PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
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ÉDIO GIACOMELLI

# AVALIAÇÃO *IN VITRO* DA CITOTOXICIDADE CELULAR DO CIMENTO DE IONÔMERO DE VIDRO MODIFICADO POR CARBONATO DE CÁLCIO DE CONCHAS MARINHAS

Profa. Dra. Luciana Mayumi Hirakata
Orientadora

Porto Alegre
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Dissertação apresentada como parte dos requisitos obrigatórios para a obtenção do título de Mestre na área de Materiais Dentários pelo Programa de Pós-Graduação da Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul.

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BANCA EXAMINADORA:

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Profa. Dra. Daniela Botega

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Prof. Dr. Hugo Mitsuo Silva Oshima

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Profa. Dra. Luciana Mayumi Hirakata

A Deus,

Por ter sempre iluminado meu caminho e ser minha fonte de Fé.

Aos meus pais Édio e Nilacir

Vocês foram essenciais na minha formação pessoal por me fazer uma pessoa de princípios. Por todo incentivo, apoio, compreensão e suporte que dedicaram a mim durante esta jornada, a vocês dedico minhas conquistas.

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**DEDICO**

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***"Não fizemos nossas escolhas em busca da aprovação alheia, mas sim em busca de nossa realização pessoal e crescimento profissional. Isto só se consegue amando o que se faz com muita dedicação, encarando as dificuldades como uma oportunidade de recomeçar e as vitórias como um incentivo."***

( Autor Desconhecido)

# RESUMO

GIACOMELLI, E. - AVALIAÇÃO *IN VITR*O DA CITOTOXICIDADE CELULAR DO CIMENTO DE IONÔMERO DE VIDRO MODIFICADO POR CARBONATO DE CÁLCIO DE CONCHAS MARINHAS

Orientadora: Profa. Dra. Luciana Mayumi Hirakata. Porto Alegre, PUCRS, Faculdade de Odontologia – Dissertação (Mestrado em Materiais Dentários), 2012.

Este estudo teve como objetivo avaliar a citotoxicidade e bioatividade do cimento de ionômero de vidro modificado com carbonato de cálcio de conchas. Para os testes de toxicidade celular foi utilizando a *Saccharomyces cerevisiae* como organismo modelo. A indução de citotoxicidade foi avaliada por dois testes diferentes usando a *S. Cerevisiae* cepa selvagem wild-type FF18733: (1) a sobrevivência da célula de levedura e (2) formação de colônias petite (mutantes respiratórios). Para os testes de bioatividade foi avaliada a variação de peso das amostras e análise de deposição de hidroxiapatita pela MEV e EDS após imersão em solução simuladora de plasma sangüíneo. Os resultados dos testes de sobrevivência mostraram que as diferentes concentrações de carbonato de cálcio adicionado ao CIV (1%, 5% e 10% em peso) induziram uma ligeira perda de viabilidade celular em *S. cerevisiae* em relação ao controle negativo, porém não foi o suficiente para ser considerado como uma indução de toxicidade significativa. Em relação às colônias petite, não foi observada a indução de formação de mutantes respiratórios em qualquer uma das concentrações testadas, indicando que o CIV modificado não induziu estresse oxidativo em células de *S. cerevisiae*. Na avaliação da bioatividade, os resultados mostraram que após a imersão todos os grupos testados apresentaram uma diminuição no peso das amostras, que pode ser atribuído à dissolução do cimento de ionômero de vidro. Na quarta semana de imersão houve uma variação significativa na percentual médio de peso para todos os grupos. A análise de MEV mostrou uma ligeira deposição de hidroxiapatita sobre a superfície dos grupos com a adição de pó de conchas em comparação ao grupo controle.

**Palavras-chave:** Citotoxicidade, Bioatividade, CIV, conchas marinhas, *S. cerevisiae*, Hidroxiapatita.

## ABSTRACT

GIACOMELLI, E. - CYTOTOXICITY "IN VITRO" OF MODIFIED GLASS IONOMER CEMENT OF CALCIUM CARBONATE OF SEASHELLS

Orientadora: Profa. Dra. Luciana Mayumi Hirakata. Porto Alegre, PUCRS, Faculdade de Odontologia – Dissertação (Mestrado em Materiais Dentários), 2012.

This study aimed to evaluate the cytotoxicity and bioactivity of the glass ionomer cement modified with calcium carbonate shells. For testing cell toxicity was using *Saccharomyces cerevisiae* as a model organism. The induction of cytotoxicity was evaluated by two different tests using wild-type strain FF18733 of *S. cerevisiae*: (1) yeast cell survival and (2) formation of *petite* colonies (respiratory mutants). For tests of bioactivity was evaluated weight change of samples and analysis of hydroxyapatite deposition by SEM and EDS after immersion in simulated blood plasma. The results of survival tests showed that different concentrations of calcium carbonate added to the GIC (1%, 5% and 10% by weight) induced a slight loss of cell viability in S. cerevisiae in relation to the negative control, but it was not enough to be considered as a significant induction of toxicity. In relation to the petite colonies, there was no induction of mutants respiratory formation in any of the concentrations tested, indicating that the GIC did not induce altered oxidative stress in cells of *S. cerevisiae*. In the evaluation of bioactivity, the results showed that after immersion all groups tested showed a decrease in weight of the samples, which can be attributed to the dissolution of glass ionomer cement. In the fourth week of immersion there was a significant variation in the average percentage of weight for all groups. The SEM analysis showed a slight deposition of hydroxyapatite on the surface of the groups with the addition of powdered shells compared to the control group.

