## Workshop: Umweltimmunologie und Umwelttoxikologie – Part I

## UTV01 In vitro cytotoxicity of resin-based VLC dental materials

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Introduction: Resin-based dental materials are used for anterior and posterior restorations, bonding agents, sealants. They can display toxic effect due to the release of poorly polymerized monomers. In this work, both the *in vitro* cytotoxicity of eight visible light-cured dental materials irradiated for different times and the distribution of the toxic components in the material bulk were tested.

Methods: Disk-shaped specimes of visible light-cured methacrylate-based materials were irradiated (time lengths: 1, 5, 10, 20, 40 sec) and soaked separately in 10% lactic acid solution for 24 h, then washed and submitted to the cellular viability assay. Cytotoxicity screening used the direct contact of the biomaterial with the cell system (monolayer of cell line L-929). After 48 h, the cells were stained with crystalviolet. The viability of the cell system was observed by optical microscope and assessed by absorbance readings. The results of the readings related to specimens irradiated for different times were analyzed statistically using the Student t-test. To examine the distribution

of toxic component, the specimens were reduced in thickness ( $\sim$ 20  $\mu$ m), and the viability test was repeated after one week of aging in bidistilled water.

Specimens were then observed by confocal fluorescent microscope using eosin as fluorescent dye. The degree of the polymerization conversion was assessed by polymerization contraction measurements.

Results: All specimens immersed in water after irradiation, showed cytotoxic effect. Both the 10 sec and the 40 sec irradiated specimens exhibited the same cytotoxic effect (Fig. 1), even with a significant difference in polymerization conversion. Cytotoxicity disappeared after the aging, but it appeared again when surface layers (~20 µm thickness) of the same specimen were removed. Confocal fluorescent microscope analysis of specimens soaked 1 week in water showed a porosity structure filled with water.

Conclusions: Specimens having different degree of the polymerization conversion (10 sec and 40 sec irradiated specimens) showed the same viability (Fig. 1), suggesting the main source of cytototoxic release as due to the air  $O_2$ , an inhibitor of polymerization reactions. For this reason the most monomers of the surface layer were unreacted and released in one week. The same inhibitory action was caused by the  $O_2$  in the porosity, that is filled with air. When specimens were scraped, the new surface bubbles absorbed water and released monomers, producing cytotoxycity.

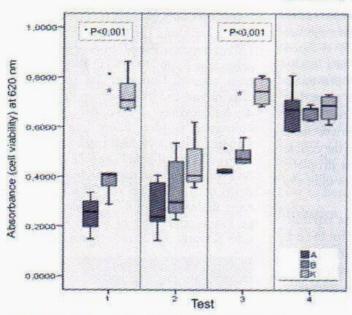


Fig. 1: Cytotoxic effects of VLC dental composite Venus

- A 10 sec irradiation, 5 specimens; B - 40 sec irradiation, 5 specimens;
- K control;
- 1 soaked 1 week in water
- 2 the same specimens soaked a second week in water
- 3 the same specimens thinned (20 µm) and soaked 1 week in water
- 4 the same specimes soaked 1 week in water