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## Evaluation of dental material cytotoxicity using direct tests in vitro

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### Introduction

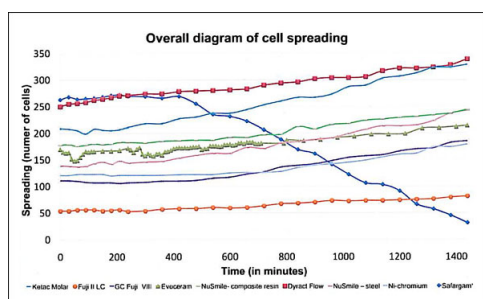
Cytocompatibility of materials can be verified by a range of tests. Elution methods, which enable to evaluate the effect of eluted substances in the defined solution on cell culture and to determine cytotoxicity of the tested materials, belong to the most frequently used methods. Another possibility offer direct methods which enable cultivation of cells in direct contact with the tested material and the cell reaction in its surrounding can be assessed. The direct methods have advantage in imaging the dynamics of material-cell interaction on living cells. The reaction of cell-culture cells in direct contact with the material surface can be observed under scanning electron microscope. The method can demonstrate the structure of contacts between the tested material and cells. All the methods mentioned quantify biocompatibility of artificial environment on the initial assays level.

### Objectives

The aim of the study is to investigate the character of contacts between cells and surface of dental materials used in paediatric dentistry.

### Material and Methods

Following dental materials were tested: Ketac Molar, Fuji II LC, GC Fuji VIII, Tetric EvoCeram, hybride composite resin of NuSmile crown, Dyract flow, stainless steel (NuSmile crown), Nichrom crown, and Safargam+ non gamma 2. Cell line HeLa was used for the tests. The cells were cultivated in MEM medium containing 10% FCS antibiotics. The sterile tested material was placed into the culture flask and cell plated. Following 72 hours of cultivation the cells covering the surface of the tested material were fixed in glutaraldehyde and transferred into absolute alcohol, dried using CPD, gold plated and photographed in Tescan Vega TS 5136 XM scanning electron microscope. The records were assessed both qualitatively and quantitatively. The cell growth inhibition test was performed on HeLa line cells. Disks of the tested materials (8x3mm) were placed into culture flasks (25cm<sup>2</sup>) containing 7 ml of the culture medium (MEM, NCS, glutamine, and antibiotics/antimycotics) with the cell suspension 60 000/ml. The growth of the cell culture was observed in the Nikon Eclipse TE 200-E microscope for 24 hours and recorded in 2 min. intervals by NIS-Elements AR programme. The cell growth inhibition is illustrated graphically (graph 1).



Graph. 1

### Results

Cells of HeLa cell culture covered continuously surfaces of most of the materials tested except of Safargam+ non gamma 2 (fig. 1-9). The cells were connected to the surfaces by large cell processes, in some places even 2 layer cover was formed. Cell surface was irregular and the cells sent numerous fine projections into their surroundings. Regularly, larger or smaller vesicles and cells in various stages of mitoses were observed. Growth inhibition was recorded only in Safargam+ non gamma 2 and stainless steel, in remaining materials the curve, after the incipient cessation, increased steadily.