**Keywords:** Cytotoxicity, bioactivity, GIC, seashells, *S. cerevisiae*, hydroxyapatite

# LISTA DE ILUSTRAÇÕES

Artigo 1



Artigo 2

# LISTA DE SIGLAS E ABREVIATURAS

et al . et alii – e outros

GIC – glass ionomer cement

CIV – cimento de ionômero de vidro

S. cerevisiae – Saccharomyces cerevisiae

FF18733 – número da linhagem da cepa selvagem da Saccharomyces cerevisiae

PTFE – polytetrafluoroethylene / politetrafluoretileno

˚C – graus Celcius

ETO – ethylene oxide / óxido de etileno

YPD – 1% yeast extract, 2% peptone, 2% glucose

ml – mililitre/mililitro

$10^{-2}$ - 0,01

$10^{-5}$ - 0,000001

$10^{-7}$ - 0,00000001

$10^{7}$ - 10.000.000

cells/ml – cells for mililitre/ células por mililitro

μl – microlitre/microlitro

CFU/ml – colony forming unities per ml

UFC/ml – unidade formadora de colônia por ml

TTC – triphenyl-tetrazolium chloride / Cloreto de Trifeniltetrazólio

ROS – reactive oxygen species / espécies reativas ao oxigênio

SBF – simulated body fluid

HA – Hidroxiapatite / Hidroxiapatita

SEM – scanning electron microscopy

MEV – microscopia eletrônica de varredura

EDS – energy dispersive spectometry / espectometria de energia dispersiva

Kv – kilovolt

mÅ – miliangström

pH – potencial de hidrogênio

m0 – media de peso inicial

m1 – media de peso final

mM – milimolar

CAPES – coordenação de aperfeiçoamento de pessoal de nível superior

PUCRS – Pontifícia Universidade Católica do Rio Grande do Sul

# LISTA DE SÍMBOLOS

% - por cento

® - marca registrada

≅ - aproximado

NaF - fluoreto de sódio

NaCl – cloreto de sódio

KCl – cloreto de potássio

$Na_2HOPO_4$ - hipoclorito de sódio

$KH_2PO_4$ - fosfato de potássio

$CaCl_2$ - cloreto de cálcio

$MgSO_4$ - sulfato de magnésio

$C_6H_{12}O_6$ – glicose

$NaHCO_3$ – bicarbonato de sódio

$Ca^{+2}$ – íon cálcio

$Mg^{+2}$ – íon magnésio

$Na^{+}$ – íon sódio

$CO_3^{+2}$ – íon carbonato

# SUMÁRIO

# 1. INTRODUÇÃO GERAL

## 1.1 CIMENTO DE IONÔMERO DE VIDRO

Desde que foram introduzidos na odontologia na década de 70 por WILSON e KENT, os Cimentos de Ionômero de Vidro (CIV's) vem sendo utilizados amplamente em diversas áreas da odontologia (WILSON *et al*, 1972). Foram vistos, inicialmente, como substitutos potencias dos cimentos de silicato que já estavam em uso por mais de 80 anos (ATKINSON *et al* 1985).

Entre as suas propriedades dos CIV’s, duas características tornaram esse material bastante aceito: a capacidade de proporcionar uma união química ao esmalte e a dentina por meio de trocas iônicas e o benefício adicional da liberação de flúor. Assim, os CIV’s combinavam as qualidades adesivas dos cimentos de policarboxilato de zinco com a liberação de flúor dos cimentos de silicato (BILLINGTON, 1990 e BERG, 2002)

Inicialmente os CIV’s foram utilizados principalmente para restaurações de lesões causadas por abrasão ou erosão ou então como agentes cimentantes. Porém, suas características tais como baixa solubilidade em fluídos orais, alta resistência à abrasão e excelente biocompatibilidade, fizeram com que novas formulações fossem estudadas para ampliar sua aplicação clínica (MOUNT *et al,* 1994 e NAASAN *et al,* 1998).

Nesse sentido, diferentes estudos avaliaram as características biocompatíveis deste material, em vista que ao longo dos anos de utilização poucos relatos de reações adversas foram registrados na literatura (LEYHAUSEN *et al,* 1998 e BROOK *et al*, 1998).

Aproveitando a resposta biológica favorável oferecida pelo CIV, algumas atenções frente as suas aplicações começaram a se voltar para utilização como um biomaterial. Apoiado nas suas propriedades, associado com perspectivas promissoras em relação ao uso de cimentos de ionômero de vidro como um novo material para substituição óssea, levantaram esperanças de avanços significativos na reparação de defeitos ósseos (HATTON *et al*, 2006).

Em 1998, BROOK e HATTON iniciaram trabalhos visando à utilização dos CIV's na medicina, apoiado nas suas propriedades. Nesses estudos iniciais, não foram observadas clinicamente reações adversas graves que tenham sido relatados.

A partir disso, numerosos modelos *in vitro* têm sido utilizados para avaliar a biocompatibilidade dos CIV's em relação aos tecidos e as estruturas ósseas. No que se refere à interpretação dos resultados destes modelos, pode se observar que os CIV's apresentam comportamento bioativo. Estas idéias estão descritas nos trabalhos de GROSS e colaboradores (1988) e mais tarde por WALLACE e colaboradores (1999).

Idealmente, a avaliação *in vitro* deve ser realizada utilizando um modelo que, na medida do possível, represente a situação clínica. Modelos simulando a condição clínica baseados na utilização de cultura de diferentes células, como, células neonatas, fibroblastos e osteoblastos que são comumente descritos na literatura (GROSS *et al*., 1988; JONCK, MARIE *et al*, 1992; MEYER, 1993; BROOK *et al;* OLIVA *et al*, 1998; COSTA *et al*, 2003).

Ainda, JONCK e colaboradores, em dois estudos nos anos de 1989 e 1992, observaram uma reação inflamatória subperiostal em fêmur de ratos, quando o CIV foi recém misturado e aplicado de forma direta, cirurgicamente, após 6 semanas. Essa reação foi observada até a 12ª semana embora tenha se constatado uma neoformação

óssea. Estes trabalhos surgiram que uma hipótese provável para ter ocorrido uma reação tecidual era a redução do pH devido ao ácido poliacrílico presente na composição do CIV, embora provavelmente não seja um grave problema para a biocompatibilidade.

