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Brief communication

Cytotoxicity of acrylic based resin compounds in a human gingival fibroblast cell line

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ABSTRACT

In vitro cytotoxicity testing of dental acrylic resins is fundamental to establish clinical safety limits, requiring suitable cell models that closely simulate physiological processes. This study's main aim is to evaluate the viability of an untransformed human gingival fibroblast cell line, as an oral cell model for acrylic resin cytotoxicity tests. For this purpose, cell viability was compared to a control cell line (Chinese hamster lung fibroblast), following exposure to increasing concentrations of methyl methacrylate and formaldehyde (acrylic resins' leachable compounds). Additionally, because of the volatile nature of these compounds and their harmful effects on the respiratory tract, a human fetal lung fibroblast line was also tested. Two-way ANOVA of generated data ($p < 0.05$) showed that all cell types are significantly affected in a dose dependent manner by these chemicals. Further characterization of the human gingival fibroblast line shall be addressed in future biocompatibility studies.

Citotoxicidade de constituintes das resinas acrílicas numa linha celular de fibroblastos gengivais humanos

Palavras-chave:
Fibroblastos humanos
Gengiva
Citotoxicidade
Resina acrílica
Metil metacrilato
Formaldeído
Etil metanosulfonato

RESUMO

Testes de citotoxicidade *in vitro* para resinas acrílicas dentárias são fundamentais para estabelecer limites de segurança clínica, requerendo modelos celulares adequados, próximos das condições fisiológicas. O principal objetivo deste estudo é avaliar a viabilidade de uma linha celular não transformada de fibroblastos gengivais humanos como modelo celular oral para testar a citotoxicidade das resinas acrílicas. Assim, a viabilidade celular foi comparada à de uma linha celular de controlo (fibroblastos de pulmão de hamster chinês), após exposição a concentrações crescentes de metil metacrilato e formaldeído (constituintes lixiviáveis das resinas acrílicas). Adicionalmente, devido à volatilidade e efeitos prejudiciais destes

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compostos no trato respiratório, testou-se uma linha de fibroblastos fetais do pulmão humano. A análise dos dados gerados com two-way ANOVA ($p < 0,05$) mostrou que todos os tipos celulares são significativamente afetados por estas substâncias químicas, dependendo da dose. Será necessário proceder a uma caracterização mais detalhada desta linha de fibroblastos gengivais humanos em estudos futuros de biocompatibilidade.

Introduction

During the prosthetic and orthodontic appliances' base fabrication, total polymerization of the monomer methyl methacrylate (MMA) is never achieved.[1] Due to the aggressive and complex oral environment, polymers undergo biodegradation and part of the trapped toxic residual monomer may leach.[1,2] Moreover, other toxic substances such as initiators, additives and byproducts (including formaldehyde) are released,[1] increasing potential exposure to these harmful products.

Both MMA and formaldehyde have been often associated with allergic local reactions in the patients' oral mucosa in contact with prosthetic and orthodontic devices.[3] Other adverse reactions reported include contact dermatitis and occupational respiratory hypersensitivity in dental professionals because of volatilization.[1,4]

Therefore, appropriate *in vitro* cytotoxicity[3] and, eventually, genotoxicity[5,6] testing is essential and requires suitable oral cell models. Non-human immortalized cell lines such as hamster cheek pouch epithelial cells, L929 murine fibroblasts[3] and Chinese hamster lung fibroblasts (V79)[6] have been frequently used in studies *in vitro* to evaluate the toxic effects of dental monomers. However, these mammalian non-oral cell types hardly simulate the clinical conditions to which mouth cells are challenged by dental materials. In addition, they have altered survival mechanisms, which might mask cellular outcomes.[7]

Primary human cells from explants such as gingival and periodontal ligament fibroblasts have also been used[8] yet, primary cells have a limited lifespan and are more difficult to maintain and work with.[7] Hence suitable cell lines should be characterized to facilitate *in vitro* cytotoxicity and/or genotoxicity studies.

The primary goal of this study is to evaluate an untransformed human gingival fibroblast (HGF) cell line response to increasing doses of MMA, formaldehyde and ethyl methanesulfonate (EMS) (positive control)[6] and compare it with the V79 cells (one of the most used non-human immortalized cell lines).[6] Additionally, a human fetal lung fibroblast (WI-38) cell line was assessed as a representative of the respiratory tract.

