Cytotoxicity of dental alginates

Citotoxicidade de alginatos odontológicos

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Abstract

Purpose: To evaluate the cytotoxicity of dental alginate (irreversible hydrocolloid), which is widely used as an impression material in Dentistry.

Methods: Four dental products were assessed: J (Jeltrate Traditional), ALG (Alga Gel), PG (Printer Gel), and AVG (Ava Gel). Three control groups were used: positive (C^+) cell detergent Tween 80, negative (C^-) PBS, and control of cells (CC – no exposure of cells to any substance). Disk-shaped specimens were immersed in Eagle minimum essential. The supernatants were collected after 24, 48, 72, and 168 hours (7 days) for analysis of the toxicity to L929 fibroblast cells after 24-h incubation. Viable cells stained with 0.01% neutral red dye were counted using a spectrophotometer. Data were analyzed by ANOVA and Tukey's test ($\alpha=0.05$).

Results: Significant differences in number of viable cells were found between the alginate groups and C^- or CC ($P<0.05$). The group J showed the highest cytotoxicity level followed by PG, ALG, and AVG.

Conclusion: All dental alginates tested showed some cytotoxic response from fibroblasts.

Key words: Cytotoxicity; dental Impression; cell culture methods

Resumo

Objetivo: Avaliar a citotoxicidade de alginatos (hidrocolóide irreversível) de uso odontológico, os quais são a categoria de material de moldagem mais utilizada em Odontologia.

Metodologia: Foram avaliadas quatro marcas de alginato: grupo J (Jeltrate Tradicional), ALG (Alga Gel), PG (Printer Gel) e AVG (Ava Gel). Utilizaram-se 3 grupos controle: positivo (C^+) com detergente celular Tween 80, negativo (C^-) com PBS, e controle de célula (CC), onde as células não foram expostas a nenhum material. Espécimes em forma de disco foram imersos em meio mínimo essencial Eagle. O sobrenadante foi coletado 24, 48, 72 e 168 horas (7 dias) para análise de toxicidade para fibroblastos L929 após 24 h de incubação. As células viáveis foram coradas com corante vermelho neutro a 0,01%, fixadas e contadas em espectrofotômetro. Os dados foram analisados por ANOVA e teste de Tukey ($\alpha=0,05$).

Resultados: Houve diferença significativa do número de células viáveis entre os alginatos e os grupos C^- ou CC ($P<0,05$). O grupo J apresentou a maior citotoxicidade, sendo seguido por PG, ALG e AVG.

Conclusões: Pode-se concluir que todos os alginatos testados mostraram resposta citotóxica para fibroblastos.

Palavras-chave: Citotoxicidade; hidrocolóide irreversível; técnica de cultura de células

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Received: January 22, 2009
Accepted: March 6, 2009

Introduction

Biocompatibility can be defined as a series of events or interactions between material and host, whose outcomes should not have undesirable response or harmful effects (1). Previous studies have studied biocompatibility of dental materials and appliances in Orthodontics (1-4), and alginate is of particular concern because it is the most widely used impression material. Alginate (irreversible hydrocolloid) has easy manipulation, good detail reproducibility, low cost, and it is comfortable for the patient (5), but perfect dental impressions are hardly achieved by non-experienced students, and often repeated impression procedures are required (6).

Some heavy metals and silica particles are present in the alginate powder and have potential toxicity risk for both the practitioner and the patient. For example, lead is added to the alginate powder to improve the material elastic properties after gelification and sometimes can be found as a contaminant (7). Basically, intoxication with alginate occurs through inhalation of the powder, accidental intake, and absorption by the oral mucosa in cases of repeated impression procedures (7-9). During the impression procedure, alginate is left in close contact with the oral mucosa for approximately 2 minutes, and this tissue is highly vascularised and has great absorption potential. Therefore, repeated impression procedures might cause a certain degree of cytotoxicity depending on the material composition and mucosal integrity (6,7).