O baixo pH durante o período de reação está relacionado a ação do ácido poliacrílico e neste estágio pode induzir a uma toxicidade celular (HATTON *et al* 2006) embora não seja uma questão que comprometa a sua biocompatibilidade (BROOK *et al*, 1998). De acordo com WILSON *et al,* 1983, o mecanismo de união do CIV à estrutura dentinária promove uma liberação de íons cálcio e fosfato sendo então capaz de neutralizar a acidez da reação.

## 1.2 BIOCOMPATIBILIDADE DOS MATERIAIS

Desde muito tempo o homem utiliza materiais naturais ou sintéticos na tentativa de substituir órgãos ou tecidos perdidos ou deteriorados na intenção de melhorar a qualidade de vida ou aumentar a longevidade. A partir da II Guerra Mundial se iniciou uma investigação e desenvolvimento de novos materiais que tiveram reflexos na evolução das soluções terapêuticas.

Atualmente pesquisas são desenvolvidas com o objetivo de desenvolver investigar ou aprimorar materiais com características mecânicas e biológicas compatíveis com a aplicação a que se destina com um desempenho satisfatório. Dentro dos objetivos buscados nesta linha de pesquisa e desenvolvimento, a biocompatibilidade é um quesito é essencial a para materiais.

Segundo o conceito de WATAHA (2000), a biocompatibilidade é a propriedade de um material em não causar injúrias ou efeitos tóxicos sobre o sistema biológico, ou induzir a uma resposta adequada do hospedeiro em situações específicas.

Dentro deste mesmo conceito, CRAIG (2002) descreveu que a capacidade de um material ser biocompatível ou não, depende da sua composição e localização, bem como da sua interação com o ambiente fisiológico.

Conseguir associar a um material, características que atendam essas necessidades se tornou o tema de inúmeros trabalhos nesta linha de pesquisa.

O crescente estudo na área de biocompatibilidade dos materiais odontológicos é uma necessidade em função da capacidade dos mesmos provocarem alterações da atividade biológica nos tecidos envolvidos. Esse fato é uma tendência observada na ciência dos materiais dentários, e de ser gradativamente substituída pela ciência dos biomateriais.

Os biomateriais apresentam uma vasta diversidade de aplicação nas diferentes áreas médicas, odontológicas e farmacêuticas devido às características que nele podem ser proporcionadas no momento de seu desenvolvimento. No entanto, indiferente à sua aplicação, a biocompatibilidade em ambiente fisiológico é um requisito fundamental para este tipo de material (MORAES *et al*, 2007).

Dessa forma, o presente trabalho propôs avaliar *in vitro* toxicidade celular do cimento de ionômero de vidro modificado por carbonato de cálcio de conchas marinhas conforme descrito no estudo de GIACOMELLI *et al*, (2011) através de testes de sobrevivência da levedura *Saccharomyces Cerevisiae*, além da avaliação de bioatividade pela imersão em solução simuladora de plasma sangüíneo.

## 2. OBJETIVOS

### 2.1 OBJETIVO GERAL

Verificação da biocompatibilidade do cimento de ionômero de vidro modificado por carbonato de cálcio de conchas marinhas por meio da aplicação de testes avaliativos de bioatividade e toxicidade celular.

### 2.2 OBJETIVOS ESPECÍFICOS

- Análise da deposição de hidroxiapatita deficiente em cálcio

  As amostras foram colocadas em imersão na Solução de Hank's em um período de 1, 2, e 4 semanas.

- Avaliação de toxicidade celular

  Análise da citotoxicidade do cimento de ionômero de vidro modificado por carbonato de cálcio de conchas marinhas utilizando como organismo-modelo a levedura *Saccharomyces cerevisiae*:

  Sobrevivência de células de *Saccharomyces cerevisiae* após exposição direta aos corpos de prova de CIV modificado por carbonato de cálcio, em meio de cultura líquido.

  Perda de metabolismo respiratório por estresse oxidativo através da análise de colônias *petite.*

## 3. ARTIGO 1

# *IN VITRO* CYTOTOXICITY EVALUATION OF MODIFIED GLASS IONOMER CEMENT OF CALCIUM CARBONATE OF SEASHELLS

**Giacomelli E[1], Vilches PFS[2], Medina RS[3], Hirakata LM[4]**

## ABSTRACT

This study aimed to evaluate the cytotoxicity of glass ionomer cement (GIC) modified with calcium carbonate from shells using *Saccharomyces cerevisiae* as a model organism. The induction of cytotoxicity was evaluated by two different tests using wild-type strain FF18733 of *S. cerevisiae*: (1) yeast cell survival and (2) formation of *petite* colonies (respiratoty mutants). The results of the survival tests showed that the different concentrations of calcium carbonate powder added to the GIC (1%, 5% and 10% by weight) induced a slight loss of cell viability in *S. cerevisiae* in relation to the negative control, which was not enough to be considered as a significant toxicity induction. In relation to petite colonies, there was no difference in terms of induction respiratory mutants in any of the concentrations tested, indicating that the modified GIC did not induce oxidative stress in *S. cerevisiae* cells.

**Keywords:** Cytotoxicity, GIC, seashells, *Saccharomyces cerevisiae*.

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## 1. INTRODUCTION

The research and development of biomaterials are shown in a field of great interest to the biomedical industry worldwide. The biomaterials have a wide range of applications in different industries medical, dental and pharmaceutical industries due to the characteristics that can be incorporated in the material at the time of its development. However, indifferent to its application, a fundamental requirement of this material is needed: the biocompatibility in physiological environment [1].

