World Journal of Stomatology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5321/wjs.v2.i4.86

World J Stomatol 2013 November 20; 2(4): 86-90
ISSN 2218-6263 (online)

BRIEF ARTICLE

Cytotoxicity of a silorane-based dental composite on human gingival fibroblasts

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Received: June 13, 2013 **Revised:** August 5, 2013
Accepted: August 20, 2013

Abstract

AIM: To evaluate the direct and indirect biocompatibility of Filtek Silorane on human gingival fibroblastic cells.

METHODS: Sixty-three standardized cylindrical specimens (8 mm diameter and 2 mm thickness) of restorative material were prepared using a light emitting diode-curing unit. The sample were built up in one increment and divided in 2 groups. In the first group, 21 samples (unpolished samples) were left without a specific polishing procedure; in the second one, 42 samples (polished samples) were polished with 4 different grains of discs. Fibroblast cultures, obtained from gingiva of 2 subjects without systemic and oral disease, were used to assess the direct and indirect biocompatibility. Cells cultured for 48 h in normal culture medium were used as a control.

RESULTS: The scanning electron microscope observations of fibroblasts cultured on the silorane samples, either polished or unpolished, confirmed the good biocompatibility of the material, favouring the cellular spreading. 3-dimethylthiazol-2, 5-diphenyltetrazolium bromide tests showed a significant reduction ($P < 0.01$) of gingival fibroblasts viability cultured both in polished samples (90.05% ± 19.00%) and unpolished samples (78.15% ± 11.00%) compared with the control. Cells growth in medium conditioned with the samples for 1 wk showed a significant viability reduction ($P < 0.01$) compared to the control. A reduction of cell viability was observed even in the groups containing the material for 3 wk (polished: 89.45% ± 10.00%; unpolished: 65.97% ± 10.00%), even if the cytotoxicity was reduced after this long time exposure.

CONCLUSION: Although the poor chromatic availability of this material remains a big limit that restricts its use to posterior sectors, the silorane-based material can be considered an option to perform restorations when aesthetic demands are not the priority, such as the class Ⅱ restorations

Key words: Silorane; Cytotoxicity; Resin composite; Fibroblasts

Core tip: The behaviour of silorane-based materials seems to be comparable to the one observed for conventional composite material, thus decreasing the cytotoxicity after long time exposure. Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry.

Orsini G, Catellani A, Ferretti C, Gesi M, Mattioli-Belmonte M, Putignano A. Cytotoxicity of a silorane-based dental composite on human gingival fibroblasts. *World J Stomatol* 2013; 2(4): 86-90 Available from: URL: http://www.wjgnet.com/2218-6263/full/v2/i4/86.htm DOI: http://dx.doi.org/10.5321/wjs.v2.i4.86

INTRODUCTION

Recently, the use of composite materials for restoring dental elements has significantly increased due to the growing aesthetic demand of patients[1].

Despite extensive improvements in mechanical and aesthetic properties of dental composites, volumetric shrinkage and contraction stress during polymerization are still a problem[1]. Contraction stress transferred to the tooth may lead to cusp deflection or enamel micro cracks; additionally, contraction stress of tooth-composite interface can determinate post-operative sensitivity, microleakage, marginal discoloration and recurrent caries[2].

In several studies different techniques have been investigated in order to minimize polymerization shrinkage and contraction stress[3-7]. At the same purpose low-shrinkage materials have been proposed, but none of them offered significant improvement to Bis-GMA-based composites[8].

In 2007, a low shrinkage dental composite based on silorane monomers has been introduced. This material contains traditional filler particles (quartz) and monomers based on a silane or a siloxane core bonded with several oxirane functional groups. The silorane monomers polymerize by a ring-opening polymerization process of the oxirane groups. According to its composition, this resin has two advantages: low polymerization shrinkage, due to the ring-opening oxirane monomer, and increased hydrophobicity, due to the presence of the siloxanes[9].

The release of substances from dental composite materials after polymerization and their possible toxicity have been widely examined during previous years[10-12]. Several *in vitro* studies have shown cytotoxic, genotoxic, mutagenic, or estrogenic effects of some monomers released by composite materials[13-17].

Limited information is available about the substance eluted from silorane composite and its cell or tissue compatibility. Kopperud *et al*[18] found no substance eluted from Filtek silorane in water, while silorane were found in ethanol solution. Krifka *et al*[19] revealed no significant signs of cytotoxicity on human pulp-derived cells caused by silorane-based materials, while a slight increase in reactive oxygen species was detected.

The aim of present study was to evaluate the biocompatibility of Filtek silorane. The maintaining of surface architecture after finishing was also investigated. These properties were investigated in polished and unpolished silorane polymerized samples.

