APPLICATION OF DIRECT CONTACT TEST IN EVALUATION OF CYTOTOXICITY OF ACRYLIC DENTURE BASE RESINS

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The use of acrylic denture base resins is widely spread in dental practice. They belong to the group of biomaterials due to their role of morphological and functional substituent in the mouth. However, clinical practice has shown that some toxic ingredients of these materials may lead to adverse local and even systemic changes. The aim of the study was to evaluate cytotoxic effect of various denture base resins on cell culture using direct contact test.

The effect of four different acrylic materials on HeLa cell structure was evaluated. Upon light microscopy analysis, MTT test was performed without previous removal of material samples.

The obtained values of MTT indicate that cell proliferation is dependant on the type of acrylic denture base resins. Cold polymerization denture base resins showed mild inhibitory effect on the cell culture growth. The signs of toxicity were not observed in heat polymerization denture base resins. Acta Medica Medianae 2012; 51(1):66-72.

Key words: acrylic denture base resins, direct contact test, HeLa cells

Introduction

Acrylic denture base resins have been used for the past eighty years in dental practice and are still indispensable in their area of indication (1). As building materials they are used for construction of plate part of mobile dentures, false teeth, temporary crowns and bridges, mobile orthodontic appliances, obturators and maxillofacial prostheses, as well as for relining and repair (2). They are referred to as biomaterials due to their role of morphological and functional substituent in the mouth (3, 4). However, clinical practice has shown that some toxic ingredients of acrylates may lead to adverse local and even systemic changes (5-8). The degree of tissue sensitivity to acrylate increases with the percentage increase of potentially toxic substances in the material (9, 10). These substances have the ability to leak from denture and diffuse in saliva by means of which they affect oral mucosa (11-14). The amount of potentially toxic substances in acrylic material is different and depends on the type and time of polymerization (11, 15-17).

Evaluation of biological properties in vitro provides adequate comparison of various acrylates commercially available, and subsequent easier selection of materials and polymerization procedure in everyday work with patients. The contact between cell culture and tested material is achieved in direct and indirect way by means of extracts (18). In case of direct contact, cytotoxicity is measured by the rate of cell death in the function of exposure time and distance from the sample which is in solid physical condition (19). The most frequent complications in vitro tests with direct contacts include bacterial contamination of culture and mechanical damage of cells by direct contact with the material (19,20).

The aim of the study was to evaluate cytotoxic effect of various denture base resins on cell culture using direct contact test.

Material and methods

Tested material

The tested material involved three hard and three soft denture base resins used in dental practice for construction and readaptation of mobile dentures. Cold, heat and light polymerization denture base resins were used in the study (Table 1).

Six samples of each tested acrylic material were made, the shape and size of which matched the half of the separate field bottom on the cultivation plate with 48 wells (semicircle with 1cm diameter). The material for cultivation was paste-like, so that the final bond was done within individual wells. After having been polymerized samples of heat polymerization acrylates were inserted in the plate wells for cell cultivation (Figure 1). Models of the samples were originally made in pink dental wax (Vomogal-S, Galenika, Serbia). Samples for evaluation were made immediately before each experiment to avoid possible changes resulting from material aging.
Table 1. Tested denture base acrylic resins

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Manufacturer</th>
<th>Acrylic type</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosforth Trusoft</td>
<td>HG Bosworth Company USA</td>
<td>soft cold polymerized acrylate</td>
<td>poly(ethyl methacrylate)</td>
</tr>
<tr>
<td>Lang Flexacryl</td>
<td>Lang Dental MFG. Co. USA</td>
<td>soft cold polymerized acrylate</td>
<td>poly(ethyl methacrylate)</td>
</tr>
<tr>
<td>Lang Immediate</td>
<td>Lang Dental MFG. Co. USA</td>
<td>soft cold polymerized acrylate</td>
<td>poly(ethyl methacrylate)</td>
</tr>
<tr>
<td>Lucitone 199</td>
<td>Dentsply International Inc.</td>
<td>light polymerized acrylate</td>
<td>poly(methyl methacrylate)</td>
</tr>
<tr>
<td>Triplex Cold</td>
<td>Ivoclar Vivadent, Lichtenstein</td>
<td>hard cold polymerized acrylate</td>
<td>poly(methyl methacrylate)</td>
</tr>
<tr>
<td>Triplex Hot</td>
<td>Ivoclar Vivadent, Lichtenstein</td>
<td>heat polymerized acrylate</td>
<td>poly(methyl methacrylate)</td>
</tr>
</tbody>
</table>

Light microscopy

Viability, density and epithelial organization of HeLa cells were observed under the invert microscope (Observer Z1, Zeiss, Germany).

