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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.