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Full Length Research Paper

In vitro assessment of cytotoxicity of giomer on human gingival fibroblasts

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Root coverage on restored root surfaces has been considered as a challenging issue. The evaluation of cytotoxic effects of restorative materials is a fundamental requirement for sustaining the cell attachment and the clinical success of root coverage. The aim of the present study was to compare the human gingival fibroblast cytotoxicity of the recently introduced giomer composite (GC) with resin ionomer (RI) restorative material. Discs (6×2 mm) of GC and RI restorative materials were prepared using sterile Teflon mold. Extracts from the materials were incubated to cell culture medium for 24, 48 and 72 h. Human gingival fibroblasts (HGF) were exposed to the extracts of the materials while the un-incubated media served as the control group. The cytotoxicity of the materials were evaluated by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In order to compare the mean values of the measured parameters a Kruskal-Walis test was carried out. MTT assay indicated that human gingival fibroblasts proliferated well in the presence of GC extract. The proliferation rate was higher in cells incubated with GC compared to RI extracts but the differences were not statistically significant (p= 0.09). This *in vitro* study indicated that GC is a non-toxic material for HGF. However, further studies are needed to assess the other biologic and clinical behavior of this material prior to it being considered as a potentially suitable restorative material to restore the carious root lesions candidated to root coverage procedures.

Key words: Root coverage material, giomer, ionomer, cell cytotoxicity.

INTRODUCTION

Gingival recession is defined as the displacement of the gingival margin apical to the cemento-enamel junction (Deliperi et al., 2006). In a study, gingival recession of 1 mm or more was reported in 11.5% of 18 to 24 year olds, 46.3% of 35 to 44 year olds, 78.3% of 55 to 64 year olds and 86.5% of people 65 or older (Brown et al., 1996). Gingival recession predisposes the affected teeth to loss of cervical structure, dental hypersensitivity and root caries (Seichter, 1987).

Root coverage procedures such as sub-epithelial connective tissue grafts (SCTG) are used successfully to cover the exposed root surfaces (Langer and Langer, 1985). However, in some complicated cases where the gingival recession and root caries are present, a combination of restorative and periodontal surgical procedures needs to be undertaken. In such circumstances, the carious lesion is removed and the resultant cavity is restored with a proper restorative material such as resin ionomer (Geristore®) before being covered by a mucoperiosteal flap and /or graft (Alkan et al., 2006). Owing to its composition and physico-mechanical properties, Geristore® can be used in treating sub-gingival defects such as root resorption and perforation (Alkan et al., 2006; Dragoo and Scherer 1995). Furthermore, in laboratory studies, Geristore was less cytotoxic to gingival fibroblasts than conventional glass ionomer (Ketac-Fil®) and Immediate Restorative Materials (IRM) (Al-Sabak et al.,

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2005).

Recently, a new fluoride releasing light cured restorative material containing pre-reacted glass ionomer fillers, known as giomer, has been introduced (Yap and Mok., 2002) based on the incorporation of surface pre-reacted (S-PRG) or full pre-reacted (F-PRG) glass ionomer fillers. In the S-PRG type, only the surface of the glass filler is attacked by polyacrylic acid leaving the glass core intact, while in the F-PRG type, the entire filler particle is attacked by polyacrylic acid (Sunico et al., 2005). According to the claims made by the manufacturer, giomer combines the advantages of a resin composite and a glass ionomer (Deliperi et al., 2006). These new hybrid materials were found to provide almost a complete seal against bacterial microleakage, cause a little mechanical and chemical pulpal irritation and inhibited demineralization (Sunico et al., 2005; Sonoda et al., 2002; ISO 10993-12., 1996). In a study, it was shown that the two year clinical performance of giomer restorations was equal or slightly better than that of resin ionomers and compomers (Sunico et al., 2005).

Due to the high success rate of giomers in cervical restorations, it is assumed that these materials can be used in combination with SCTG for the coverage of carious root surfaces. Since the knowledge around the cytotoxicity of giomers towards human gingival fibroblasts is limited, this study aimed to assess the citotoxicity of giomer (Beautifil®) on human gingival fibroblasts and compare it with the citotoxicity of a resin ionomer (Geristore®).

METHODS AND MATERIALS

Sample preparation

6 mm diameter and 2 mm thick discs of Beautifil® (Shofu Dental Co-orporation, Osaka. Japan) and Geristore (DEN-MAT Co-orporation, Santa Maria. CA. USA) were fabricated under aseptic conditions by packing the materials in a Teflon mold and compressed between two glass plates to create a even thickness disc. The discs were sterilized by suspending in 70% ethanol for 10 min before the experiment.

Cell culture

Human gingival fibroblast cells were cultivated and maintained in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal calf serum, 100 U ml-1 penicillin, 100 lL ml-1 streptomycin and 2 mmol L-1 l-glutamine (Cambrex Bio Science, Verviers, Belgium) at 37ºC in a humidified atmosphere of 95% air and 5% CO2 as described (Farajnia et al., 2008). Sub-cultivation was performed with cells from confl-uent cultures treated with 0.2 g L-1 ethylenediaminetetraaceticacid in phosphate-buffered saline (PBS).

