam restorations and that 70 million new restorations are placed annually. Indeed, as reported by Jarup [12], so far there are no studies showing any association between dental amalgams and illness. A contact hypersensitivity reaction to Hg is the most common adverse effect [13]. Regarding Hg carcinogenesis, the International Agency for Research on Cancer (IARC) [14] includes elemental Hg as well as inorganic Hg compounds in Group 3, being not classifiable as to carcinogenicity to humans. However, several lines of evidence, e.g., from results of chromosome aberration tests, micronucleus assays and SCE measurements demonstrate the genotoxicity of Hg [15,16]. The effect is imputable to the interaction of Hg with soluble and protein bound sulfhydryl groups and in particular to glutathione [17–19]. This induces a general collapse of antioxidant mechanisms and an increase in reactive oxygen species (ROS), causing DNA damage [20].

Based on the concern about the environmental and occupational aspects of dental treatment with amalgam [21], in the last 30 years resin-based materials, named composites or methacrylates, have been introduced in restorative treatments. More than 30 different compounds have been extracted from polymerized dental composites, including major monomers, additives, adhesive components and reaction products [22]. During the last decade, in addition to reports on hypersensitivity reactions to composites [23,24], many of these compounds have been identified as cytotoxic agents [25–28]. The biological response to dental restorative composites has been ascribed mainly to monomer release. This is due to incomplete polymerization, probably only during a short time after setting, and to degradation processes in the oral environment [29,30]. It has been hypothesized that the released monomers can diffuse into the tooth pulp and gingival tissue and then reach salivary glands, saliva and circulating blood [31]. Several new composite formulations have a lower un-polymerized residual mass in comparison with traditional formulations, which reduces but does not eliminate their cytotoxicity [32]. Usually, the low amounts of compounds released into aqueous solutions by resin-based materials are not enough to cause acute cytotoxicity but are sufficiently high to modify essential cell functions. These include induction of heat-shock proteins, modifications of cell-mediated immune responses and genetic effects such as gene mutations or chromosomal aberrations [33,34]. Both dental materials [35] and methacrylates [36] are categorized by IARC as not classifiable as to their carcinogenicity to humans (Group 3). However, in vitro studies have shown the genotoxicity of some of the released compounds. In fact, the (co)monomer triethylene glycol dimethacrylate (TEGDMA) induces mutations probably by covalent binding to DNA via Michael addition, while the mono-functional monomers methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA), as well as some of their metabolic intermediates, increase the frequency of micronuclei in cultured cells [37,38]. In addition to this experimental evidence for the mutagenic potential of dental resinous materials, an increase of ROS has been observed in human cells exposed to HEMA [39]. Besides activation of NF-kappa B and apoptosis pathways, this composite effect causes depletion of the intracellular glutathione pool and oxidative DNA damage [40,41].

The alkaline version of comet assay (single-cell gel electrophoresis, SCGE) is one of the most frequently used tests for assessing the genotoxic effects caused by agents in environmental, occupational and iatrogenic settings [42]. The high sensitivity of the technique is a great advantage in human biomonitoring, where a small genotoxic effect can be usually expected but implies the necessity of a careful sample selection. Recently, Möller [43,44] has summarized some biological, environmental and lifestyle factors that can interfere in the analysis. Test variability has to be added to these factors, which make it necessary to conform carefully to the guidelines and recommendations for appropriate use [45,46].

As reported above, experimental data show that both amalgams and resin-based dental materials enhance intracellular ROS, which cause DNA oxidative damage and are a well-known potential genotoxic factor implicated in many human chronic degenerative diseases, including cancer [20].

Considering the high number of subjects continuously exposed for many years to dental filling constituents, the aim of the present biomonitoring study was to assess the potential genotoxicity of dental restorative material in lymphocytes of exposed subjects, as compared with matched controls. The high sensitivity of comet assay to oxidative DNA lesions, due to alkali-labile sites other than strand breaks, prompted us to use this test for the present biomonitoring study.

2. Materials and methods

2.1. Selection of samples

One hundred and ten students attending the University of Messina, a homogeneous group with respect to socio-economic status and age, voluntarily agreed to participate in this study. An a priori selection was made according to a variety of exclusion criteria, including exposure to diagnostic X-rays during the last 5 months, previous radiotherapy or chemotherapy, presence of infectious diseases and/or flogistic conditions during the last 2 weeks, presence of dis-metabolic diseases and/or cancer. Moreover, competitive sport activities, exposure to materials used in orthodontic practices or to known genotoxic compounds, and the presence of permanent or mobile dental prosthesis were considered as exclusion criteria also.