As regards biocompatibility, we studied the viability of human fibroblastic cells both after direct contact with silorane composite and after cells conditioning using a medium exposed to silorane.

MATERIALS AND METHODS

Sixty-three standardized cylindrical specimens (8 mm in diameter and 2 mm in thickness) were prepared using a transparent plastic molds. The molds were positioned on a glass plate and filled with Filtek silorane (3 mol/L ESPE, Seefeld, Germany). The samples were built up in one increment. The specimens were polymerized using a diode unit with a power of 1100 Mw/cm^2 for 60 s (LE Demetron I; Kerr, Bioggio, Switzerland). Forty two of these samples were polished using a slow speed handpiece using 4 polishing discs of different grains (Sof-Lex discs, 3 mol/L ESPE; Seefeld, Germany), from the most (2382 C) to the least (2382 SF) abrasive. The remaining samples were left unpolished. All the samples were processed for observation under a scanning electron microscope (SEM: Philips XL20; FEI, Milano, Italy).

Cell culture

Cultured fibroblasts were obtained from subjects without systemic and oral disease, after signing informed consent. Biopsies (2 cm × 2 cm were taken from the gingiva of 2 subjects (40 years old), rinsed twice with phosphate buffered saline (PBS) at pH 7.4, containing penicillin (100 U/mL), streptomycin (100 μg/mL) and amphotericin B (2.5 μg/mL; all from Sigma Aldrich, Milan, Italy) and cut in small pieces with a sterile blazer. The tissue fragments were placed in culture flasks of 25 cm^2 with Dulbecco Modified Essential Medium (DMEM), containing 1 mg/mL of collagenase (all from Sigma Aldrich), and incubated for 3 h at 37 ℃. Afterwards, fragments were incubated at 37 ℃ (5% CO_2) in Petri plates of 35 mm containing DMEM supplemented with 10% of fetal bovine serum (FBS, Life Technologies, Monza, Italy), 4.5 g/L of glucose, penicillin (100 U/mL) and streptomycin (100 μg/mL) all from Sigma Aldrich. The first fibroblast cells were visible after 3-4 d. Culture medium was changed twice a week until cells confluence (2 wk). Using a trypsin/EDTA treatment (0.25% trypsin, 0.02% EDTA; Sigma Aldrich), the cells were detached and cultured in flasks of 75 cm^2 until a new confluence was achieved. Cells between the 2nd and the 4th passage of subculture have been used.

For direct toxicity test, silorane samples have been disinfected with alcohol at 70% for 3 h and washed with PBS for 24 h after the alcohol removing. After a conditioning treatment in DMEM containing 10% FBS and penicillin (100 U/mL) and streptomycin (100 μg/mL) for 24 h, the medium was discarded and samples considered suitable for cell seeding. Specimens were placed in ultra-low attachment 24/well plates (Corning, Tewksbury, MA, United States) and seeded with 1×10^4 $cells/cm^2$.

To assess indirect toxicity assay, samples disinfected as previously described were placed in agitation in DMEM containing 10% FBS and penicillin (100 U/mL) and streptomycin (100 μg/mL) for 1 and 3 wk. The conditioned medium was placed in contact with fibroblasts (1×10^4 $cells/cm^2$) seeded in 24/well polystyrene plates for 48 h. Cells cultured for 48 h in normal culture medium were used as a control.