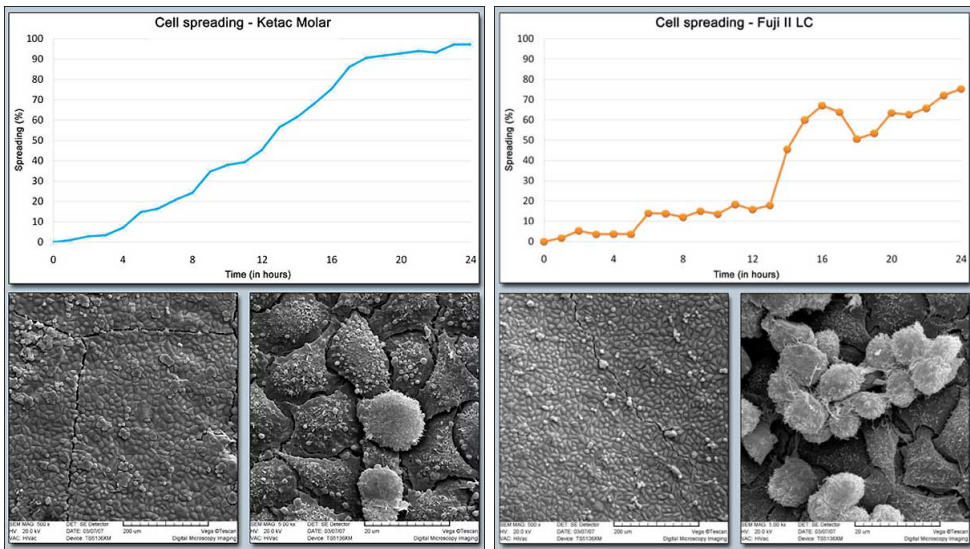


Fig. 1 and 2: HeLa line cells cover continuously the surface of the material

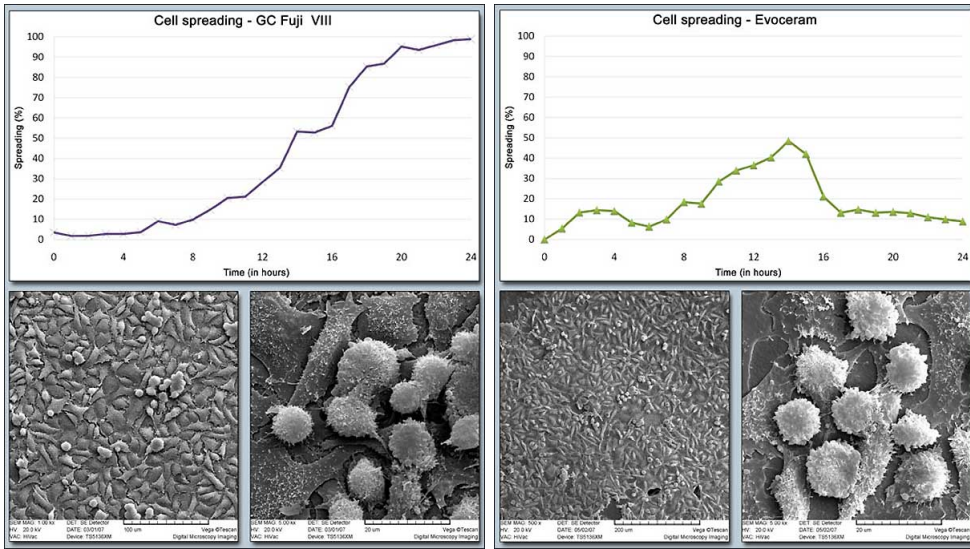


Fig. 3 and 4: HeLa line cells cover continuously the surface of the material

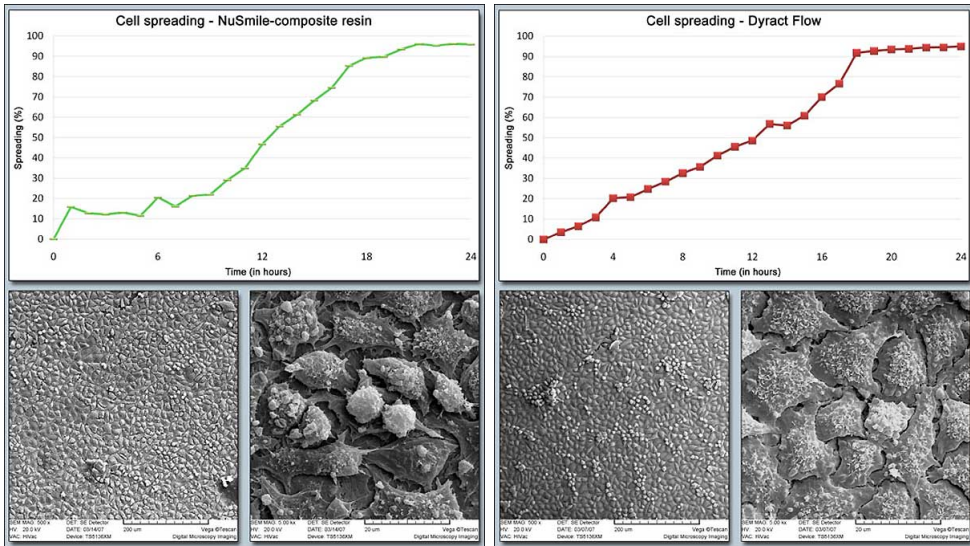


Fig. 5 and 6: HeLa line cells cover continuously the surface of the material

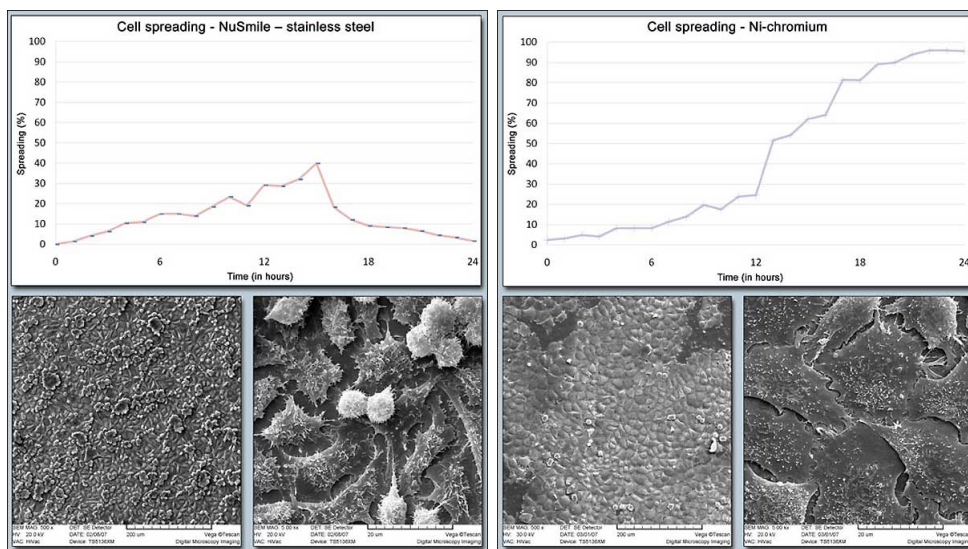


Fig. 6 and 7: HeLa line cells cover continuously the surface of the material

## Conclusions

Presence of irregular cell surface suggests that the materials tested evoke in cells of HeLa line reaction of the surface membrane. The dental material was not inert and in most instances its influence was not cytotoxic. No substantial differences were found between the materials