In order to minimize the variability due to exogenous factors, such as climate and seasonal dietary habits [44], all blood specimens of the 68 selected subjects, who had previously given their informed consent, were collected during the 4th week of November and the 1st week of December 2005. A detailed questionnaire was submitted in order to collect information about age, gender,
smoking, drinking and dietary habits, physical activities, therapeutic drug intake and chewing gum use [47]. Since the percentage of subjects affected by allergies is high among young people, it was impossible to exclude them from our study, and, therefore, a question concerning this pathological condition was added. After oral observation, a dentist recorded the number and material of the fillings of each subject.

2.2. Biochemicals and reagents

All chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

2.3. Blood sampling

Peripheral blood lymphocytes were collected from heparinized whole blood obtained by finger drawing (minimum 50 μl). The samples were placed in dark-glass test-tubes and refrigerated immediately. In the laboratory, 100 μl of a suspension 1:1 sample/RPMI were carefully layered over 100 μl Lymphoprep Separation Medium3M (Axis-Shield Oslo, Norway) and centrifuged at 2000 g for 3 min. The buffy layer was removed, the cells washed with phosphate-buffered saline (PBS, 500 μl) and collected by centrifugation at 14,000 g for 5 min. The pellets were suspended in 10 μl PBS and used for preparing the slides.

2.4. Single-cell gel electrophoresis (SCGE)

The comet assay was performed according to the method proposed by Tice et al. [45], with some modifications. Samples were assayed in duplicate using 5 μl of the cell suspension for each spot and, after slide preparation, lysis was performed at 4 °C for 1 h in cold solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl, pH 10) to which 10% DMSO and 1% Triton X100 were added just before use. The slides were rinsed for 20 min in 3 changes of an alkaline solution (0.3 M NaOH, 1 mM EDTA, pH 13) and placed side by side in a horizontal electrophoresis tank (Sub-Cell GT BioRad, Segrate, Milan, Italy), filled with ice-cold fresh alkaline rinse solution. After 20 min to allow DNA unwinding and the expression of alkali-labile damage, electrophoresis was carried out for 30 min at 300 mA and 25 V (0.86 V cm−1) by use of a Power Pac 3000 electrophoresis power supply (BioRad). The slides were rinsed with 0.4 M Tris–HCl, pH 7.5, dehydrated during 20 min in cold 100% ethanol, and stored in dry air up to the end of sampling. Before stain with ethidium bromide (2 μg/ml in H2O), the spots were re-hydrated with cold distilled water for 30 min and within 24 h after staining analyses were carried out by use of a DM IRB fluorescence microscope at 400× magnification (Leica Mircosystem, Heidelberg, Mannheim, Germany), equipped with a digital camera (Canon Power Shot S50, Milan, Italy). For each coded spot, with exclusion of the outer area, images of at least 100 randomly selected nuclei were acquired and submitted to an automated image analysis system CASP (Comet assay software project) (http://www.casp.sourceforge.net). The considered parameters were tail length (TL), percentage of DNA in the tail (TDNA%), tail moment (TM) and Olive tail moment (OTM).

2.5. Statistical analyses

The analyses were performed by use of the STATISTICA program (version 6.0) for the Windows operating system. A probability level of 0.05 was considered as cut-off criterion for significance. In order to assess the distribution patterns of the data sets, the collected values for each parameter were examined by the Lilliefors’ and Shapiro–Wilk normality tests. Since the data showed a non-normal distribution and their variability coefficients (VC%) were generally very high, median and percentiles were used in order to describe the observed data and the analysis of independent variables was performed by nonparametric tests of Spearman, Kruskall–Wallis and step-wise multiple regression.

3. Results

The examined population consisted of 33 males and 35 females. The age ranged between 18 and 27 years. The 44 subjects carrying dental fillings included 20 males and 24 females, aged 21.2 ± 1.99 and 20.9 ± 1.84 years (means ± S.D.), respectively. The 24 individuals forming the control group included 13 males (aged 20.9 ± 2.50 years) and 11 females (aged 20.6 ± 2.01 years). Within the exposed group, 45.5% had composite fillings only, 22.7% had amalgams only, and 31.8% had fillings of both materials. The number of restored surfaces per individual ranged between 1 and 12. The higher prevalence in females than in males (Table 1) was not statistically significant.