Cell density was determined by their counting in three vertically placed quadrants of visual field for each place of the tested sample and the control group. Cells with altered phenotype separated from the base were counted as dead.

Since the tested material occupied a quarter of each observed quadrant in the visual field, the density of cells in the control group was presented as 75% of its total cell growth.

MTT test

The enzyme succinate dehydrogenase that is an integral part of mitochondrial respiratory chain is active in viable cells. This enzyme reduces yellow tetrazolium salt - MTT (3-(4,5-di methylthiazol-2)-2,5-diphenyltetrazolium bromide) to formasan, a blue colored compound that is deposited in the cells in the form of crystals. Isopropanol damages cell membrane and formasan dissolves. There is a direct reciprocity between the number of viable cells and the intensity of blue colour (Figure 2).
The medium in which cells were incubated was drawn at the end of the incubation and the cells were washed with 100 μl PBS (phosphate buffered salt) and 20 μl of MTT was added. After 4 h incubation at 37 °C, the formasan crystals were dissolved with 100 μl of isopropanol. Spectrophotometric measurement of the intensity of MTT reduction was carried out at optical density of 540 nm on the multi-channel photometer (Multiskan Ascent No354, Thermo Labsystems, Finland).

Interpretation of results

Quantitative changes in cell proliferation are presented descriptively: non-cytotoxic (> 90%), slightly cytotoxic (60-90%), moderately cytotoxic (30-59%), severely cytotoxic (<30%) viability and growth of cells compared to the control group (22). Regarding the one-day incubation, the results are presented as percentage of cell viability and cell culture proliferation of tested groups compared to controls. Since HeLa cells are epithelial, adherent cells, the reduction of adherent phenotype was considered as the sign of toxic effect.

For the values obtained by MTT test statistical analysis of cytotoxicity of the tested materials compared with the control testing and mutual testing of samples was performed. We used ANOVA (p <0.05) and Post Hoc Analysis.

Cell viability and proliferation of untreated control groups are presented as 100% cell growth.

Results

Figures 3-6 show cell viability, proliferation and epithelial organization in direct contact with the samples of tested materials. It may be noted that cells change shape when close to material.

Round cells separated from the base are regarded dead.

The greater the distance from the sample material the greater the density of HeLa cells that are organized in clusters characteristic for epithelial tissue.

Graph 1. shows the percentage of live HeLa cells cultured in contact with the samples of tested acrylic materials compared to controls. The control group is presented as 75% of cell growth.
Graph 1. The percentage of viable cells compared to control, after one day direct contact with the acrylic materials.

Graph 2. The percentage of epithelial organization compared to control, after one day direct contact with the acrylic materials.

Table 2. Procenat intenziteta redukcije MTT-a ispitivanih akrilatnih materijala u odnosu na kontrolu

