Cytotoxic Study of Three Cements in Gingival Fibroblasts

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INTRODUCTION

Biocompatibility is defined as a relationship between the material and the host, in which neither produces undesirable effects on the other. It has been a subject of study in many areas of health and it is known that all the materials and devices used in the oral cavity can trigger adverse effects and they are not today fully characterized. So arises the problem of not biocompatibility of biotechnological materials, which include dental cements.

Dental cements, essential for the success of oral rehabilitation, may be responsible for the release of substances that can induce negative effects on cells, including their death. They are present in the transition between restoration and tooth and, although in small areas, remain in contact with the oral tissues of patients for long periods of time.

It is described that there is a higher tendency to recession or gingival inflammation in areas where irritating products are released by materials. The aim of this study was to compare the cytotoxic effect of three luting cements from 3M ESPE (St. Paul, MN, USA) on the cells from gingival fibroblast cell line: Ketac™ Cem Easymix (glass ionomer cement), Ketac™ Cem Plus (resin-modified glass ionomer cement) and Relyx™ Unicem 2 Automix (resin cement). To determinate the cytotoxicity effect were used MTT and crystal violet assays, which determine cell viability by evaluating the cytotoxicity of the cements, based on this induced damage to the cells.

MATERIALS AND METHODS

The cements, Ketac™ Cem (KC), Ketac™ Cem Plus (KP) and Relyx™ Unicem 2 (RU) were prepared according to the manufacturer's indications and inserted into silicone molds shapped cylinder, with 5 mm diameter and 2 mm thick. RU was prepared in two different ways: light curing 20 seconds (RU1) and auto curing (RU2). After 24h in contact with culture medium (DMEM supplemented), were made 5 dilutions (table 1) that were in contact with 3T3 fibroblasts cells. After an incubation-period of 24 hours, cell viability was measured by MTT and crystal violet assays. Experimental tests were performed two independent and each experimental point was obtained in 8 replicate cultures for each concentration.

Dilution	µl of culture medium + µl of cement extract
1	0+200
1:2	100+100
1:4	150+50
1:8	175+25
1:10	180+20

Table 1. Dilutions made.

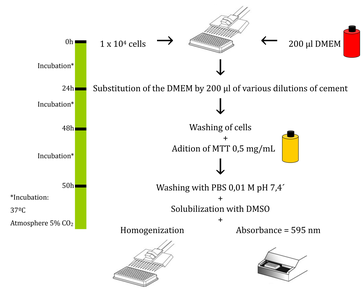


Image 1. Schematic illustration of the experimental test procedures performed.

RESULTS

Figure 2. a) Control; b) KC; c) KP; d) RU1; e) RU2. These images translate microscopically the results. In the control plate (a), the cells are confluent and adherent, representing a high percentage of viable cells. From image b) to e) there is a decrease of the percentage of adherent cells (viable cells) with an increase of the percentage of non-adherent cells (non-viable cells).

%		Cement KC	KP	RU1	RU2	*p-value*
Dilution	1:10	81,7 ± 10,0	80,6 ± 6,5 a,b	84,6 ± 8,1 a	74,0 ± 15,5 a	0,153 °
	1:8	81,6 ± 11,9 1	85,6 ± 5,4 b,1	82,2 ± 10,2 a,1	64,8 ± 9,4 a,2	< 0,001 **
	1:4	78,9 ± 6,4 1	71,9 ± 4,4 a,2	63,5 ± 10,6 b,2	39,7 ± 3,3 b,3	< 0,001 °°
	1:2	76,6 ± 13,0 1	63,1 ± 5,5 c,2	30,2 ± 5,8 c,3	17,1 ± 4,4 c,4	< 0,001 °°
	1	73,9 ± 10,1 1	48,4 ± 10,9 d,2	7,9 ± 3,0 d,3	5,3 ± 1,6 d,4	< 0,001 °°
p-value		0,294 *	< 0,001 °°	< 0,001 °°	< 0,001 °°	

* ANOVA one-way; ** ANOVA one-way + teste *post-hoc* Scheffe; ° ANOVA one-way c/ correcção de Brown-Forsythe; °° ANOVA one-way c/ correcção de Brown-Forsythe + teste *post-hoc* Tamhane

Table 2. Average value of the percentage of MTT reduction ± standard deviations (representing the percentage of cell viability) of all cements and dilutions. *p-value* corresponds to the level of significance between the values of the various cements tested for each dilution and between the values of various dilutions tested for each cement. (1), (2), (3) and (4) correspond to homogeneous subgroups of average values of cements for each dilution and (a), (b), (c) and (d) correspond to the mean values of the subgroups homogeneous dilutions for each cement.