Table 2 reports the SCGE data as mean values of percentiles calculated within exposed subjects, independently of the number and types of fillings, and within control subjects. In addition to presenting the classic parameters TL and TDNA%, taken into account in order to allow comparison of our data with those reported in other studies, we report the results as TM and/or OTM also, being the most sensitive indicators of DNA damage in a preliminary data analysis. Starting from the 5th percentiles, the values recorded in exposed subjects were consistently and significantly higher than in controls. The correlation between iatrogenic exposure and DNA damage was also shown by the highly significant differences assessed by Spearman test (data not shown).

Fig. 1 gives TL, TDNA% and TM values obtained by stratifying the examined group in relation to filling constituents and shows how both composites and amalgams increased DNA migration. The subjects with both kinds of fillings, having the highest number of restored surfaces (see Table 1), had the highest levels of damage. Since the comparison of filling constituents did not show any significant difference in the Kruskal–Wallis test, the overall sample was stratified into 3 subgroups according to the number of fillings in order to evaluate dose-effect.

<table>
<thead>
<tr>
<th>Dental filling</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composites</td>
<td>2.4 ± 2.2</td>
<td>2.7 ± 2.10</td>
<td>2.6 ± 2.09</td>
</tr>
<tr>
<td>Amalgams</td>
<td>3.0 ± 2.89</td>
<td>3.0 ± 3.46</td>
<td>3.0 ± 2.87</td>
</tr>
<tr>
<td>Both materials</td>
<td>3.8 ± 1.30</td>
<td>5.4 ± 3.24</td>
<td>4.9 ± 2.77</td>
</tr>
</tbody>
</table>

The data are means ± S.D. within each group.

Table 2

<table>
<thead>
<tr>
<th>SCGE parameters</th>
<th>Group</th>
<th>Percentile</th>
<th>5th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>Control</td>
<td>11.5</td>
<td>23.1</td>
<td>38.5</td>
<td>63.3</td>
<td>131.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>17.4a</td>
<td>36.9b</td>
<td>62.9b</td>
<td>112.1b</td>
<td>208.4b</td>
<td></td>
</tr>
<tr>
<td>%TDNA</td>
<td>Control</td>
<td>0.9</td>
<td>2.8</td>
<td>5.7</td>
<td>10.9</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>1.7a</td>
<td>4.5a</td>
<td>8.7a</td>
<td>17.8a</td>
<td>42.1a</td>
<td></td>
</tr>
<tr>
<td>TM</td>
<td>Control</td>
<td>0.2</td>
<td>1.1</td>
<td>3.2</td>
<td>9.3</td>
<td>46.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>0.6b</td>
<td>2.3b</td>
<td>7.3b</td>
<td>25.5b</td>
<td>101.6b</td>
<td></td>
</tr>
<tr>
<td>OTM</td>
<td>Control</td>
<td>0.7</td>
<td>2.3</td>
<td>5.2</td>
<td>10.7</td>
<td>35.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>1.4b</td>
<td>3.7b</td>
<td>8.3b</td>
<td>21.4b</td>
<td>65.8b</td>
<td></td>
</tr>
</tbody>
</table>

aP <0.05 and bP <0.01 vs. controls, as assessed by Kruskal–Wallis test.
correlations. DNA migration increased with the number of restored surfaces, with significant values of the Spearman test for all parameters studied. Fig. 2 shows a box plot relative to the TDNA% parameter, with $P$ values of 0.003 and 0.01, as assessed by Spearman and Kruskal–Wallis tests, respectively. The latter test detected significant differences among the subgroups, but only the TM parameter showed $P$ values <0.001 for all percentiles.

Due to the effect of dental restorations on lymphocyte DNA migration, we carried out the analysis in the exposed and control groups separately in order to assess the weight of other independent variables. Statistical analyses did not show associations between DNA damage and biological variables such as gender and age in the control group. The exposed group also showed no differences in relation to gender, even though damage tended to be higher in females. The medians (25–75% quartile) of the TDNA% parameter were, for instance, 7.72 (4.36–17.25) in females vs. 7.00 (3.61–12.64) in males. However, the DNA damage was related to the age in the exposed group, stratified in 18–19, 20–22 and ≥23 years subgroups. Fig. 3, showing the box plot referred to TL, highlights the significant gradient in DNA damage as related to age (Spearman test, $P=0.007$; Kruskal–Wallis test, $P=0.03$). The confounding factor “restored surface number” was excluded, being very similar among the 3 age groups considered (means ± S.D. of 3.6 ± 3.24, 3.1 ± 1.83 and 3.8 ± 3.73, respectively).