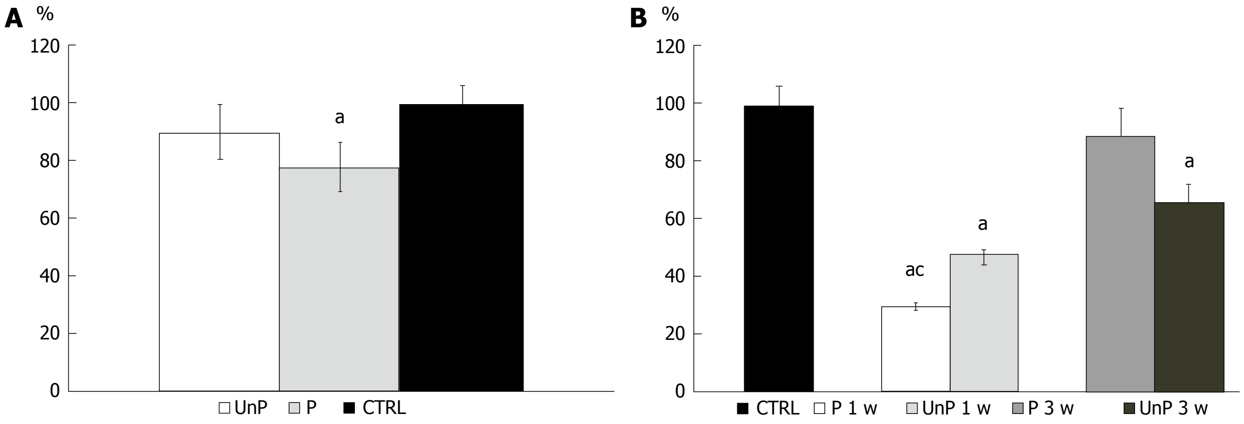

Figure 1 Histogram of cell viability. A: Cell viability of fibroblast cultured directly on unpolished samples (UnP), polished samples (P: finished surface using polishing discs) and control (CTRL); B: Cell viability of fibroblasts in CTRL, polished samples at 1 wk (P 1 w), unpolished samples at 1 wk (UnP 1 w), polished samples at 3 wk (P 3 w), unpolished samples at 3 wk (UnP 3 w); $^{a}P < 0.05$ *vs* CTRL; $^{c}P < 0.05$ P 1 w *vs* P 3 w.

Cell culture processing for SEM analysis

The obtained monolayer cells were fixed in 2% glutaraldehyde in cacodylate buffer for one hour at 4 ℃. After fixation, cells were rinsed in cacodylate buffer 0.1 mol/L, pH 7.4 and 7% sucrose; cells were then post-fixed using 0.1% OsO_4 in cacodylate buffer 0.1 mol/L, at 7.4 pH (1 h in dark at 4 ℃). After a second rinse in cacodylate buffer for 10 min, samples were dehydrated using a growing grade of ethanol (from 25% to 100%) at 4 ℃ with Critical Point Drying at 31.3 ℃ and 72.9 Atm. The samples were placed on aluminium stubs with a graphite-based glue, covered with gold, using an Edwards sputtering device, and observed with a SEM operating at 20 kV.

Cell culture processing for 3-dimethylthiazol-2, 5-diphenyltetrazolium bromide test

After 48 h of culture, medium was removed and 200 μL of a solution (5 mg/mL in medium without phenol red) containing 3-dimethylthiazol-2, 5-diphenyltetrazolium bromide (MTT; Aldrich, Sigma) and 1.8 mL of medium was added to the monolayer cells. The plates were incubated at 37 ℃ for 4 h. The supernatant was removed, the blue-violet formazan crystals were dissolved adding 2 mL of solvent (HCL 4% in isopropanol) and quantified with the spectrophotometer (Secoman; Anthelie light, 3.8 version, Contardi, Italia) at 570 and 690 nm. The results have been reported as viability percentage compared with the control culture.

Statistical analysis

Statistical analysis of the data was performed using two-ways analysis of variance. In detail, cell viability was evaluated on fibroblasts: (1) directly cultured on polished samples (P), unpolished samples (UnP) and control (CTRL); and (2) in contact with the eluates of P, UnP and CTRL samples at 1 and 3 wk.

Levels of $P < 0.05$ were considered to be statistically significant. The results were also evaluated in accordance with ISO standard 10993-5[20] which describes less than 25% inhibition as non-cytotoxic, 25% to 50% inhibition as slightly cytotoxic, 50% to 75% inhibition as moderately cytotoxic and more than 75% inhibition as highly cytotoxic[21].

RESULTS

Biocompatibility

MTT tests showed a significant reduction ($P < 0.01$) of gingival fibroblasts viability cultured both in P (90.05% ± 19.03%) and in UnP (78.15% ± 11.01%) compared with the CTRL (100.00% ± 6.00%), as shown in Figure 1A.

As regards to indirect toxicity, the viability of fibroblastic cells incubated in a medium conditioned with both P and UnP, for 1 or 3 wk, respectively, was studied using MTT test.

Cells growth in medium conditioned for 1 wk showed a significant viability reduction ($P < 0.01$) compared to the CTRL: the group conditioned with P showed a viability of 29.83% ± 1.92%, the one with UnP: 47.06% ± 1.87% (Figure 1B).

A reduction of cell viability was also observed in both groups conditioned for 3 wk (P: 89.45% ± 10.11%; UnP: 65.97% ± 9.89%), but only in the second group this reduction was statistically significant (Figure 1B).

SEM evaluation

As shown in Figure 2, SEM observations of fibroblasts cultured on the silorane samples, either P or UnP, confirmed the good biocompatibility of this material, which favoured cell spreading. These observations showed that the surface of the silorane-based material is able to absorb a big quantity of the serum component from the culture medium.

