RESEARCH ARTICLE

Could night-guards be used as a simple method to detect leached-elements from dental restorations intra-orally? A study on amalgam restorations [version 1; peer review: 1 approved, 1 not approved]

Rasha Mohamed Abdelraouf ^{1,2}

¹Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt

²Dental Family Clinic, Cairo, Maadi, Egypt

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Abstract

Background: Detection of leached-elements from dental restorations intra-orally has been a subject of prime importance in dental research. However, this is challenging as most of the present techniques have some limitations. In this study, a new simple method was proposed via using night-guards. Thus, the aim of the study was to verify if night-guards could detect leached-elements from restorations as dental amalgam.

Methods: Ten upper custom-made night-guards were fabricated for patients suffering from bruxism, who had amalgam-restorations in their upper molars. The night-guards were delivered to the patients and they were instructed to wear the night-guards during when they were asleep. After six months, the night-guards were taken from the patients to be analyzed. A new unused night-guard was fabricated from the same material to be used as a control. In the used night-guards, two areas were studied: the fitting surfaces contacting the amalgam restorations and the fitting surfaces not contacting amalgam restorations. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDXA) were used to examine the structural and elemental changes in the night-guards.

Results: SEM of the unused night-guard revealed a homogenous structure, and the composition was carbon and oxygen, as shown using EDXA (C=88.9wt% and O=11.1wt%). By contrast, the fitting surfaces of the night-guards contacting amalgam restorations showed numerous lustrous particles. Elemental analysis of these areas showed the presence of mercury and sulfur, in addition to carbon and oxygen (Hg=21.2wt%, S=2.5wt%, C=67.1wt% and O=9.2wt%). The night-guards' fitting surfaces not contacting amalgam restorations showed slight cracking, and the composition was carbon and oxygen (C=88.3wt% and O=11.7 wt%).

Open Peer Review

Approval Status

	1	2
version 1		
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1. Sabry A. El-Korashy , Suez Canal University, Ismailia, Egypt		
2. Brian W. Darvell , University of Birmingham, Birmingham, UK		
Any reports and responses or comments on the article can be found at the end of the article.		

Conclusions: Analyzing fitting surfaces of night-guards contacting dental restorations, such as amalgam, could aid in understanding the nature of leached-elements from these restorations intra-orally. However, further studies about its application upon dental-restorations other than amalgam are recommended.

Keywords

Night-guard, Amalgam, Mercury, Elemental Analysis, leached Elements

Corresponding author: Rasha Mohamed Abdelraouf (rasha.abdelraouf@dentistry.cu.edu.eg)

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Introduction

Determination of leached-elements from dental restorations has been investigated as a result of exposure to oral conditions¹. Several methods have been performed both *in vitro* and *in vivo*. Most of the *in vitro* techniques relied on storing specimens in surrounding media, such as distilled water or artificial saliva¹⁻³. Various analytical means had been used to analyze the leached-elements, including inductively-coupled-plasma, atomic-absorption, Fourier-transform-Infrared spectroscopies and chromatographic methods^{1,2,4}.

On the other hand, *in vivo* methods have been used to assess the actual situation intra-orally⁵. *In vivo* techniques have been performed either in animals or humans. Cavities are prepared in the teeth of animals, such as monkeys⁶, pigs^{7,8} or rats⁹, then filled by dental-restorations. The released-elements are either detected after sacrificing the animals⁷ or by analyzing secretory products, such as urine⁹ or fecal samples⁶.

Yet, measuring the released elements in humans may be best. Several studies quantified the excretion of mercury in urine, hair and nails of patients with dental amalgam-restorations and found a positive correlation^{10,11}. In contrast, others did not find a correlation between mercury content in hair and amalgam-restoration, but mercury in hair has been correlated with fish consumption¹². Thus, assessing the leached-elements in the oral-cavity may be more specific to eliminate other possible systemic sources. Thus, several studies examined released ions in patients' saliva^{13,14}. However, these ions may be affected by salivary secretions or washed out by swallowing. In addition, saliva collection and storage may be technique sensitive¹⁵. Thus, a new simple method was proposed in this study through using night-guards (polymeric occlusal-splints used to decrease bruxism and clenching teeth) in gathering leached-elements from dental-restorations.

Methods

Ten upper dental alginate impressions (Tropicalgin, Zhermack, Italy) were taken as part of routine treatment at the Dental Family Clinic (Cairo, Egypt), between February and December 2016, for ten patients suffering from bruxism, and who also had amalgam-restorations in their upper molars. This was performed after all the patients signed written informed consent forms agreeing to participate in the study. The participants were six females and four males with the following inclusion criteria: adult (>18 years), non-periodontal-affected patients suffering from bruxism, and had amalgam restoration. Medically-compromised patients were excluded. Custom-made night-guards (example shown in Figure 1) were fabricated for each patient from 2mm soft polymeric sheets (Easy-Vac-Gasket, 3A-Medes, Korea), using a vacuum forming machine (Pro-Form, Keystone, Germany). The night-guards were delivered to the patients in the clinic after one week from first visit

and they were instructed to wear the night-guards when they were asleep (≈ 7 hours ± 2). Recall visits were given to the patients in the regular check-up schedule after six months. In this visit, the night-guards were taken from the patients to be analyzed. A new unused night-guard was fabricated from the same material to be used as a control.