Although our study involved a small population sample, it allowed us to detect the influence of some lifestyle variables on DNA damage. In particular, non-competitive physical activity, carried out by 58.8% of the examined subjects, was inversely correlated to the DNA damage parameters considered and, as shown in Fig. 4, it was able to cause a decrease in DNA migration also in control subjects. However, only in subject carrying dental fillings the differences were significant ($P<0.01$) according to the Spearman and the Kruskal–Wallis tests.
Fig. 5. Percentile values of tail length in control subjects (empty symbols) and exposed subjects (full symbols), as related to alcohol intake (circles, non-drinkers; diamonds, occasional drinkers; squares, regular drinkers).

The daily intake of ethanol was moderate in all subjects and almost all of it was attributable to consumption of wine and beer. As reported in Fig. 5, regular alcohol consumption caused a decrease in DNA damage, which was statistically significant ($P < 0.05$) in the control group only.

The other considered lifestyle variables were not correlated with lymphocyte DNA damage. Since the subjects had very similar dietary habits these results are predictable. Less expected, instead, are the data concerning smoking habits. The non-significant effect of latter variable, as assessed on the basis of cigarettes/day (mean $±$ S.D. $= 7.1 ± 5.72$) and pack-years (cigarettes/day $×$ smoking years), is probably attributable to the absence of heavy smokers and to the young age of subjects, 32.8% of whom were smokers. In particular, in the group of subjects having dental restorative materials the values of median TDNA% (25–75% quartile) were 9.65 (4.36–15.65) and 6.72 (3.24–16.32) in smokers and non-smokers, respectively. In the control group, which included 6 smokers only, the values were 1.79 (0.84–3.85) and 5.21 (2.4–9.29), respectively.

Chewing gum use, which favours salivation, may be expected to increase the solubilization of dental restorative compounds. However, the differences between chewers and non-chewers were not statistically significant, the median TDNA% (25–75% quartile) being 9.74 (5.25–17.25) and 6.98 (3.91–15.43), respectively.

Finally, the lymphocyte DNA damage related to allergies, which affected 22.1% of the investigated subjects, was significant in the control group only. The median TDNA% (25–75% quartile) was 9.13 (4.18–17.64) in subjects suffering from allergies vs. 4.29 (2.25–7.12) in allergy-free subjects. In contrast, in exposed subjects the allergies increased DNA damage parameters only weakly, with median TDNA% (25–75% quartile) of 9.25 (4.47–19.95) vs. 6.93 (3.43–15.03).

Although bi-variate analysis highlighted several protective as well as adverse relationships among biological and lifestyle variables, the multiple regression test did not reveal important associations. In particular, as shown by the $\beta$ values reported in Table 3, the multivariate analysis pointed out the role of dental filling number and age, which accounted for approximately 65% of lymphocyte DNA damage on the whole. Moreover, as suggested by the bi-variate analysis, the type of filling had no significant effect, while the protective effect of physical activity was weak and able to decrease the DNA migration of only the less severely damaged lymphocytes.

### Table 3

Results of multivariate analysis in the group of subjects with dental restorative fillings

