Effects of Current Provisional Restoration Materials on the Viability of Fibroblasts

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ABSTRACT

Objectives: The aim of the present study was to evaluate the cytotoxic effects of three different provisional restoration materials on fibroblasts. Two bis-acrylic based [Tempofit Duomix (Detax), Protemp 3 Garant (3M ESPE)] and one urethan dimethacrylate [Revotek LC (GC Corporation)] based provisional restoration materials used.

Methods: Materials were prepared according to the manufacturers' instructions in standard teflon disks (2x5 mm) and four samples were extracted in 7 ml of Basal Medium Eagle with 10% new born calf serum and 100 mg/ml penicillin/streptomycin for 24 hours. The L929 fibroblast cells were plated (25.000 cells/ml) in well plates, and maintained in a CO_2 incubator at 37°C for 24h. After 24 hours, the incubation medium was replaced by the immersed medium in which the samples were stored and the L929 fibroblasts were incubated in contact with eluates for 24 hours at 37°C for 24h. The fibroblast cell viability was analyzed by measuring the mitochondrial activity with the methyltetrazolium test (MTT). Twelve well used for each specimen and experiment repeated for two times. The data was statistically analyzed by Mann-Whitney U tests.

Results: The results showed that, Revotek LC and Protemp 3 Garant were not cytotoxic for fibroblast cells when compared to control group ($P>.05$). However, Tempofit duomix was cytotoxic for L929 fibroblasts when compared to control group and other tested materials ($P<.05$).

Conclusions: Taking into consideration the limitations of an in vitro study, our study indicate that provisional restoration materials might have cytotoxic effects on fibroblasts and should be selected carefully for clinical applications. (Eur J Dent 2009;3:114-119)

Key words: Provisional restoration materials; Cytotoxicity; Fibroblast; MTT.

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INTRODUCTION

Dental materials contain a great variety of different monomers and additives.[1] Because of the complex chemical composition and the incomplete monomer–polymer conversion, several components are leached out from each resin-based restorative material into the oral environment.[2,3] This in turn may cause some adverse effects.[4] Previous studies have used in vitro cytotoxicity tests to evaluate the biological risks of resin composites used in dentistry.[1,5] Cytotoxicity tests have primarily focused on restorative materials such as glass ionomers, dental adhesives and composite resins.[6-8] However, fewer studies on prosthodontic materials have been published, and investigations regarding the cytotoxicity of provisional prosthodontic materials are even more limited.[5,9]

Provisional restorations are used in the interim between tooth preparation and fitting a definitive restoration. The length of time between preparation of teeth and cementation of final restorations can vary from a few days for straightforward cases, to several weeks or even, in the case of complex reconstruction, several months. Provisional restorations are generally essential to cover freshly cut dentine, stabilize the position of the prepared tooth, regain chewing function and phonation, maintain esthetic appearance and evaluate the minimal thickness of the definitive restoration. They can also help stabilize the periodontal condition prior to definitive restoration.[10]

Provisional materials can be classified by the type of resin. Acrylic polymethyl or polyethyl methacrylates belong to the oldest group of provisional materials. The latest class of materials is formed by bis-acryl composite resins, which are comparable to composite resins used for direct restoration therapy.[10] They consist of an organic matrix and inorganic fillers. Bis-acryl composites produce less heat and shrinkage during polymerization than other resins, resulting in a better marginal fit.[11] Aesthetically they are reasonable and are more color stable than polymethyl or polyethyl methacrylates.[12] Most recently, visible light cured resins have been introduced based on urethane dimethacrylate. These resins have good mechanical properties, being light cured, the operator has some control over the material's working time and colour is relatively stable but marginal fit can be poor.[10,13]

Acrylates and mainly methacrylates were found to cause cytotoxic effects.[14] Evaluation of the cytotoxicity of dental resin materials showed a relationship between their composition and the degree of cytotoxicity.[15] Continuous cell lines, like L929 mouse fibroblasts are being routinely used for the testing of cytotoxic properties of dental materials because of their reproducible growth rates and biological responses.[1] The purpose of this in vitro study was to evaluate the effect of current bis-acryl and urethane dimethacrylate based provisional materials on the fibroblast cell viability.

MATERIALS AND METHODS

The provisional restoration materials tested in this study are shown in Table 1. Two of the tested materials were bis-acryl based (Tempofit Duomix, Detax, Germany & Protemp 3 Garant, 3M ESPE, Germany) and one was urethane dimethacrylate based (Revotek LC, GC Corporation, Japan) provisional restoration materials. Test specimens were prepared according to the manufacturers' instructions in standard teflon discs, 5 mm in diameter and 2 mm of height. All specimens were prepared and handled under aseptic conditions to limit the influence of biological contamination on

Table 1. Material name, company, lot number and composition.