The increase in the study in area of biocompatibility of dental materials is a necessity in the assess capacity of cause changes in biological activity in the tissues involved. This fact is a trend in dental materials science and should be gradually replaced by the science of biomaterials.

According to the concept of Wataha and collaborators, the biocompatibility is a property of a material does not cause injury or toxic effects on a biological system, or be able induce an adequate response on the host in specific situations [2]. Also, the ability of a material to be biocompatible or not, depends on its composition and location, as well as its interaction with the physiological environment [3].

Certain dental materials are made of chemical elements that are related to adverse biological effects in humans. In physiological environment many of them interact with the tissues it comes in contact, releasing specific molecules, and/or suffering corrosion or dissolution [4]. This release can cause a variety of phenomena including: transport, metabolism and accumulation of this material in different organs, which can lead to the induction of disorders ranging from allergies to carcinomas [4,5].

In this context, to produce a material with biocompatible characteristics have been the subject of several studies.

The glass ionomer cement (GIC) is widely used since its implementation in dentistry in the 70's [6]. Because the glass ionomer cement has an affinity for calcium, allows a chemical union with that structure, and has an excellent biocompatibility, showing no significant adverse reactions, much attention is focused on the use of this cement to repair bony structures [7].

Several in vitro models have been proposed to evaluate the biocompatibility of GICs in bony structures or tissues. In conclusion the vast majority of these studies indicate a bioactive behavior of GICs, as described in the previous works and Gross *et al* and Wallace *et al* [8-10].

One aspect of dental materials biocompatibility is its potential cytotoxicity. Currently, there are many in vitro tests that can be used to evaluate the cellular toxicity of dental materials. Among these, the cultivation of human cells tends to be the top choice in dentistry research. However, human cells cultures have high costs, are difficult to keep, have a long life cycle and, in many cases, do not allow satisfactory quantitative analysis. [11-14]. Moreover, the results from citotoxicity tests of dental materials using human cell cultures were sometimes conflicting and not conclusive [15].

*In vitro* tests using the yeast *Saccharomyces cerevisiae* as a model organism have shown great success for this purpose [16-21]. The use of this organism offers some advantages compared to conventional cell tests such as a low costs, easy handling, and a large sample, allowing quantitative analysis in a short period of time. Moreover, *S. cerevisiae* is the most investigated and described unicellular eukaryote, in biochemical and genetic aspects. Furthermore, Animals and Fungi are proved to be phylogenetically closely related, since both are grouped in the eukaryotic supergroup called "Opisthakonta". Thus, they have biochemical and genetic similarities that justify the use

of yeast cells as an experimental model [22,23]. However, there are few studies using this microorganism as a model to evaluate the cytotoxic effects related to clinical materials [18-24]. Specifically for dentistry, one study described *S. cerevisiae* as a reliable and useful model organism to evaluate the cytotoxicity of orthodontic materials [24].

The assessment of the cytotoxic potential of dental materials is of great importance, since this knowledge is the base to ensure a safe and effective treatment of the patients. In this context, this study aimed to verify the citotoxicity of GICs modified by calcium carbonate from sea shells**,** using *in vitro* tests with *S. cerevisiae*.

## 2. MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Catholic University of Rio Grande do Sul (Brazil). The cytotoxicity tests were performed at the Laboratory of Microbiology and Immunology at the Faculty of Biosciences of the same University.

### 2.1 Calcium Carbonate (seashells)

The calcium carbonate used in this study was obtained from the grinding of seashells. For this purpose, black and white seashells were selected and collected at Rio Grande do Sul (Brazil) the coast. After this selection, the seashells were subjected to a rigorous cleaning process with water and mild soap. For a complete removal of impurities, the shells were placed in an ultrasonic tub (Plus Ultra Sonic 1440 - Odontobrás, Ribeirão Preto, Brazil) with distilled water for thirty minutes. In the

sequence, were crushed in a mechanical mill (ANM-1000, Nittokataku Co., Japan), to obtain a fine powder, and then mixed.

## 2.2 Glass Ionomer Cement

For the selection of glass ionomer cement (GIC), several tests with different brands and forms of prey of the product (only acid-base reaction, light curing and dual). In turn, the material that showed the best results was the glass ionomer cement only conventional acid-base reaction (Vidrion R - SS White, Rio de Janeiro / RJ, Brazil).

## 2.3 Samples

Seashell calcium carbonate powder was mixed with the GIC, at the proportion of 1%, 5% and 10% (w/w). Samples without the addition of shell carbonate were used as the control group. Shortly after obtaining the different GICs samples, 10 specimens were prepared for each group. The mixture of carbonate powder and glass ionomer cement followed the manufacturer's recommendations. After the reaction, the material was placed in a Polytetrafluoroethylene (PTFE) matrix (10 mm in diameter and 3 mm thick) and removed after completing its prey. The specimens were packed in a moisture-fee airtight container and placed in an incubator at 37˚C (OOL CB - ANEN Ltda, São Paulo, Brazil) for a period of 24 hours. Following this, samples were subjected to sterilization by Ethylene Oxide (ETO). The distribution of samples and related groups are described in Table 1.

2.4 *S. cerevisiae* strain and culture conditions

For both survival and petite tests the *S. cerevisiae* wild type strain FF18733 (*mat-a*, ura3-52, his7-3, leu2-1, trp1-289, lys1-1) was used. The cultures of *S. cerevisiae* were done in broth YPD medium (1% yeast extract, 2% peptone, 2% glucose) or agar YPD medium (broth YPD with agar 2%). In all experiments, pre-cultures of *S. cerevisiae* were prepared in 5 ml of broth YPD and incubated at 30°C until the cell cultures reached the exponential phase (~$10^{-7}$ cells / ml).

