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Cytotoxicity of post and core composites as a function of environmental conditions

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ABSTRACT

Objectives. In the revised version of ISO 7405 there are so far no detailed recommendations concerning temperature and humidity during specimen production for light curing and chemically setting dental materials. The main objective of the present study was to observe if different environmental conditions during specimen production influence cytotoxicity and degree of conversion of four post and core composite materials and to investigate if cytotoxicity of post and core materials is influenced by their corresponding bonding substances.

Methods. Specimens of four different post and core composite materials (LuxaCore – Dual, Core X-Flow, Flow White and MultiCore Flow) were produced in a climate test chamber at 23 °C/50% relative humidity or 37 °C/95% relative humidity and were dual-cured or self-cured, with or without their corresponding bonding substances. Specimens were added to cell cultures immediately after production or after preincubation for 7 days. Specimens were incubated with L-929 fibroblasts for 72 h and cell numbers determined by a flow cytometer. FTIR spectroscopic measurements of post and core materials were performed at the same temperature conditions as for the cytotoxicity assay (23 °C or 37 °C).

Results. Dual-cured specimens of all post and core composites exhibited less cytotoxicity under both environmental conditions than self-cured specimens. All self-cured specimens manufactured at 37 °C/95% showed less cytotoxicity than specimens produced at 23 °C/50%. All dual-cured specimens showed similar cytotoxicity at both environmental conditions. After 7 days of preincubation most dual-cured specimens produced at 23 °C/50% showed less cytotoxicity than self-cured specimens (with the exception of Flow White). Compared to fresh specimens, 7-day aged specimens of most materials showed reduced cytotoxicity.

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Materials already showing low cytotoxicity as fresh specimens did not further reduce their cytotoxicity after 7 days of preincubation. For dual-cured materials the degree of conversion was higher compared to self-cured materials.

Significance. Different temperatures during specimen production have an impact on cytotoxicity and degree of conversion of dual-curing composite materials. Detailed recommendations for standardization concerning environmental conditions during specimen production are required.

1. Introduction

Biocompatibility of dental materials has gained increasing interest among dentists and patients during recent decades. Cytotoxicity is one important aspect of biocompatibility [1] and has been tested with varying protocols and therefore results are not generally comparable between laboratories [2–8]. It has been shown that, for example, the color of specimen molds and different ratios of specimen sizes to cell culture parameters produce different results [9]. Therefore in the revised version of ISO 7405 [10] specimen production has been identified as a critical factor regarding the comparability of results. Recommendations for specimen production have been introduced. For light curing materials and chemically setting materials specific recommendations exist concerning light and oxygen exposure, but not for temperature and humidity.

We have shown earlier that the degree of conversion of dentin bonding substances is significantly influenced by an air inhibition layer which is generated when specimens are cured in the presence of oxygen [11]. A lower degree of conversion of dentin bonding agents results in increased cytotoxicity [11]. Therefore in ISO 7405 recommendations were introduced to avoid air inhibition during specimen production to ensure internationally comparable results for cytotoxicity testing [10].

It has been known for a long time that temperature and relative humidity influence the quality of composite restorations. Some studies indicate that the bond strength is influenced by temperature and relative humidity. It has been shown that bond strength increases with increased temperature and decreases with increased relative humidity [12–17]. However, contradictory results have been found in that some studies report that relative humidity does not influence bond strengths of self-etch adhesives [18]. The influence of environmental conditions on the cytotoxicity of composite materials has not been studied so far, although in the recently revised version of the standard ISO 7405, dealing with the evaluation of biocompatibility of medical devices used in dentistry, it is recommended that temperature and humidity should be taken into account during specimen production [10].

Other factors that might influence cytotoxicity of composite materials are: (i) aging of specimens, e.g. fresh specimens vs. 7-day preincubated specimens [8], (ii) curing mode of specimens [19] and (iii) bonding substances [11]. With regard also to these criteria the following null-hypotheses were formulated with respect to conversion and cytotoxicity of post and core materials:

(1) The ambient temperature during specimen production does not influence degree of conversion of post and core materials.
(2) Fresh specimens are not cytotoxic (i.e. cell cultures incubated with fresh specimens do not show reduced cell numbers compared to negative controls).
(3) Bonding substances do not influence the cytotoxicity of post and core materials.
(4) Additional light curing (i.e. dual curing) of post and core materials does not influence cytotoxicity compared to chemically setting only.
(5) Certain environmental conditions (i.e. combinations of temperature and relative humidity) during specimen production do not influence cytotoxicity.
(6) 7-days preincubated post and core specimens do not show reduced cytotoxicity in comparison to fresh specimens.

2. Materials and methods

2.1. Post and core materials

The following four post & core materials: LuxaCore – Dual (LXC), Core X-Flow (CXF), Flow White (FW) and MultiCore Flow (MCF) and their corresponding bonding substances are listed in Table 1.

2.2. Cytotoxicity assay

2.2.1. Preparation of specimens

All specimens were produced in a climate test chamber (type VC 0018, Vötsch Industrietechnik GmbH, Balingen-Frommern, Germany) at two different environmental conditions (23 °C and 50% relative humidity [23/50] and 37 °C and 95% relative humidity [37/95]) and were dual-cured (dc) or self-cured (sc), with or without the corresponding bonding substance. Cylindrical specimens were prepared in Teflon blocks containing 5 mm diameter cylindrical holes (cylinder height 2 mm), covered with a polyethylene foil (Hostaphan®, Mitsubishi Polyester Film GmbH, Wiesbaden, Germany; film thickness 75 μm). Dual-cured specimens (dc) were light cured from one end according to the manufacturers' instructions. Self-cured specimens (sc) were produced in the same manner as described for dual-cured specimens but instead of light curing the specimens they were allowed to set for 7 min.