Materials	Company	Lot #	Composition
Tempofit Duomix	Detax, Ettlingen, Germany	315185	Mixture of methacrylic resins and silane treated glass with auxiliary matters and pigments
Protemp 3 Garant	3M ESPE, Seefeld, Germany	51200	Dimethacrylate, Silicic acid, Initiators, Diacrylate, Stabilizers, Synthetic resins, Pigments,Dyes, Strontium glass powder
Revotek LC	GC Corporation, Tokyo, Japan	704091	Urethane, Silica powder, Camphorquinone

the cell culture tests. Specimens were prepared between mylor and glass slabs to minimize the oxygen inhibition and maximize the surface smoothness. Tempofit Duomix is a two-part base/catalyst, hand-mix, self-curing and bis-acrylic composite based provisional restoration material. Base and catalyst were extruded equal amounts by pressing onto piston in the dispenser onto mixing pad. Both components mixed with spatula within 20 – 30 sec. homogeneously. Then applied into the teflon disc and after 2 min – 2 min 30 sec curing completed. Protemp 3 Garant is a two-part base/catalyst, auto-mix, self-curing and bis-acrylic composite based provisional restoration material. Using the Garant dispenser, the base and catalyst were extruded directly into the teflon disc and after 2 min 30 sec curing completed. Revotek LC is a light cure single component sculptable composite resin for temporary restorations. Using a spatula required amount of material dispensed and applied into the teflon disc. The specimen was light-cured for 6 sec by LED light curing unit (LED, Bluephase, Ivoclar Vivadent, Liechtenstein, Austria).

Four samples prepared for each group for cytotoxicity test. The samples immersed in 7 ml culture medium for 24 hours at 37°C to extract residual monomer or cytotoxic substances. The culture medium containing material extracts were sterile filtered to use on the cell cultures.

Cytotoxicity testing

L929 fibroblast cell line (ATCC CCL 1) cultured in Basal Medium Eagle (BME), Biological Industries, Israel) containing 10% new born calf serum (Biochrom AG, Berlin, Germany) and 100 mg/ml penicilin/streptomysin (Biological Industries, Israel) at 37°C in a humidified atmosphere of 95% air / 5% CO_2. Cell cultures between the twelve and fifteen passages were used in this study. Confluent cells were detached with 0.25% trypsin and seeded at a density of 5×10^3 well in 96-well plate at 37°C under 5% CO_2 for 24h and. After 24 hours incubation, culture medium was replaced with 200 µl of culture medium containing material extracts of provisional restoration materials. Original culture medium was served as control in this study. Cultures were incubated for 24 hours at 37°C and 5% CO_2 for 24 hours. The viability of cells exposed to material extracts was assessed using succinic dehydrogenase activity. The succinic dehydrogenase activity has been shown to be reasonably representative of mitochondrial activity in the cells and reflects both cell number and activity.[16] The old medium removed and cell cultures were rinsed with phosphate buffer saline (PBS) and 200 µl aliquots of freshly prepared MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Sigma Aldrich, Germany] solution (0.5 mg/mL in BME) were added to each well. After a 2h incubation period (37°C, 5% CO_2) the supernatant was removed and the intracellulary stored MTT formazan was solubilized in 200 µl dimethyl sulfoxide for 30 min at room temperature. The absorbance at 540 nm was spectrophotometrically measured. Twelve replicate cell cultures were exposed to a constant concentration of a single material in at least two independent experiments. The treated groups compared to cell survival in untreated controls. Differences between mean values were statistically analyzed using the Mann-Whitney U test.

RESULTS

The results of cytotoxicity test with provisional restoration materials are summarized in Figure 1. Reduced cell density is shown for Tempofit in Figure 2(b). In contrast, Protemp 3 Garant group demonstrate full cell density in Figure 2(c).

The results showed that, eluates of the Revotek LC and Protemp 3 Garant lead to 99% and 101% cell survival. Statistically Revotek LC and Protemp 3 Garant were not cytotoxic for cells when compared to control group (P>.05). Eluates from Tempofit duomix lead to 88% cell survival. Tempofit duomix

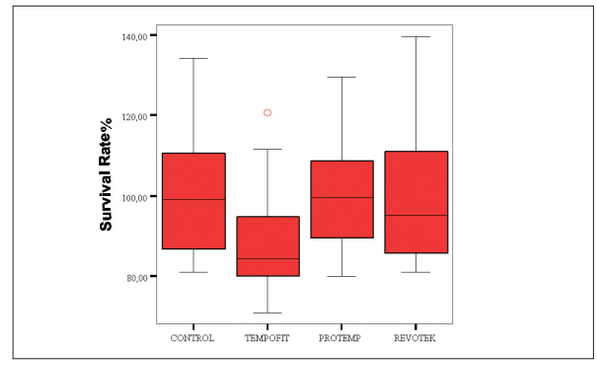

Figure 1. Cell survival of L929 cells in a methyltetrazolium test after exposure to provisional restoration materials. Data are expressed as percentage of the control cultures. Cell survival rates were calculated from independent experimental cultures: Control (n=24), Tempofit Duomix (n=24), Protemp 3 Garant (n=24), Revotek LC (n=24).

was cytotoxic for cells when compared to control group and other tested materials (P<.05).