Materials and methods

Three types of adherent commercial fibroblast cell lines were studied: HGF (AG09429, Coriell Cell Repository, Camden, NJ, USA), WI-38 (90020107, Sigma, St. Louis, MO, USA) and V79 (603371, Cell Lines Service, Eppelheim, Germany).

Cells were seeded in α-minimal essential medium (α-MEM) containing 10% fetal bovine serum, 100 IU/ml penicillin, 2.5 μg/ml streptomycin, 2.5 μg/ml amphotericin B and 50 μg/ml ascorbic acid, which was replaced twice a week, and incubated in a 5% CO_2 humidified atmosphere at 37 °C. Passages were performed at least twice with 0.05% trypsin in 0.5 mM EDTA, when the monolayer cultures were 70–80% confluent.

MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) was performed in 3 independent experiments for each cell type, according to the general guidelines in ISO 10993-5:2009.[9] During the exponential growth phase, the cultures were seeded in 96-well plates in 100 μl of complete α-MEM. HGF and WI-38 were seeded with a cell suspension of 3×10^4 cells/cm^2 and V79 at a lower concentration (9×10^3 cells/cm^2) due to its higher proliferation rate.

After 24 h of incubation, the chemical agents EMS (0, 600, 1200, and 2400 μg/ml), MMA (0, 40, 80, and 160 mM) and formaldehyde (0, 400, 800, and 1600 μM) (Sigma–Aldrich, St. Louis, MO, USA) were diluted in fresh complete α-MEM, just before replacing the initial culture medium with 100 μl of treatment medium.

Subsequently, 24 h later, 10 μl of the MTT solution (Sigma-Aldrich, St. Louis, MO, USA) were added to each well and incubated for 3–4 h in standard conditions. Then, the culture medium was removed and 100 μl of dimethyl sulfoxide (DMSO, Panreac Quimica) was added to each well, even as two blanks of DMSO in each plate. The plates were agitated for 5 min before being introduced in a microplate reader (Synergy HT, BioTek Instruments, Winoosky, VT, USA). The absorbance was read at a wavelength of 550 nm.

The data were analyzed using SPSS® V.20 (Chicago, IL, USA). The percent of cell viability was calculated for each well ($n = 18$) in relation to the mean absorbance of control wells (no chemical). The normal distribution of the sample was confirmed by the Kolmogorov–Smirnov test ($p > 0.05$). Two-way ANOVA test was performed to assess if there were differences between the three cell types with increasing doses of each separate chemical substance. The Dunnett *post hoc* tests were performed considering as control groups 0 for the chemicals concentrations and the V79 for cells. Though the general significance level was $p < 0.01$, a Bonferroni correction was applied, resulting in a $p < 0.05$ significance level, since 5 comparisons were performed. Additionally, agreement in controls' viabilities between different experiments was calculated with the Cronbach's Alpha.

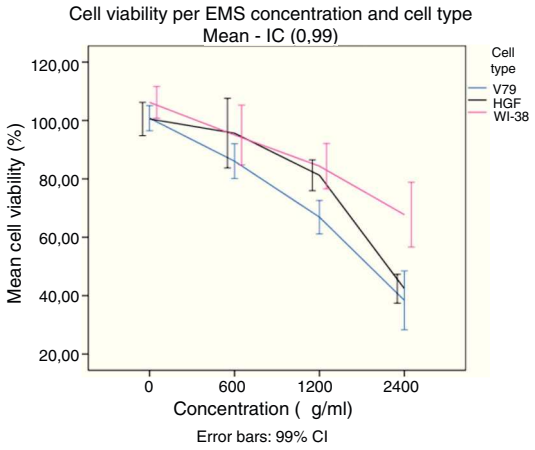

Fig. 1 – Cells viability for ethyl methanesulfonate (EMS) with 99% of confidence levels.

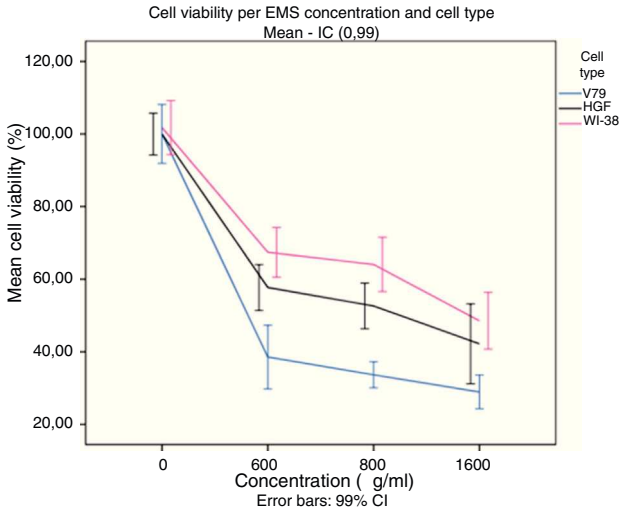

Fig. 3 – Cells viability for formaldehyde (Form) with 99% of confidence levels.