2.5 Survival experiments for cytotoxicity analysis

The cytotoxicity analysis was carried out through *S. cerevisiae* survival experiments under direct exposure of yeast cells to modified GIC samples in broth YPD.

For these experiments, one sample of each GIC group was introduced into 5 ml of broth YPD inoculated with 100 µl of pre-cultured cells (as described above). For positive control, silver amalgam disks (composition: 40% Silver, 31.3% Tin, Copper 28.7% and 47.9% Mercury – DFL , Rio de Janeiro/RJ, Brazil) were used, since the amalgam is a very cytotoxic material used in dentistry. One culture without any sample was used as a negative control. These inocula were incubated at 30°C until the cell cultures reached the exponential phase (~ $10^7$ cells /ml).

An aliquot of each culture was serially diluted (in sterile saline 0.9%) and 5µl of each dilution ($10^{-2}$ to $10^{-5}$) were placed on YPD agar and incubated at 30°C for two days to emergence of small colonies, which allowed a qualitative analysis. For quantitative analysis, 100 µl of final dilutions were placed on YPD agar (two plates for each

dilution). After two days of incubation at 30°C the emerging colonies were counted and the number of colony forming unities per ml (CFU/ml) was estimated. Four complete independent experiments were performed.

## 2.6 Petite colonies test

To assess the induction of respiratory loss in *S. cerevisiae* cells, which is an indication of oxidative stress, the formation of petite colonies by the modified GICs was investigated.

For this test, the colonies of the quantitative analysis plates were covered with a top agar (0.7%) containing triphenyl-tetrazolium chloride (TTC) 0.05%. The resulting color of the colonies can distinguish between those that lost their respiratory metabolism (white), the so called petite colonies, from those that keep their aerobic metabolism (red).

## 2.7 Cytotoxicity and petite colonies data analyses

In all survival experiments the values of CFU/ml of each modified GIC group were compared with the corresponding negative control, to verify the occurrence of differences in cell survival. Moreover, the average and standard deviation of CFU/ml of each GIC group from three independent experiments were transformed in log values and compared to negative controls (100%) to verify any significant difference. If there is at least one log of difference (considering de standard deviation) in terms of CFU/ml in treatments in relation to controls it is assumed a significant difference, which is an indication of cellular toxicity in *S. cerevisiae.*

To measure the induction of oxidative stress, we compared de average values of petite frequency from GIC groups in relation to those from the ne negative controls. To verify significant differences the *Student* (T) Test was applied.

## 3. RESULTS

The results from survival experiments are shown in figures 1 and 2. Firstly, figure 1 shows the qualitative analysis from one survival experiment with GIC groups 1, 2, 3 and 4. One can visually observe that no differences in terms of colony formation was induced by GIC groups 1, 2 and 3 (1%, 5% and 10% shell powder respectively) compared to group 4 (0% shell powder). This lack of colony forming differences was also observed in relation to negative controls in all survival experiments.

Figure 2 shows the average values from four independent survival experiments, which were based on CFU/ml counts. These data indicated that the positive control (silver amalgam) and all tested GIC groups induced a mild cell viability loss in *S. cerevisiae* compared to the negative control. Interestingly, the GIC group 2 (5% seashell) showed a degree of cell survival similar to that observed for the positive control, which was even lower than the GIC group 3 (10% seashell). Nevertheless, the loss in cell viability observed for all GIC groups did not reflect a significant difference in terms of survival, since their difference in relation to the negative control was below one log. These data indicate a lack of actual citotoxicity induction by the modified the GICs tested in *S. cerevisiae* cells.

In relation to petite colonies tests, illustrated in figure 3, the frequencies of petite colonies from GIC groups were the same as those from the corresponding negative controls (data not shown). Thus, no respiratory loss was induced by the modified GICs, in any concentration tested, suggesting that this biomaterial do not cause oxidative stress in *S. cerevisiae* cells.

## 4. DISCUSSION

To verify the induction of citotoxicity by GICs modified with calcium carbonate from seashells cell survival experiments using *S. cerevisiae* were performed. Additionally, loss of respiratory metabolism was evaluated by the petite colony test, which indicates the induction of cellular oxidative stress.

Analysis of cellular toxicity using models such as the yeast *S. cerevisiae* as an experimental model has been currently performed in biomedical sciences. This microorganism is easy to handle and allows quantitative analysis with large samples in a short period of time [25]. Moreover, it bears unique metabolic properties, like the so called "petite" cells, which can be properly used for respiratory toxicity analysis. It is also a eukaryotic microorganism with genetic and biochemical characteristics similar to those of animal organisms [26,27].

An average of the CFU/ml values of each treatment was calculated and compared to the negative control to check for any significant differences in terms of survival of *S. cerevisiae*. The results revealed that the CFU/ml values of all GIC groups induced a slight reduction in cell viability of *S. cerevisiae* in relation to the negative control. Although the positive control and the GIC group 2 showed the higher loss of cell viability among the tested samples, their differences in relation to control were not

significant. These results were similar to those observed for conventional GICs tested *in vitro* for cytotoxicity using different cell cultures [28-30].

Regarding the TTC overlay technique applied to check the loss of respiratory capacity in *S. cerevisiae* cells, the results revealed the lack of mitochondrial injury induction by the modified GICs tested in this study. It suggests that these materials may not induce oxidative cellular stress in yeast cells.

Other *in vitro* methods are also used to elucidate the cellular mechanisms which lead to toxic effects induced by oxidative stress. In this context, special attention has recently been paid to the disruption of the cellular redox balance as a result of increased generation of reactive oxygen species (ROS) [31]. An imbalanced redox state of the cell caused by resin monomers has been previously associated with cell cycle delay and apoptosis [31-33]. Moreover, a consequence of material-induced cellular redox alteration may also be genotoxic and mutagenic effects [34].