Based on this premise, the objective of the present study was to assess the cytotoxicity of four commercial brands of alginate for dental impression by means of a fibroblast cell culture method.

Methods

The research protocol followed the regulations to conduct scientific experiments with cell cultures. This study used mouse L929 fibroblasts obtained from the American Type Culture Collection (ATCC, Manassas, EUA) and cultivated in Eagle's minimum essential medium (MEM) (Cultilab, Campinas, SP, Brazil). The cell culture was supplemented with 2 mM of L-glutamine (Sigma, St. Louis, USA), 50 mg/mL of gentamicin (Schering Plough, Kenilworth, NJ, USA), 2.5 mg/mL of fungizone (Bristol-Myers-Squib, New York, NY, USA), 0.25 mM of sodium bicarbonate solution (Merck, Darmstadt, Germany), 10 mM of HEPES (Sigma, St. Louis, USA), and 10% of foetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil), and was stored at 37 °C in 5% CO_2 environment.

Specimen Preparation

Four dental alginates fabricated by different manufacturers were assessed: Group J (Jeltrate Tradicional, Petrópolis, Brasil, Lot 017484A), Group ALG (Alga Gel, Tecknew, Rio de Janeiro, Brasil, Lot 08276), Group PG (Printer Gel, Euroda, Magé, Brasil, Lot 012\06), and Group AVG (Ava Gel, Petrópolis, Brasil, Lot 024471A). The basic composition of the materials, as informed by the manufacturers, was: diatomite, potassium alginate, calcium sulphate, sodium phosphate, magnesium carbonate, clorhexidine, and artificial flavours and pigments. Each material was manipulated during 1 min by using a rubber bowl and plastic spatula according to the manufacturer's recommendations, and the mixture was inserted into silicon rings (4 mm diameter × 4 mm height) until full gelification

To verify the cell response to extreme situations, other 3 control groups were included: Group CC (cell control), consisting of cells not exposed to any material; Group C^+ (positive control), consisting of Tween 80 (Polioxietileno-20-Sorbitan); and Group C^- (negative control), consisting of PBS solution (phosphate-bufferid saline) in contact with the cells.

Assessment of the Material Cytotoxicity

The materials were previously sterilized by exposing them to ultra-violet light (Labconco, Kansas, USA) during 1 hour. Next, three samples of each material were placed in 24-wells plates containing Eagles' MEM (Cultilab, Campinas, SP, Brazil). The culture medium was replaced with fresh medium every 24 hours, and the supernatants were collected after 24, 48, 72, and 168 hours (7 days) for analysis of the toxicity to L929 cells. The supernatants were placed in a 96-well plate containing a single layer of L929 cells and then incubated at 37 °C for 24 h in 5% CO_2 environment. After the incubation period, cell viability was determined using the "dye-uptake" technique described by Neyndorff et al. (10), which was slightly modified. After the 24-h incubation period, 100 µL of 0.01% neutral-red staining solution (Sigma, St. Louis, USA) were added to the medium within each well of the plates, and these were incubated for 3 h at 37 °C to allow the dye to penetrate into the living cells. After this period, the cells were fixed using 100 µL of 4% formaldehyde solution (Reagen, Rio de Janeiro, Brazil)) in PBS (130 mM NaCl; 2 mM KCl; 6 mM Na_2HPO_4 $2H_2O$; 1 mM K_2HPO_4, pH = 7.2) for 5 min. Next, 100 µL of 1% acetic acid solution (Vetec, Rio de Janeiro, Brazil) with 50% methanol (Reagen, Rio de Janeiro, Brazil) were added to the medium to remove the dye. Absorption was measured after 20 min by using a spectrophotometer (BioTek, Winooski, USA) at a wavelength of 492 nm.

Statistical Analysis

Statistical analyses were performed by using the SPSS v.13.0 software (SPSS Inc., Chicago, USA), and means and standard deviations were calculated for descriptive statistical analysis. The values for the amount of viable cells were submitted to analysis of variance (ANOVA) and Tukey's test. The level of significance was set at 0.05.