Scanning-Electron-Microscopy (SEM) and Energy-Dispersive-X-ray-Analysis (EDXA) were used to examine structural and elemental changes in the used night-guards, and compared to the unused one. In the used night-guards, two areas were studied: the fitting surfaces contacting amalgam-restorations and the fitting surfaces not contacting amalgam-restorations. The patients' records were used to identify teeth that had restorations. Parts from these areas of interest were cut using a scissor (Singer, Germany), mounted on coded brass stubs and sputter coated with 10Å gold platinum and observed at 20000 $\times$ magnification. The SEM and EDXA (Supra40, Carl-Zeiss-NTS-GmbH, Germany) were used with an accelerating voltage of 20.0–30.0kV.

Results

The SEM of the unused night-guard revealed a homogenous structure (Figure 2), with its composition consisting of carbon and oxygen (C=88.9wt% ± 0.3 and O=11.1wt% ± 0.4 ; Figure 3). By contrast, the night guard surfaces contacting amalgam-restorations showed numerous lustrous particles (Figure 4). Elemental analysis of these areas showed the presence of mercury and sulfur, in addition to carbon and oxygen (Hg=21.2wt% ± 0.6 , S=2.5wt% ± 0.5 , C=67.1wt% ± 0.3 and O=9.2wt% ± 0.5 ; Figure 5). The surfaces not contacting amalgam-restorations under SEM showed slight cracking, but were still homogenous without lustrous particles (Figure 6). Their composition was carbon and oxygen (C=88.3wt% ± 0.6 and O=11.7wt% ± 0.6 ; Figure 7).

Figure 1. Example of custom-made night-guard.

Figure 2. Scanning electron micrograph of an unused night-guard. Magnification: 20000x.

Figure 3. Energy-Dispersive-X-ray-Analysis of an unused night-guard.

Figure 4. Scanning electron micrograph of a night-guard surface contacting amalgam-restoration. Magnification: 20000x. Image representative of 10 night guards.

Figure 5. Energy-Dispersive-X-ray-Analysis of night-guard surface contacting amalgam-restoration.

Figure 6. Scanning electron micrograph of night-guard surface not contacting amalgam-restoration, Magnification: 20000x. Image representative of 10 night guards.

Figure 7. Energy-Dispersive-X-ray-Analysis of night-guard surface not contacting amalgam-restoration.

Dataset 1. SEM images for night guard surfaces contacting and not contacting amalgam restorations

<http://dx.doi.org/10.5256/f1000research.12311.d177383>

Dataset 2. Raw EDXA data for night guard surfaces contacting and not contacting amalgam restorations

<http://dx.doi.org/10.5256/f1000research.12311.d177384>

Discussion

The oral-cavity is an aggressive environment, which may affect the integrity of dental restorations^{16–18}. It is essential to examine the resultant leached-elements to assess the degradation products of the filling materials in the oral-cavity and investigate if these materials could provoke an adverse systemic effect¹⁹.

Several studies have examined released elements by *in vitro* testing through fabricating specimens and soaking them in medium^{1,2,20}. However, simulating the oral-cavity is difficult due to its complex nature with multifactorial variables¹⁷. Thus, some studies have analyzed patients' saliva^{21–23}. However, saliva is composed of various components and its analysis has shown variation in methodology. In addition, contamination of saliva may occur¹⁵.

Accordingly, in this study, a new technique was introduced via examining night guards contacting dental restorations. Night guards are polymeric materials designed to fit the occlusal-surfaces of patients to decrease signs and symptoms of bruxism and teeth clenching. Consequently, the study was performed by providing patients who suffered from bruxism with custom-made night guards. An additional inclusion criterion was the presence of amalgam fillings in their upper molars. Amalgam restorations were selected rather than any other dental restoration, as it has been well proven in the literature that mercury is released from such material¹⁹. Therefore, utilizing night guards in gathering leached-elements from amalgam-restorations intra-orally was evaluated in this study.

Both the unused night guard and the used surfaces not contacting amalgam-restorations were homogenous, yet the latter showed cracking, which may be due to bruxism. This consistent structure

may be attributed to the following: these areas were not contacting dental restorations and no leached elements were deposited. This was confirmed by the EDXA results, which were only carbon and oxygen in both. The night-guards are polymeric materials, with their basic structure consisting of carbon, oxygen and hydrogen²⁴. However, hydrogen was not detected as it did not have core electron, only one valence electron that enters in chemical bonding²⁵. Analysis relied on excitation of electrons in lower shells, not the valence electron, which was shared in covalent bonding in the case of hydrogen²⁵.

The night guard surfaces contacting amalgam restorations showed numerous lustrous particles, which were identified by EDXA as mercury. This is in agreement with numerous studies that have detected mercury released from amalgam restorations^{19,26}. Sulfur was also detected, which may be due to tarnish and corrosion of the amalgam²⁷.