The data presented here revealed that the GIC modified by calcium carbonate from seashells did not induce significant cytotoxicity or oxidative stress in *S. cerevisiae* cells, indicating that the tested materials bear an adequate biocompatibility. Nevertheless, there is still a great need for further *in vitro* and *in vivo* analysis to verify the feasibility of their clinical use.

## 5. ACKNOWLEDGEMENTS

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**Table 1: Distribution of GIC groups.**

| Groups distribution | Proportions (wt%) |
|---|---|
| Group 1 | 1% shells powder<br>99% GIC powder |
| Group 2 | 5% shells powder<br>95% GIC powder |
| Group 3 | 10% shells powder<br>90% GIC powder |
| Group 4 | 100% GIC powder |

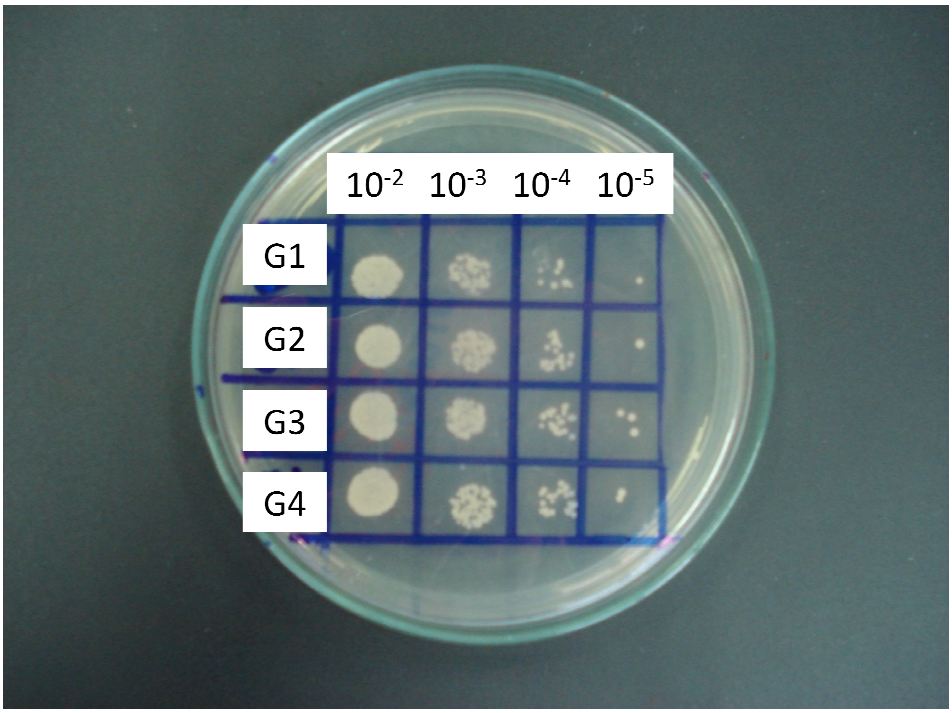


**Fig. 1: Qualitative analysis on YPD-agar from one survival experiment of *S. cerevisiae* FF18733 strain exposed to GIC groups 1, 2, 3 and 4, in different dilutions ($10^{-2}$ to $10^{-5}$). No significant difference in terms of cell survival was observed from groups 1, 2 and 3 (1%, 5% and 10% shell powder respectively) to group 4 (0% shell powder), neither to negative control (data not shown in this figure).**

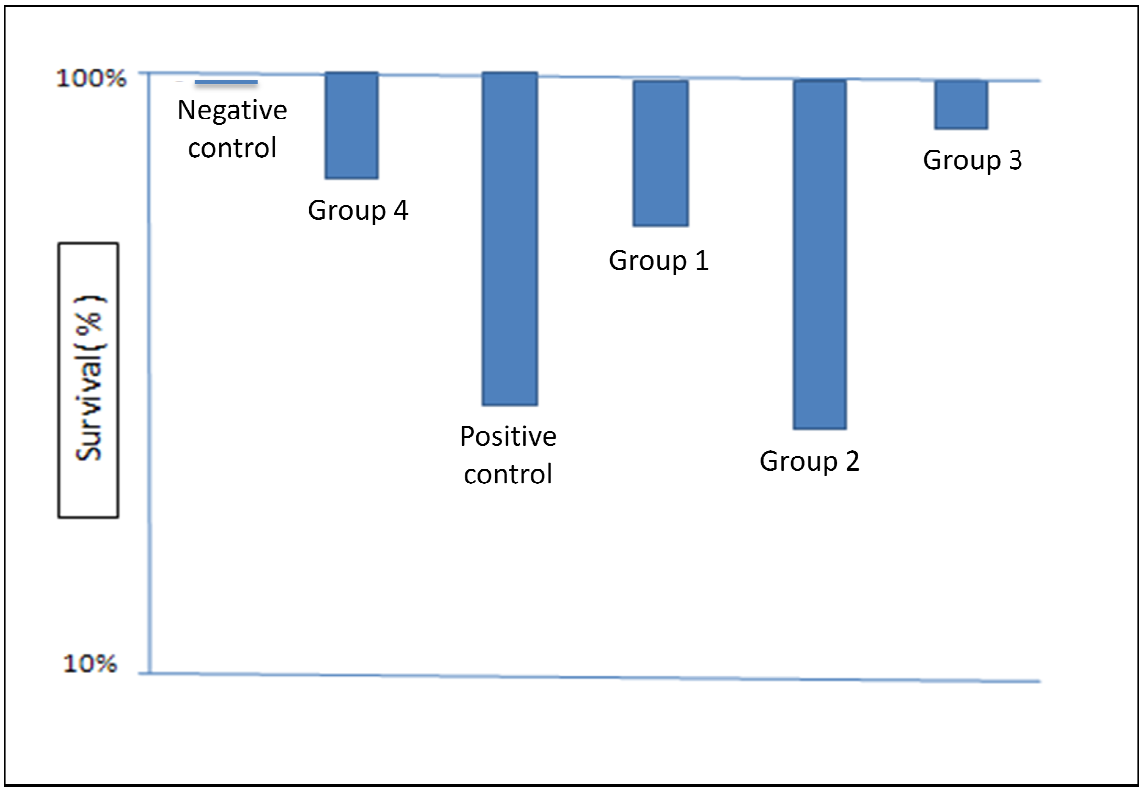


**Fig. 2: Average values from four survival experiments using F18733 *S. cerevisiae* cells submitted to GIC groups 1, 2 3 and 4, as well as to the positive control, in relation to negative control (100%). The survival values were based on CFU/ml calculations. All materials showed a tendency to induce cell viability loss comparing to the negative control, but this was not significant, since their survival values were over 10%.**