tested, however, surfaces of Safargam+ non gamma 2 were not covered continuously with the cell culture, the material elicited unfavourable reaction which finally led to cell destruction and the cell growth inhibition test proved the growth depression with cell death. The study demonstrated that commonly used dental materials are not always fully cytotoxic, the reaction of Safargam+ non gamma 2 and stainless steel had even the cytotoxic character.

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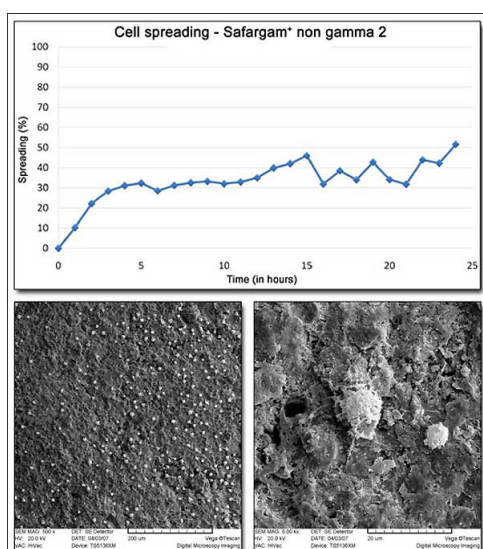


Fig. 10: Safargam+ non gamma 2, the surface of the material covered by dead cells

## Literature

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## Abbreviations

CPD - Critical Point drying

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