Results

The results showed statistical differences between control groups (CC and C^-) and other groups ($P<0.05$). However,

no statistical differences were observed between Groups J and PG as well as between Groups ALG and AVG ($P>0.05$) (Table 1). All alginates were found to be cytotoxic compared to Groups CC and C^-.

Table 1. Comparison of the amount of viable cells (mean, standard deviation (SD), and percentage) among the experimental groups.

Groups	Amount of viable cells			Stat*
	Mean	SD	%	
J	334.875	82.92	26.3	A
ALG	530.125	74.29	41.64	B
PG	387.62	41.60	30.44	A
AVG	545.87	100.2	42.88	B
C+	67	2.20	5.26	C
C-	1111.5	67.85	87.31	D
CC	1273.75	125.71	100	D

* Stat - Same letters indicate no statistically significant difference.

Regarding cell viability, Group CC had the best results, followed by Groups C^-, AVG, ALP, PG, and J. On the other hand, Group C^+ had the worst cell viability and was statistically different from the other groups.

Discussion

Alginate is one of the most used materials in Dentistry. The alginate manufacturers produce a powder containing various compounds for different aims. However, many elements, such as zinc, barium, cadmium, lead, silicates, and fluorides, are added to their basic formulations in order to improve the physical, chemical, and mechanical properties, but this also raises some concern in terms of toxicity (11). Alginate can affect the reproduction capacity of the cells (9), *i.e.*, the substance may not be toxic enough to kill the cell, but it is sufficiently toxic to inhibit cell growth or, to a lesser extent, affect the normal function of the cell. Clinically, this means that although a single contact with the material may not cause clinical symptoms, repeated contact can change or affect the cell viability, thus resulting in delayed toxic or allergic reaction. Therefore, the objective of the present work was to assess the cytotoxicity of four alginates by using cell cultures.

Cell cultures have been used as part of a series of recommended tests to evaluate the biological behavior of materials in contact with human tissues (12-14). In the present study, cytotoxicity tests were conducted to evaluate alginates for dental use by employing mouse fibroblasts (L929 cell line), which are largely used for testing dental materials (15-18). Two minutes of evaluation time was adopted in the present study, which is the maximum length of time that the alginate material remains within the oral cavity during a single impression procedure. After the contact between samples and cell culture medium, the supernatant was collected and put into contact with the cells. The indirect contact method was chosen in order to avoid cell damage from the samples, which might interfere with the results as suggested by Costa (19).

The results obtained in the present study demonstrated that all alginates were cytotoxic compared to the cell control and negative control groups. Avagel (AVG) showed the best results for cell viability, followed by Alga Gel (ALG), Printer Gel (PG), and Jeltrate (J). The differences found in the cytotoxicity of each material may be related to the concentration of heavy metals in their compositions (7).

In order to evaluate the cell response to extreme conditions, a positive control group (C+) consisting of damaged cells was included in the study. The material used for positive control was Tween solution, a non-ionic surfactant consisting of polyoxyethylene sorbitol fatty acid esters that is toxic to cell membranes (20), whose main characteristic is to stimulate protein secretion in microorganisms (21) and to change both morphology and surface of the cell wall (22). As expected, the control positive group showed high cytotoxicity as statistical differences were observed in comparison with all the other groups. The negative control group consisted of PBS (phosphate-buffered saline) solution, known to be non-toxic to cells, and was used only to evaluate the physical effect on the cells. As expected, low cytotoxicity was observed in this group, with no statistical difference with the cell control group.

A successful dental treatment involves not only mastering the clinical technique, but also requires that the practitioner follows biosafety norms regarding the proper use of dental materials and clinical procedures. The results of this *in vitro* study cannot be directly extrapolated to a clinical situation, but caution should be taken to use dental alginates when there is a potential risk for accidental ingestion by young children or prolonged contact in persons with mucosa rupture.

Conclusions

According to the results obtained in the present study, one can conclude that all alginates evaluated have some cytotoxic effect to fibroblasts.

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