Attachment assay

For analysis of cell attachment, the test discs were placed in the center of a 24-well-cell culture plate under sterile conditions and then the cell suspension was added (104 cells/well). After 24 and 48-h-incubation periods, the test discs were transferred into the 96-well plate and washed 3 times with media to remove unattached cells. The attachment of viable cells on the surfaces of discs was assessed using the MTT assay. A total of 20 □l MTT dye 3-(4, 5-dimethyl-thiazoyl)-2,5-diphenyl-SH-tetrazolium bromide) (5 mg/ml in PBS) was added to each well and the microplates were incubated at 37ºC for additional 2 h. After the incubation period, 100 µl of acidified isopropanol (0.04 N HCl in isopropanol) was added to the wells and mixed thoroughly to dissolve the dark blue crystals of formazan. The absorbance values of each well were determined with a microplate enzyme-linked immuno-assay (ELISA) reader at a 570-nm wavelength. Untreated cultures were used as control groups and their survival rates were set to represent 100% viability. The results were calculated as 100–(absorbance of test well/absorbance of positive control well) × 100 and presented as a percentage of cell proliferation. Each experiment was repeated 3 times and the mean value was calculated.

Preparation of discs extracts and cell cytotoxicity assay

Eight discs from each product were immersed in RPMI 1640 media and agitated for 48 and 72 h at 37ºC according to ISO Standard 10993-12 (Gonzales et al., 2004). The control samples containing only medium were treated similarly. HGF cells were diluted in a fresh medium containing 2, 5 and 10% of FCS and seeded into 96-well plates (10-4 cells well-1). After incubation for 24 h, the medium was aspirated from all wells and replaced by 100 □l well-1 extraction or control medium and incubated for another 24 h before cytotoxicity was checked. The colorimetric assay developed by Mosmann (Mosmann, 1983) and modified by Edmondson et al. (Edmondson et al., 1988) was used as a test for cell proliferation and survival assay. A total of 20 □l MTT dye (5 mg ml-1 in PBS) was added to each well and incubated at 37ºC, in air containing 5% CO2 and at 95% relative humidity for 4 h in the dark. After incubation, the MTT was aspirated and the formazan product was dissolved in 100 µl of the acidified isopropanol (0.04 N HCl in isopropanol). The plates were shaken before the optical densities (OD) were measured at a 570 nm wavelength. Three tests of each extract and control were performed in each experiment. All assays were repeated at least twice to ensure reproducibility.

Statistical analysis

In order to compare the mean values of the measured parameters a Kruskal-Walis test was carried out. Two sided values of $p<0.05$ were accepted as significant.

RESULTS

Attachment assay

The attachment of human gingival fibroblasts on Beautifil® and Geristore® based discs was assessed by MTT assay after 24 and 48-h incubation. The results are given in Table 1. More HGF cells were attached to Beautifil® discs than for the Geristore® discs but the difference was not statistically significant ($\chi^2= 1.33$; $p=0.24$).

Cell cytotoxicity assay

The cytotoxicity of the Beautifil® based discs on human

Table 1. Assessment of the attachment of human gingival fibroblasts on Beautifil® and Geristore® discs.

	Cells + Beautifil® disc			Cells + Geristore® disc			Positive control			Negative control		
% FCS	2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10
OD	1.17 ±0.21	1.47 ±0.12	1.62 ±0.32	1.43 ±0.11	1.7 ±0.23	1.80 ±0.63	1.06 ± 0.47	1.39 ±0.41	1.62 ± 0.50	0.43 ±0.02	0.47 ± 0.03	0.56 ±0.02

OD, optical density; FCS, Fetal Calf Serum.
Data are expressed in mean ± SD.

Table 2. Assessment of the cytotoxicity of Beautifil® and Geristore® based discs on human gingival fibroblasts.

	G 24 h	G 484 h	G 72 h	B 48 h	B 48 h	B 24 h	Cell	Media
OD	1.43±0.25	1.58±0.11	1.5±0.13	1.64±0.21	1.4±016	1.68±0.31	1.55±0.2	0.38±0.06

OD = Optical density, G = Geristore®, B = Beautifil®.
Data are expressed in mean ± SD.

gingival fibroblasts were assessed by proliferation analysis of cells incubated with Beautifil® disc extracts using Geristore discs extracts as a control. As shown in Table 2, Beautifil® discs 24, 48 and 72 h extracts did not show any cytotoxicty and there were no statistically significant differences between the proliferation rate of cells cultured in the culture medium and that of cells incubated with Geristore and Beautifil® extracts (χ^2= 4.75; p=0.09).

