Journal of Dental Sciences (2013) **8**, 37—43

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

Ex vivo evaluation of genotoxic effects of four dental adhesives on human leukocytes

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Received 19 December 2011; Final revision received 11 December 2012
Available online 20 February 2013

KEYWORDS
biocompatibility;
comet assay;
dental adhesives;
genotoxicity;
leukocytes

Abstract *Background/purpose:* The use of dental adhesives in everyday dental practice has raised questions about their biologic safety. Their biocompatibility is a relevant aspect of the clinical success of these materials. The objective of this study was to evaluate the genotoxicity of dental adhesives *ex vivo* using a cytogenetic assay.
Materials and methods: Four materials (AdheSE, G-Bond, Excite, and Adper Single Bond 2) were tested on human peripheral blood leukocytes using a comet assay. Prepared materials were eluted in a saline solution for 1 hour, 1 day, and 5 days. The comet assay was used to evaluate primary DNA damage by measuring the tail length and tail intensity. A Kruskall-Wallis nonparametric test was used for the statistical analysis, with the significance level set to $P < 0.05$.
Results: None of the tested dental adhesives revealed a statistically significant increase in the tail length or tail intensity in treated leukocytes, independent of the applied dilution, elution duration, and polymerization form. A slight increase in the tail length and intensity of DNA molecules was observed after 1 and 5 days of the elution period at the lowest dilution ($1:10^2$) for all tested adhesives, only in their nonpolymerized form; however, these results were not statistically significant.
Conclusion: Under the conditions used in this study, all adhesives had acceptable biocompatibility in terms of genotoxicity.

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http://dx.doi.org/10.1016/j.jds.2012.12.001

Introduction

The use of composite filling materials along with adhesive techniques has revolutionized today's dental practice. Dental adhesives have evolved at a rapid rate over the past decade. Significant advances in dentin bonding technology have contributed to a large part of this success, at least in the short term.[1] The use of these materials in everyday dental practice has raised questions about their biologic safety. The biocompatibility of dental adhesives is an important aspect of the clinical success of these materials.[2]

Biocompatibility and biologic tolerability define the absence of any negative material properties that can damage biologic systems by manifesting themselves through various parameters (cytotoxicity, genotoxicity, mutagenicity, carcinogenicity, histocompatibility, and microbiologic effects).[3] Measuring the biocompatibility of a material is not simple. It is not possible to biologically characterize a material using a single test. Different characteristics can be explored via both *in vitro* and *in vivo* tests.[4–6] *In vitro* studies are primarily performed to evaluate the cytotoxicity (cell damage)[7–9] or genotoxicity (specific DNA damage or chromosomal aberrations)[10–12] of dental materials. A comet assay or single-cell gel electrophoretic assay is an uncomplicated and sensitive technique for detecting DNA damage at the level of individual eukaryotic cells. This technique does not require cell cultivation; it detects primary DNA damage *in situ* at the level of each individual cell. The comet assay is used to detect single- and double-strand breaks and other alkali-labile sites on DNA. The high sensitivity of the comet assay was used to evaluate the genotoxic potential of various chemical and physical agents.[13,14] To quantify DNA damage by the comet assay, the tail length (μm) and tail intensity (% DNA) are most frequently used. The tail length determines the length of DNA migration and is directly related to the DNA fragment size and extent of DNA damage. Theoretically speaking, a higher damage rate would produce smaller-sized fragments that will be pulled during electrophoresis to greater distances from the core resulting in a longer tail of the comet. It is calculated from the center of the nuclear core. The tail intensity denotes the amount of DNA fragments which directly indicates the proportion of the genome affected by the damage.[13–15]

Different cell cultures are commonly used for cytotoxicity and genotoxicity evaluations.[16,17] Due to their cultivation *in vitro* for many generations, those cells undergo several genomic transformations. Therefore, in studies attempting to record even minimal effects on the DNA level, primary cultures of isolated diploid cells, like human leukocytes, are preferable. Normal diploid cells have mitotic rates and mitochondrial functions relatively similar to *in vivo* conditions and differ from those of transformed or tumors cells.[18] Therefore, the response and susceptibility of leukocytes toward treatment with a genotoxin will more likely correspond to cells which are directly exposed to the particular harmful substance.

Dental adhesives that create a stable relationship with biologic tissues and allow both healing and tissue differentiation are considered biocompatible. The scientific evidence on adhesives is contradictory. Some authors claimed that they are very safe and can be used even in direct contact with the pulp,[19,20] while others believe that they are not suitable for direct pulp capping due to reported associated symptoms of persistent inflammation.[21–23] Some also claim that dental adhesive systems contain certain components that can be released into the oral environment and show biologic activities (cytotoxicity, carcinogenicity, mutagenicity, genotoxicity) in the body.[24–27] Acidic and nonacidic components of nonpolymerized adhesives are considered responsible for the cytotoxic effects on the dentin–pulp system. Certain components of dental adhesives such as hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) can be dissolved in water and therefore leach out and cause different effects in the body. So far, many cytotoxic reactions have been attributed to these components, but in high concentrations (HEMA concentrations of $>10^{-6}$ M and TEGDMA concentrations of $>10^{-3}$ M), they have been also identified as genotoxic or mutagenic *in vitro*.[28,29]

In our work, we focused on four commercially available adhesives widely used in restorative dentistry. The aim of the study was to test their possible genotoxicity *ex vivo* in human leukocytes in relation to the duration of the elution period and the polymerization form. The potential genetic risk was evaluated by a comet assay as a standard and sensitive cytogenetic method.

Materials and methods

Blood sampling

The potential genotoxicity of dental adhesive systems was evaluated on leukocytes obtained from three young, healthy, nonsmoking voluntary donors. The donors included one man and two women with ages ranging from 25 to 28 (mean, 26.7) years. They had not been exposed to any physical or chemical agents that might have interfered with the results of the genotoxicity testing within a period of 1 year prior to blood sampling. The volunteers were acquainted with the purpose of the study and signed permission for the blood samples to be used for scientific purposes. A peripheral blood sample (5 mL) was collected under sterile conditions by venipuncture into heparinized tubes (Becton Dickenson, Plymouth, UK) on November 21, 2011 and January 23, 2012. The study was approved by the Ethical Committee of the School of Dental Medicine, University of Zagreb, Croatia.

Preparation of materials and cell culture treatment

In the present study, four dental adhesives were tested: AdheSE (Ivoclar Vivadent, Schaan, Liechtenstein), G-bond (GC, Tokyo, Japan), Adper Single Bond (3M ESPE, St. Paul, MN, USA), and Excite (Ivoclar Vivadent). Their compositions, as provided by the manufacturers, are presented in Table 1. Under aseptic conditions, systems were prepared in accordance with the manufacturers' instructions.

To test the genotoxicity of nonpolymerized materials, the dental adhesives were placed in previously weighed bottles (Sartorius BLG10S, Goettingen, Germany). The mass of each dental adhesive was calculated from the difference