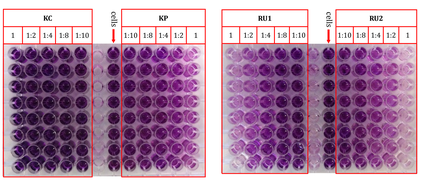
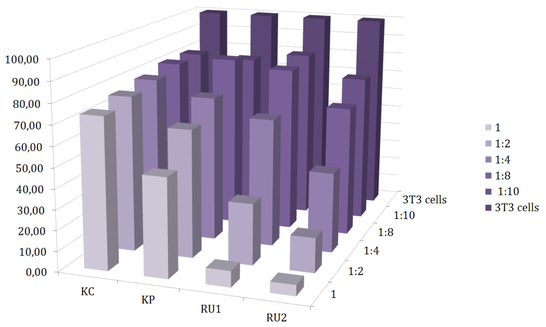


Figure 3. Microplates.



Grafic 1. Ratio of cement distribution with distribution by dilution. Values are presented as a percentage of MTT reduction (representing the percentage of cell viability) of all tested dilutions cements. 3T3 cells = control cultures without cement (100% cell viability).

Table 2 presents the averages of the percentages of cell viability obtained from the three experimental tests performed with data from the statistical analyzes.

To verify if the results were statistically significant compared to control cultures, a Student´s t-test was performed for each percentage of cell viability, with a significance level of 5%.

The highest cell viability occurs for KP at 1:8 dilution (85.6%) and lowest in the dilution 1 for RU2 (5.3%).

For all values was obtained *p-value*<0,05, meaning that all cements at all concentrations tested exhibit statistically significant differences in terms of cell viability.

Analyzing the results by cement, we found that only KC caused no decrease in cell viability statistically significant with increasing concentration of cement applied (p> 0.05). In other cements, the increase of cement concentration led to a significant decrease in cell viability (p <0.05).

Comparing the various cements in all dilutions except in 1:10 dilution, there are significant differences between them, and RU2 was the cement that caused a decrease in cell viability more meaningful.

In graphic 1, we see that all cements compared with control cells, cause a decrease in cell viability. Denotes a correlation between the dose of the compound and reduction of the percentage of cell viability, in particular from 1:4 dilution. For all cements, there is a decrease in the percentage of cell viability.

It is also clear that the cells have varying degrees of sensitivity to the cements tested, resulting in a higher or lower cytotoxicity. These results are especially noticeable in dilutions 1:2 and 1, in which the concentration of toxic compounds is substantially higher. It is observed that cements the RU1 and RU2 are obviously more cytotoxic than the cements KC and KP.

CONCLUSIONS

- Gingival fibroblasts are sensitive to all cements concentrations equal or higher than 10%.
- The results suggest the following order of cytotoxicity: KC<KP<RU1<RU2.
- The Ketac™ Cem Easymix (CIV) is the less cytotoxic cement, without a significant increase in its cytotoxicity, when its concentration increases.
- The cement Relyx™ Unicem 2 Automix (CR) is the most cytotoxic cement, whatever their mode of polymerization.
- The photo-activation of Relyx ™ Unicem 2 Automix (CR) reduces its cytotoxic effect. This is, its cytotoxic potential is higher if not cured.
- The cytotoxic effect of Ketac™ Cem Plus (RMGIC) and Relyx™ Unice 2 (CR) is dose-dependent, i.e. the increase in concentration results in an increase in their cytotoxic potential.
- Cements that have in its composition resin monomers are more cytotoxic than those that do not.

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