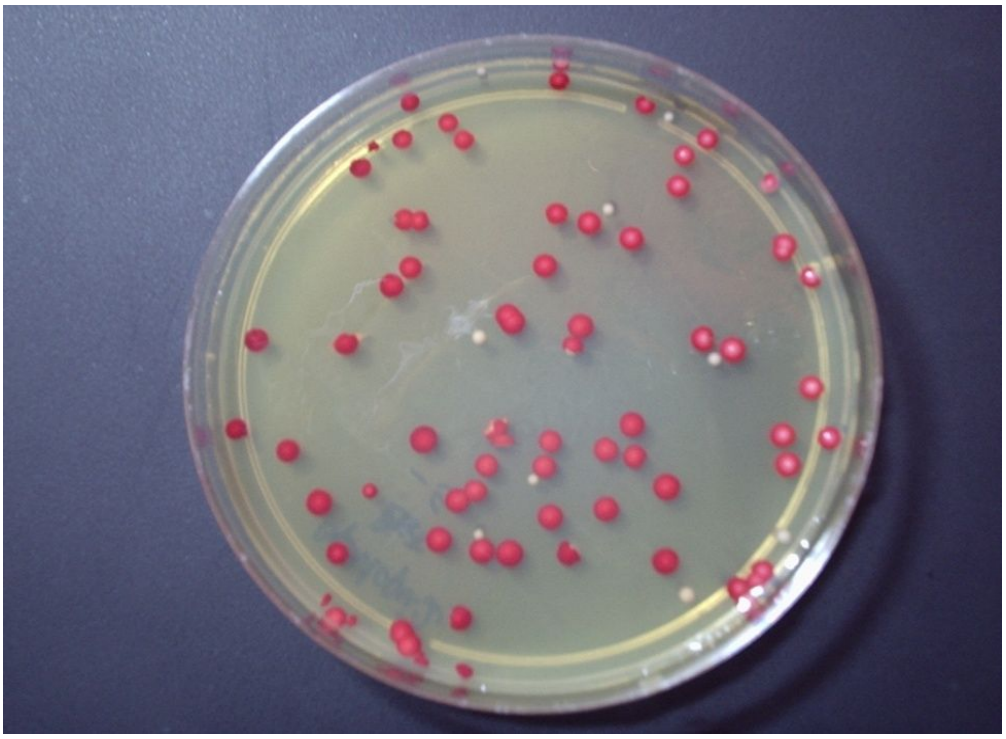

**Fig. 3: TTC overlay technique with *S. cerevisiae* FF18733 strain (illustrative). The large and red are respiring colonies; the small and white are petite (non-respiring, exclusively fermentable) colonies.**

## 4. ARTIGO 2

# *IN VITRO* EVALUATION OF BIOACTIVITY OF GLASS IONOMER CEMENT MODIFIED BY CALCIUM CARBONATE OF SEASHELLS.

**Giacomelli E[1] , Mota EG[2], Oshima HMS[3], Hirakata LM[4]**

## ABSTRACT

This study aimed to evaluate the bioactivity of glass ionomer cement modified by seashells powder. The samples were analyzed by SEM and by variation in weight after immersion in simulated plasma solution. The results showed that after immersion in simulated body fluid samples from all groups showed a decrease in weight that can be attributed to the dissolution of glass ionomer cement. In the fourth week of immersion there was a significant variation in the average percentage of weight for all groups. The SEM analysis showed a slight deposition of hydroxyapatite on the surface of the groups with the addition of seashells powder compared to the control group.

**Keywords:** GIC, calcium carbonate, hydroxyapatite, simulated body fluid.

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## INTRODUCTION

The glass-ionomer cements (GIC's) were first developed for use as a restorative material. Are constituted of two phases, a liquid and a powder that when mixed, produce a gel that soon after becomes rigid. The GIC's were developed in the 70's and were seen initially as potential substitutes of silicate cements that were already in use for over 80 years [1].

Among the properties of the GIC's, two characteristics make this material widely accepted: the ability to provide a chemical union to enamel and dentin by ion exchange and the additional benefit of fluoride release. Thus, the GIC's combined the qualities of adhesive cements, zinc polycarboxylate with fluoride release of silicate cements [2,3].

Initially, the GIC's were mainly used for filling by abrasion or erosion or as cementing agents. However, characteristics such as low solubility, high abrasion resistance and excellent biocompatibility, new formulations have been studied to expand its clinical application [4,5].

Accordingly, some studies have evaluated the characteristics of biocompatible of this material, given that over the years of use have been few reports of adverse reactions were reported in the literature [6,7].

Taking advantage of the favorable biological response offered by the GIC, some attention their applications began to come back to use as a biomaterial. Supported in their properties, associated with promising prospects in relation to the use of glass ionomer cements as a new material for bone replacement, raised hopes for significant progress in the repair of bone defects [8].

Studies evaluated the behavior of bone structures in GIC observed a bioactive behavior, presenting osteoconductive properties [9]. These studies also observed the formation of a chemical bonding between the glass particles and calcium ions of bone structure [10].