Results

The Cronbach's Alpha test showed that there was concordance between the three independent experiments (84.4%). The two-way ANOVA test presented a high observed power (approximately 1.000). The cells' viabilities with the doubling toxic concentrations are plotted separately for each chemical agent (Figs. 1–3), with 99% confidence intervals.

It was verified that for every chemical agents there was a significant decrease ($p < 0.05$) between the viabilities of the control groups and the other concentrations for all cell lines. On the other hand, despite an overall tendency of HGF and WI-38 to show higher viability values than V79, the differences on the three cell types are not statistically significant ($p > 0.05$). However, if the interactive effect of the chemical doses on the cells is considered, the ANOVA model shows statistically significant differences ($p < 0.001$).

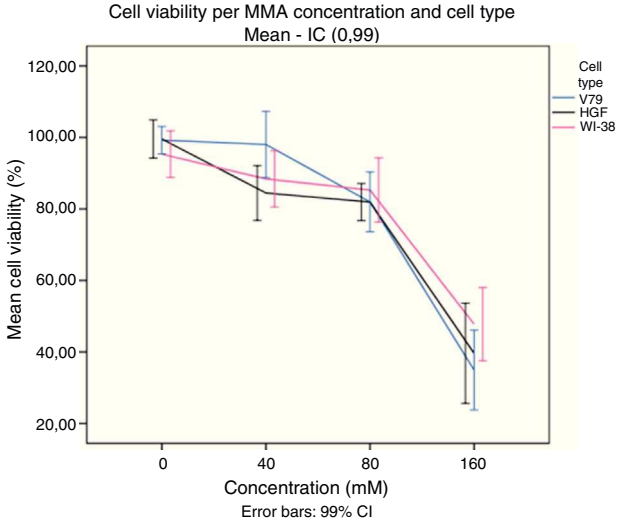

Fig. 2 – Cells viability for methyl methacrylate (MMA) with 99% of confidence levels.

Discussion

Since most *in vitro* models used to evaluate toxicity of dental base polymers rely on non-human immortalized cell lines from a variety of tissues, namely the V79 cell type, we decided to evaluate HGF and WI-38 cell lines response to dental acrylic resins' derivatives, which may be closer to physiological conditions. Both WI-38 and V79 are recommended by the ISO10993-5:2009 standard as cell line models for cytotoxicity testing.[9] V79 cells have been frequently used in cytotoxic and genotoxic testing,[6] and in this study served as a term of comparison. When analyzing solely in terms of the cells, the outcomes of the present study show that the viability behavior of HGF and WI-38 cells is not significantly different from V79 cells. However, our data also suggest that the human untransformed fibroblasts tend to be more resistant to MMA and formaldehyde than V79 cells, which should be further assessed in subsequent studies with other cytotoxicity tests.

To the best of our knowledge, this is the first report where EMS is tested in a commercial untransformed HGF cell line, therefore various concentrations had to be tested. Also, though cytotoxic testing using primary HGF is very common, untransformed HGF cell line use is very limited and there are no reports regarding dental polymers.[5] In this study, we observed a dose dependent loss of cell viability of HGF in response to MMA and formaldehyde. Interestingly, similar results have been reported in experiments using commercially available primary HGF cells, in response to amalgam and to methacrylic co-monomers from restorative dental composites.[10]

Likewise, it has been described that co-monomers from inhaled composite resins' vapors induce cytotoxicity in lung epithelial cells[4] and in the present study WI-38 fibroblasts were affected by MMA and its subproduct formaldehyde.

For further characterization of the HGF cell line, other features related to the cell viability, for instance cell morphology, cell cycle disturbances, apoptosis and necrosis events and the

activity of specific genes need to be evaluated. The present results may be useful for future biological risk assessment tests, such as genotoxicity, of leachable residual monomer and by-products from acrylic resin pieces in simulated oral conditions.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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