DISCUSSION

Silorane-based composite is a candidate for use in conservative dentistry due to its low polymerization shrinkage. However, it cannot be excluded that the potential release of remaining monomer substances may exert harmful effects on cells of periodontal tissues[22]. The current

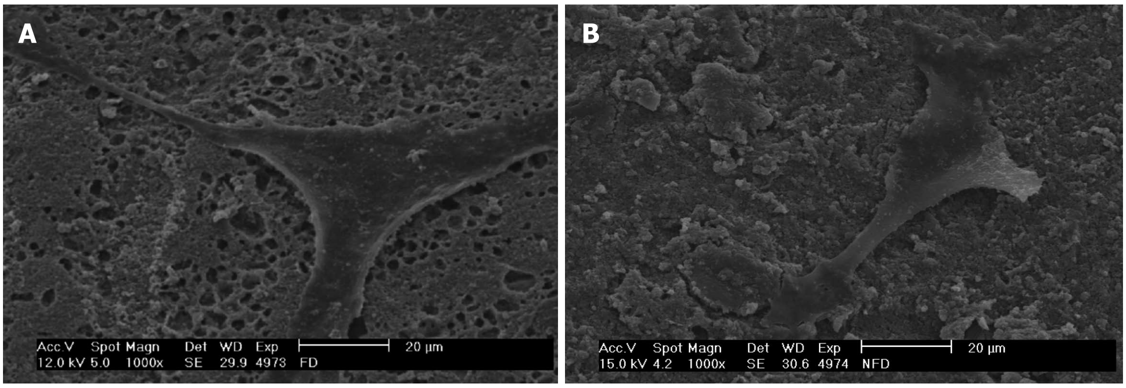

Figure 2 Scanning electron micrograph (x 2000, magnification). A: Gingival fibroblasts cultured directly on polished sample; B: Gingival fibroblasts cultured directly on unpolished sample.

limited literature indicates that silorane-based composite has a low toxicity presumably due to the low rate of free monomers released after polymerization[18]. In order to ensure a safe use of silorane-based materials, studies on the biocompatibility of this material are still needed.

Biocompatibility of a dental material can be studied exposing tissue directly to the material (direct toxicity) or placing it in a medium (conditioning), which will be used for additional tests (indirect toxicity)[23].

The results obtained in our study show a low direct cytotoxicity of both samples: P and UnP. The percentage of survival is lower in UnP than in P probably due to the larger surface contact area between composite and fibroblasts. Furthermore, the presence of oxygen inhibits the polymerization, resulting in a higher percentage of unreacted composite on the composite surface. Incomplete polymerization not only causes a decrease in the mechanical properties, but it can cause tissue reaction, as shown by Spangberg *et al*[24]. Composite finishing and polishing may indeed decrease the toxicity, as hypothesized in the study of Mohsen and Vankerchoven[25,26]. A moderate (with a few peaks of high toxicity) indirect cytotoxicity was observed in the samples placed in culture medium conditioned for 1 wk with silorane eluates (being the UnP slightly less cytotoxic than the P ones). Slight indirect cytotoxicity values were obtained for the samples placed in culture with medium conditioned for 3 wk. Under this condition, the fibroblast cultures show a different behaviour, since cell viability was slightly greater in case of contact with P than with UnP ones. These findings are in agreement with Sheridan *et al*[27], reporting that the cytotoxic effect of acrylic resin was greater after polymerization and decreased with time for many resins. The authors concluded that the longer a prosthesis is soaked, the less cytotoxic effects it is likely to have regardless of the denture base resin it is manufactured from[27]. Due to the not univocal data among P and UnP, the surface roughness does not seem to be a determining factor in the study of indirect toxicity. Indirect toxicity can be determined by release of substances from silorane as widely described in scientific literature[22].

Scanning electron micrographs allow observing the characteristic fibroblastic spreading. This is consistent with a study of Balcells *et al*[28], which states that the adsorption of serum proteins present in the culture medium is the first event that occurs when cells are seeded on a material and the adsorbed protein layer influences cell adhesion, spreading and proliferation.

In conclusion, although the poor chromatic availability of this material remains a big limit that restricts its use to posterior sectors, the silorane-based material can be considered an option to perform restorations when aesthetic demands are not the priority, such as the class Ⅱ restorations[29]. The behaviour of silorane-based materials seems to be comparable to the one observed for conventional composite material[30], thus decreasing the cytotoxicity after long time exposure. Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry.

ACKNOWLEDGEMENTS

Dr. Marcantoni, Dr. Morici and Dr. Kyriakidou are kindly acknowledged for technical assistance.

COMMENTS

Background

Despite extensive improvements in mechanical and aesthetic properties of dental composites, volumetric shrinkage and contraction stress during polymerization are still a problem.

Research frontiers

In several studies different techniques have been investigated in order to minimize polymerization shrinkage and contraction stress at the same purpose low-shrinkage materials have been proposed but none of them offered significant improvement to Bis-GMA-based composites.

Innovations and breakthroughs

The behaviour of silorane-based materials seems to be comparable to the one observed for conventional composite material, thus decreasing the cytotoxicity after long time exposure.

Applications

Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry.

Peer review

The authors considered and concluded that the materials are biocompatible.

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