<table>
<thead>
<tr>
<th>Ispitivani materijal</th>
<th>N</th>
<th>X</th>
<th>±</th>
<th>SD</th>
<th>Cv</th>
<th>SE</th>
<th>95%</th>
<th>CI</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosworth Trusoft</td>
<td>6</td>
<td>98,71</td>
<td>±</td>
<td>8,72</td>
<td>11,08</td>
<td>3,56</td>
<td>69,56</td>
<td>87,85</td>
<td>90,71</td>
<td>11,08</td>
</tr>
<tr>
<td>Lang Flexacryl</td>
<td>6</td>
<td>98,42</td>
<td>±</td>
<td>22,72</td>
<td>23,08</td>
<td>9,27</td>
<td>74,58</td>
<td>122,26</td>
<td>118,58</td>
<td>23,08</td>
</tr>
<tr>
<td>Lang Immediate</td>
<td>6</td>
<td>79,94</td>
<td>±</td>
<td>15,05</td>
<td>18,82</td>
<td>6,14</td>
<td>64,15</td>
<td>95,73</td>
<td>105,24</td>
<td>18,82</td>
</tr>
<tr>
<td>Lucitone 199</td>
<td>6</td>
<td>96,35</td>
<td>±</td>
<td>7,04</td>
<td>7,31</td>
<td>2,88</td>
<td>88,95</td>
<td>103,74</td>
<td>106,13</td>
<td>7,31</td>
</tr>
<tr>
<td>Triplex Cold</td>
<td>6</td>
<td>96,40</td>
<td>±</td>
<td>13,80</td>
<td>14,31</td>
<td>5,63</td>
<td>81,91</td>
<td>110,88</td>
<td>120,95</td>
<td>14,31</td>
</tr>
<tr>
<td>Triplex Hot</td>
<td>6</td>
<td>99,70</td>
<td>±</td>
<td>9,51</td>
<td>9,54</td>
<td>3,88</td>
<td>89,73</td>
<td>109,68</td>
<td>110,57</td>
<td>9,54</td>
</tr>
</tbody>
</table>

* p<0,05 vs Bosworth Trusoft

The lowest intensity of MTT was found in Bosworth Trusoft samples. Tamhan test showed that this difference was very close to the threshold of statistical significance (p=0,0505) compared to the tested Lucitone 199.

Table 2. shows the intensity of reduction of MTT in relation to the type of tested acrylic material.

Discussion

The analyses of cell culture are of great importance for understanding biological action of the material. However, they are prone to certain limitations referring primarily to impossibility of
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The presence of cold HeLa cells was observed in direct contact of culture material. The lowest percentage of clustering of cells is characteristic of HeLa culture, any deviation from the normal phenotype organization could be considered dead. The greater the distance from the sample material the greater the density of HeLa cells that are organized in clusters characteristic for epithelial tissue.

On the basis of the obtained data, it is obvious that there is mild toxic effect of soft acrylates and Triplex Cold on HeLa cell culture, that is, their clinical application immediately leads to reduction of proliferation of epithelial cells (73-87%). Conversely, the direct contact test showed non-toxic effect of Lucitone and Triplex Hot 199 (97%).

Regarding the fact that epithelial clustering of cells is characteristic of HeLa culture, any deviation from the normal phenotype organization could be considered to have negative effect on applied material. The lowest percentage of clustering of HeLa cells was observed in direct contact of culture with soft acrylates (35-60%). The presence of cold polymerized Triplex Cold (85%) showed a slightly lower level of cell organization in relation to light-polymerized Lucitone 199 (92%). The greatest degree of epithelial organization was observed in Triplex Hot (96%), which indicates its negligible cytotoxicity.

The growth of cells in culture is presented by the intensity of MTT reduction as well. The obtained MTT values indicate that cell proliferation is dependent on the type of acrylic denture base resins. The lowest values are present in Trusoft Bosworth, and the highest values are present in Triplex Hot. Excellent biological characteristics of Triplex Hot may be attributed to its flat surface structure, and minimal porosity (16). The results obtained are in accordance with the results of in vitro examination of potential acrylic toxicity on culture using the indirect contact test (22-29). On the other hand, in vitro examinations by Huang et al. and Melili et al. confirmed greater toxicity of the heat polymerized acrylic denture base resins in comparison to light polymerized acrylic denture base resins (30, 31). Cold polymerized Triplex Cold had higher inhibitory potential as compared to heat and light polymerized resins, which is in accordance with the results of other authors as well (28,29, 32,33). The study results showed greater inhibitory effect of soft resins compared to other studied groups, which correlates with the findings of Okita et al. (34). Increased cytotoxicity of cold polymerized denture base resins can be explained by their weaker and more porous structure (35,36).

Conclusion

Acrylic denture base resins may be considered to have biocompatible features, which justifies their everyday use in clinical practice. Cold polymerized denture-based resins showed more toxic effect on cell structure in comparison to heat and light polymerized acrylic denture-based resins.

References

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PRIMENA TESTA DIREKTNOG KONTAKTA U ISPITIVANJU CITOTOKSIČNOSTI STOMATOPROTETSKIH AKRILATA

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Ključne reči: akrilati, test direktnog kontakta, HeLa ćelije