DISCUSSION

Restorative materials must be biocompatible to minimize their adverse effects on periodontal tissues induced by direct contact (Souza et al., 2006). *In vitro* tests are often preferred to measure biocompatibility aspects in the early stages of the assessment of a newly introduced material, in the interest of time, cost and ethics. While *in vitro* studies are easier to conduct, their validity can only be substantiated by careful and meticulous *in vivo* research (Schmalz, 1994; Sidhu and Schmalz, 2001).

In the present study, the method of MTT-colorimetric assay was used as an *in vitro* method to assess the fibroblast cell survival and proliferation rate measuring dehydrogenase activity as described in previous studies (Mosmann, 1983; Huang et al., 2002).

Like other tissues, normal fibroblast function is critical to maintain the periodontal tissue function for optimal healing. Gingival fibroblasts were chosen due to their availability and culturing characteristics (Huang et al., 2002; Hou and Yaeger, 1993).

The results of the present study revealed that fibroblasts attached well on the surface of the both tested materials. In addition, there was no statistically significant difference between cell proliferation rate of control group (culture medium alone), resin ionomer (Geristore) and giomer (Beautifil®) extracts. Our results were in agreement with the previous study in which fibroblasts grew and spread well over Geristore with a morphology close to that of the control groups in SEM evaluation (Al-Sabak et al., 2005). Moreover, in a study carried out by Camp et al. (Camp et al., 2003) on Geristore group, fibroblast attachment occurred significantly greater than for other investigated root-end filling materials (Camp et al., 2003). Based on histologic evidence, Dragoo (Dragoo, 1997) demonstrated that both epithelium and connective tissue can adhere to resin ionomer placed in a subgingival environment. Resin ionomers have also been used successfully in subgingival areas to treat furcation lesions and restore resorption cavities (White, 1998; Breault et al., 2000; Anderegg, 1998).

It is really difficult to clarify why these two materials especially giomer showed acceptable results in cytotoxicity tests because investigators often do not know the exact composition of materials being tested. However, there might be some possible reasons; favorable cellular response in Geristore® and Beautifil® might be attributed to their surface characteristics as the surface structure of the final restoration can often determine the biocompatibility of the material (Yap and Mok, 2002; Dunkin and Chambers, 1983). The amount and nature of leachable compounds in resinous materials can influence their biocompatibility (Huang et al., 2002), as a result, less eluted toxic substances into the medium by those restorative materials is another possible explanation for the good cell attachment and proliferation. In resin base materials, free monomers and additives are extracted especially during the first 24 h. Therefore, monomer-polymer conversion is a very important factor in biocompatibility of resinous materials (Ferracane and Condon, 1990).

Even though there were not any statistically significant differences between the two materials, giomer exhibited higher fibroblast attachment compared with the resin ionomer. This might be due to its better polishability compared to the resin ionomer (Yap and Mok, 2002). Also, it has been revealed that the low initial pH of dental mate-

rials may lead to cytotoxic reactions (Sidhu and Schmalz, 2001; Meryon et al., 1983). Since giomer employs pre-reacted glass ionomer technology, the fluoroalumino silicate glass reacts with polyalkenoic acid in water prior to the inclusion into silica-filled urethane resin (Yap and Mok., 2002), it seems that the initial pH in giomer does not decrease as much as that of resin ionomer. A study demonstrated that the resin modified glass ionomers maintained a low surface pH for at least the first 60 min of setting (Woolford and Chadwick, 1992).

Unlike the results of Al-Sabak et al. (2005), Camp et al. (2003) and the findings of this current study, Huang et al. (2002) had demonstrated that resin-modified glass ionomer cement was cytotoxic to cultured human gingival fibroblasts by inhibiting cell growth, attachment and proliferation (Huang et al., 2002). The differences in the results between the study by Huang et al. (2002) and our study might be due to the different materials and methods (such as specimen preparation). In addition, materials within the same category may not have behaved similarly. Variations in the release of ions occur in different products (Sidhu and Schmalz, 2001). For instance, differences in the pattern and amount of fluoride released were shown among different commercial products (Forss, 1993; Oliva et al., 1996; Kan et al., 1997).

Some studies have investigated the pulpal effects of resin modified glass ionomers and reported different results (Sidhu and Schmalz, 2001; Gaintantzopoulou et al., 1994). The different outcomes may be due to the disparities in the chemical compositions of the materials, particle size, curing time and setting properties.

In vitro results extrapolating to the clinical situation should only be done with extreme caution. Lack of simulation of the *in vivo* situation and the difficulties in extrapolating the data to the patient are the main limitations of cell culture toxicity tests (Schmalz, 1994). Furthermore, due to the lack of defense and repair mechanisms under in vitro conditions, cells may show less tolerance to the materials, which are biocompatible *in vivo* (Müller et al., 1990).

Further studies are recommended to evaluate cytotoxicity of these restorative materials for longer period of time to resemble clinical conditions. In addition, SEM growth assay would seem to be useful in evaluating the morphology of human gingival fibroblasts.

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