DISCUSSION

The literature contains descriptions of cell-culture tests with various cell types to establish cell damage caused by dental materials.[17] In the present study the effect of two bis-acryl and one urethane dimethacrylate based commercially available provisional restoration materials on fibroblast cells were investigated by MTT test. Fibroblasts are the targets of any chemical components that may be released from the dental restorative materials. L929 fibroblast cells were selected due to its availability, popularity and efficiency to grow in vitro.[18] MTT assay is a well-established method for analyzing cell viability.[16] The viability and proliferation of the cells are assessed by means of the functional state of the cell mitochondria.[19] Mitochondrial dehydrogenases in living cells reduce the yellow tetrazolium salt, MTT (3-(4,5-dimethyl) thiazol-2-yl) 2,5 diphenyl-tetrazolium bromide) to blue MTT formazan, which is then retained in the cell. Formation of the formazan product has been found to correlate well with number of viable cells.[8,19,20]

Today, bis-acryl composites possess considerable amount of the market share for tooth colored provisional material. Main advantages of bis-acryl provisional materials include a lower curing temperature, reduced polymerization shrinkage (5%) with improved marginal fit, and minimal odour and taste.[13,21] The low setting temperature of these materials allows them to be used directly with decreased risk of pulpal injury.[22] In addition, bis-acryls are gaining in popularity, in part because of their cartridge delivery system. This dispensary method not only is convenient but also may allow for a more accurate and consistent mix.[21] Dental practitioners have clearly welcomed these products and very limited data can be available about their cytotoxicity and biocompatibility.

In present study, two of the tested provisional restoration material was bis-acryl based which are chemically very similar to bisphenol-A-glycidyl methacrylate (Bis-GMA) composites. According to our results, eluates from Tempofit duomix lead to 88% cell survival and when compared to control group and other tested materials it was cytotoxic for cells (Figure 2a-b). On the hand Protemp 3 Garant, the other bis-acryl based provisional material, was not cytotoxic for L929 fibroblast cells (Figure 2c). Interestingly slightly increased cell vitality was observed with Protemp 3 Garant (101%). Differences in cytotoxicity can be partly attributed to differences in chemical composition. Protemp Garant has been modified and marketed as Protemp 3 Garant. The modifications include a newly developed monomer system, not with the rigid intermediate chain characteristic of some bis-GMA homologues, but with a somewhat flexible chain in comparison to other synthetic resins (ESPE Technical Product Profile). This modification in the monomer system may limit the cytotoxic potential of the material.

However, manufacturer of Tempofit duomix do not state any difference in monomer formulation. Probably as most other bis-acryl based provisional materials, the organic polymer matrix of Tempofit duomix is composed of traditional monomers such as Bis-GMA, triethylene glycol dimethacrylate (TEGDMA) or similar monomer systems. But one must keep in mind that resin materials may contain rather 'unknown' monomers and generally these monomers protect by patents. Patents may also hinder objective research.[23] Only available composition of the resin cements tested in this study. They may also contain such unknown monomers.

Current investigations reported the cytotoxic effects of some resin monomers, such as BIS-GMA,

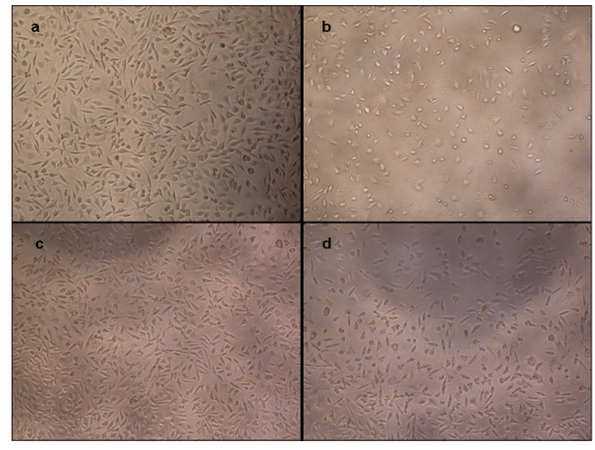

Figure 2. Effects of provisional materials on L-929 fibroblasts: (a) control group (Original culture medium), (b) culture medium containing material extracts of Tempofit, (c) culture medium containing material extracts of Protemp 3 Garant and, (d) culture medium containing material extracts of Revotek. Cells were incubated with these mediums for 24 hours (x10).

TEGDMA and urethane dimethacrylate (UDMA).[24,25] These resin monomers are able to deplete intracellular glutathione as well as interfere with the expression of some proteins, such as collagen I, osteonectin, and dentin sialoprotein, which play a fundamental role in the pulp repair.[26,27]

Among the tested materials, Revotek LC is the only UDMA based and light cure provisional material. Geurtsen et al[1] reported that UDMA is as cytotoxic as BIS-GMA and TEGDMA. Elution of residual monomers from resin materials related to degree of their polymerization, properties of resin composition, and chemistry of organic solvents in vitro situation.[28] Altıntas et al[29] demonstrated that leaching of UDMA was lower than BIS-GMA and TEGDMA from a resin cement. Consequently, in present study, eluates of the Revotek LC showed similar cytotoxicity with control group.

CONCLUSIONS

The results of this study demonstrated that cytotoxic potential may vary among provisional materials. Taking into consideration the limitations of this in vitro study, provisional restoration materials may have cytotoxic effects and should be selected carefully for clinical applications.

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