<table>
<thead>
<tr>
<th>Variable</th>
<th>TDNA% (95th percentile) $R = 0.601$, $R^2 = 0.361$</th>
<th>TL (5th percentile) $R = 0.533$, $R^2 = 0.284$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ $\pm$ SE $\beta$ $\mid P$</td>
<td>$\beta$ $\pm$ SE $\beta$ $\mid P$</td>
</tr>
<tr>
<td>Intercept</td>
<td>$P = $ NS</td>
<td>$P = $ NS</td>
</tr>
<tr>
<td>Gender</td>
<td>0.13 $\pm$ 0.14 $\mid$ NS</td>
<td>$-0.0025$ $\pm$ 0.15 $\mid$ NS</td>
</tr>
<tr>
<td>Age</td>
<td>0.32 $\pm$ 0.15 $\mid$ &lt;0.05</td>
<td>0.15 $\pm$ 0.16 $\mid$ NS</td>
</tr>
<tr>
<td>Filling kind</td>
<td>0.07 $\pm$ 0.15 $\mid$ NS</td>
<td>0.15 $\pm$ 0.16 $\mid$ NS</td>
</tr>
<tr>
<td>Filling number</td>
<td>0.32 $\pm$ 0.15 $\mid$ &lt;0.05</td>
<td>0.16 $\pm$ 0.16 $\mid$ NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>$-0.15$ $\pm$ 0.15 $\mid$ NS</td>
<td>0.16 $\pm$ 0.16 $\mid$ NS</td>
</tr>
<tr>
<td>Physical act.</td>
<td>$-0.11$ $\pm$ 0.15 $\mid$ NS</td>
<td>$-0.33$ $\pm$ 0.16 $\mid$ &lt;0.05</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>$-0.19$ $\pm$ 0.14 $\mid$ NS</td>
<td>$-0.04$ $\pm$ 0.15 $\mid$ NS</td>
</tr>
<tr>
<td>Allergies</td>
<td>0.27 $\pm$ 0.16 $\mid$ NS</td>
<td>0.04 $\pm$ 0.16 $\mid$ NS</td>
</tr>
</tbody>
</table>

NS, not significant.
Hg vapour as well as composite compounds caused significant DNA damage. Further, the association was reinforced by the observed correlations between dose, exposure time and DNA damage. In particular, the effect due to the dose was also confirmed by the higher values observed in females, since their number of fillings was higher than in males. The role of the exposure time was shown by the relation age-DNA damage, observed in the exposed group only. The young age of the subjects emphasized the importance of the release of genotoxic compounds, even when the dental restorations had been carried out few years earlier. Since the confounding factor “filling number” had been excluded, the harmful systemic effect caused by the release of dental filling constituents clearly showed the role of wearing out.

Regarding the influence exerted by lifestyle habits on the DNA-damaging effect due to dental fillings, our study emphasizes the protective role of a moderate physical activity, contrasting significantly the effect in the exposed group. Similar results were observed by Kasai et al. [52] who used the urinary 8-oxo-dG levels as a genotoxicity marker, while several studies showed an increased DNA damage in peripheral blood lymphocytes after agonistic physical activity [43,44,47]. Although smoking causes a relevant exposure to various genotoxins, no significant difference was observed between smokers and non-smokers. Conflicting results have been obtained in comet assay studies regarding the role of smoking habits [53–55]. Hoffmann et al. reported in their meta-analysis [56] that smoking rarely causes significant DNA damage when it is considered a potential confounder, as was the case in our study, rather than the main factor examined.

Lastly, our results show that the presence of dental fillings masked the DNA damage exerted by allergies and, therefore, underlines their confounding role. This suggests the need to consider the “presence of dental filling” in biomonitoring carried out to assess various harmful factors.

As was mentioned in Section 1, several experimental data show the capability of both dental filling constituents to trigger cellular ROS generation [16,17,39,41]. In particular this behaviour, observed at the low doses released by dental fillings also, is underlined by depletion of GSH, a known scavenger of radicals. The prevailing role of radical species in genotoxicity elicited by amalgams and methacrylates has been confirmed by the use of ROS scavengers, such as N-acetylcysteine (NAC), ascorbate and vitamin E, able to inhibit the biological effects of these compounds [57–59]. On this basis a biologically plausible mechanism explaining why both amalgams and composites cause the observed DNA damage could be related to an impairment of the cellular pro- and anti-oxidant redox balance. This hypothesis is strengthened by the marked electrophoretic alterations in the comet assay observed in human lymphocytes following ex vivo and occupational exposure to low doses of resin monomers and Hg [16,50,51,60]. Lastly, the ROS-induced DNA damage is confirmed by the increase in 8-hydroxydeoxyguanosine, which is a widely used marker to evaluate DNA oxidation [49].

In conclusion, our biomonitoring study, using an exposure marker able to detect strand breakage, transient repair sites and alkali-labile sites, confirms the potential adverse impact of both dental filling constituents – amalgams and methacrylates – on human health. Considering the large number of people involved in this kind of iatrogenic exposure, further investigations are desirable. We intend to apply, in a further biomonitoring study, a more specific indicator of DNA oxidation, in addition to the comet test.

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