Since leached elements detected by night guards matched with that reported in the literature, night-guards could be used as a simple method to detect released elements from dental restorations such as amalgam intra-orally.

Data availability

Dataset 1. SEM images for night guard surfaces contacting and not contacting amalgam restorations. [10.5256/f1000research.12311.d177383](https://doi.org/10.5256/f1000research.12311.d177383)²⁸

Dataset 2. Raw EDXA data for night guard surfaces contacting and not contacting amalgam restorations. [10.5256/f1000research.12311.d177384](https://doi.org/10.5256/f1000research.12311.d177384)²⁹

Author contributions

It should be noted that all the work, including the clinical steps, was performed by the author.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Brian W. Darvell

School of Dentistry, University of Birmingham, Birmingham, UK

On the face of it, the concept has some attraction, but it is predicated on something unstated: that the mouthguard material will adsorb any ions arising from the amalgam. Here we have the first problem: dissolution is not leaching, so the premise is faulty. Secondly, there is no evidence that such adsorption could occur - certainly it is unlikely but it is, more to the point, untested. Where is the positive control? However, what is actually detected, it would seem, are particulates (apparently HgS, but not specifically demonstrated). These are either the result of mechanical action, which seems likely, embedding them in the polymer surface, or the result of reaction with mercury ions forming a precipitate on the polymer. The latter would be very serious in its implications, and for which I know of no precedent. The fact that the chemistry is not considered in any way is major deficiency. (The naïvety of the remarks in para.4 of the Discussion suggests a lack of such comprehension.)

At the very least, elemental mapping should have been used, identifying the restoration borders carefully and looking for migration beyond them. Particulates should have been studied in more detail, but the experiment fails to achieve what it sets out to do, but it seems that there is no comprehension of this or why. For example, was the sulphur already on the amalgam surface as sulphide (which is likely) and simply transferred? I can think of two ways of testing this - both very simple. But, without this information, we have no idea what is going on. Indeed, why is there no silver?

The English is pretty poor, with failures of logic and sense in many places. At the very least it should have been copy-edited by a native English speaker versed in technical writing before submission. It is in the best interests of all authors that this be done where English is not the first language. It would save referees an awful lot of trouble, and authors a lot of time.

Scientifically, this paper is below the standard required in several respects. Division of the content into the proper sections is required. However, the 'review' section is of no value, and the discussion completely empty except for repetition of introductory material and other material of no value.

With some sensible direction, something useful might have been achieved with the basic idea, but this has not occurred.

Some few comments. A comprehensive list would take too long.

Abstract

verify if >> verify whether
could detect : logically wrong - night-guards cannot detect anything
leached-elements >> leached elements
restorations as >> restorations such as
during when >> while
analyzed >> analysed (the z is always illiterate)
homogenous >> homogeneous
the composition was carbon and oxygen : no ...
wt% >> mass%

Introduction

Several studies ... : there is no trace here of a critical review so the value of the various reports cannot be assessed or the present attempt actually justified.

Methods

polymeric sheets : what material?
instructed to wear the night-guards when they were asleep : ... while ...
10Å >> SI equivalent, please, and with a space between value and symbol: but does this not obscure the test surface and prevent elemental detections?
homogenous >> homogeneous

Discussion

para 1: >> introduction
para 2: repetition, delete.
para 3: repetition!
para 4:
This was confirmed ... : no, this is the only evidence you have, the assertion does not count.
The remainder of this paragraph is padding.
para 5: results, delete.
para 6: is the totality of the discussion? Actually, a conclusion, but very weak.

Fig. 2 and 6 are near pointless - the contrast is bad.

There is no point in Figures 3, 5, and 7 - this is raw data.

Fig. 4 : elemental maps for Hg and S (as well as Ag, Sn, Cu, if ever detected locally) would make more sense. "Lustrous" is quite inappropriate for a description from an SEM image - this character is not detectable.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 11 October 2017

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Sabry A. El-Korashy

Faculty of Science, Department of Chemistry, Suez Canal University, Ismailia, Egypt

Different methods are used in determination of the leached-elements from dental restorations intra-orally are reported. Some of these methods have been performed both *in vitro* and *vivo*, using different analytical techniques. They were carried out via surrounding media, such as distilled water or artificial saliva. However, these methods still having some reservations and almost take a great effort. In the present article, the author used very simple and new technique

for measuring the leached elements from amalgam restorations via fabricated polymeric night guards for the tested patients suffering from bruxism. The concentrations of the leached elements after removing the night guards were measured using SEM with EDAX spectroscopy.

In my opinion, the article is accepted for publication after answering the following questions:

1. The results of EDAX (Fig. 5) have detected the Hg, O, S and Ca elements. The author did not address the sources of sulfur and calcium where they came from?
2. It is known that the amalgam contains some other metals such as copper and silver and yet none of them appeared in the analysis. Why?
3. It is well known that hydrogen has no spectrum in analysis by using EDAX technique and hence the paragraph in the discussion section (page 6) begins with " However, hydrogen was not detected..... case of hydrogen ²⁵" must be deleted.
4. In the future, the author must do the same study on patients who do not have bruxism for comparison.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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