*In vivo* experiments were performed in which the surgical implantation of GIC in the femur of baboons immediately after handling the material, there is a demonstrated osteoblastic action without being noticed significant inhibitory effects [11,12].

Different formulations have been developed in the quest to improve the characteristics and properties of the GIC. The addition of calcium carbonate powder to the GIC promotes a structural change with the formation of scaffolds in its structure. This modification can be considered of great importance since it may provide a receptacle to host cells, antibiotics or other materials, thereby providing an increased cell proliferation and promoting an improvement in the ability of osseointegration of the material [13].

Front exposed to above, this study was to evaluate the bioactive characteristics of a possible new biomaterial developed from the addition of calcium carbonate seashells of the powder of glass ionomer cement.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of the samples

The preparation of calcium carbonate derived from seashells was carried out as described in the previous study [13].

The calcium carbonate powder was added to the GIC in proportions of 5 and 10%. Samples without the addition of shell were designed for the control group.

Shortly after obtaining the different proportions were prepared 15 specimens for each group. The mixture of powder and liquid of glass ionomer cement following the manufacturer's recommendations. After handling, the material was placed in a matrix of polytetrafluoroethylene (PTFE) with dimensions of 10 mm in diameter and 3 mm thick, being removed after completion of the reaction (10 min).

After the reaction of the specimens, they were packed in an airtight container free of moisture and placed in an incubator at 37˚C (OOL CB - ANEN Ltda, São Paulo, Brazil) for a period of 24 hours and distributed randomly into groups according to Table 1.

## 2.2 Immersion in simulated body fluid (SBF – Hanks' solution)

For analysis, the samples were immersed in simulated body fluid (SBF, Hanks' solution) as the composition described in Table 2. Each group of samples (n = 5) was immersed in 100 ml of Hanks' solution, remaining in this condition for 1, 2 and 4 weeks. During this period, the samples were stored at 37°C, and the solution was refreshed every 2 days.

The specimens were weighed before and after immersion time, the samples were removed from the solution, rinsed in distilled water, dehydrated in an oven of constant temperature of 37°C and free of moisture, and subjected to a weighing precision balance (AG-204 Mettler, Toledo, São Bernardo do Campo, SP, Brazil). For each time, the dissolution was assessed by the average percentage change of weight of samples.

2.3 Surface analyses by scanning electron microscopy (SEM)

To evaluate the surface, photomicrographs of the structures were performed by scanning electron microscopy and HA crystal deposition was assessed by analysis of energy dispersive spectrometry - EDS (Phillips XL 30, Eindhoven, Holland - accelerating voltage of 20kV and a filament current of 0.8 mÅ).

In this analysis, we obtained micrographs of the surface of the samples at different concentrations, as well as EDS analysis before and after being subjected to immersion in a Hanks' solution.

2.4 Statistical analysis

The results were statistically analyzed using ANOVA ($\alpha$ = 5%) to assess the variation in weight of samples after different periods of immersion in a Hanks' solution. It was considered as a parameter the percent change in weight (m0-m1) / m0, where m0 is the average initial weight of the sample and the final average m1 weight of the samples after immersion at different time.

## 3. RESULTS

After immersion in Hanks solution, samples from all groups had a decrease in the average weights when compared to measurements taken before immersion. The decrease in weight of samples was caused dissolution of the GIC.

Through statistical analysis it was observed that there was no statistical difference between the average percentage change in weight between the first and second weeks of immersion of samples from different groups. However in the fourth

week of immersion there was a significant variation in the mean percentage of weight for all groups.

At 4 weeks the group 2 showed the largest weight decrease, while the group 3 (control) showed the smallest weight decrease during this period (Figure 1).

The addition of groups containing calcium carbonate in the proportions of 5 and 10% had a higher amount of HA crystals deposited on its surface compared to the control group (without addition of calcium carbonate).

In the EDS analysis (Figure 3) revealed the presence of peaks of calcium, magnesium and sodium present in the formation of HA crystals.

## 4. DISCUSSION

The decrease in the average weight of the samples after immersion of samples in simulated body fluid (SBF) is related to the leaching of inorganic GIC. These leachate components found are silica, calcium phosphate, fluoride and aluminum [14].

As these elements, silica is naturally present in food and water, although their effect on humans is not well established, has not been associated with problems of biocompatibility. Calcium phosphate is the main inorganic constituent of bones and teeth. The effects of leaching could be considered to be biologically useful, even considering the small concentration of this compound released from the material [14,15].

The release of fluoride by GIC's is for a period of time that can last at least 18 months when used as a restorative material [16]. Despite the long period of fluoride

release and concern about the possible toxicity of fluoride, several studies suggest that fluoride may exert a beneficial effect on new bone formation [17-22]. *In vivo* and *in vitro* demonstrate that fluoride stimulates osteoblast proliferation, which is why the sodium fluoride (NaF) and widely used in the treatment of osteoporosis [23,24].

Aluminum proved to be a substance biologically harmful in some concentrations and a certainly could affect the biocompatibility of GIC's. Really, when tested in vitro, the effect of aluminum seems to be more important in determining the biocompatibility. However, despite the accumulation of aluminum observed in osteoblasts cultured in vitro in the presence of GIC's, the cells had apparently normal physiological activity and no signs of toxicity [25]. This result suggests that the amount of aluminum released from the GIC could be biologically acceptable under such circumstances.

The low pH during the reaction is related to the action of polyacrylic acid and at this stage may induce a cellular toxicity [7] although it is not an issue that compromises their biocompatibility [8]. According to Wilson *et al*. [26], the mechanism of union of the GIC dentin structure promotes a release of calcium and phosphate ions are then able to neutralize the acidity of the reaction.

However, when applied after complete reaction the results were very promising. Studies have reported activity and potential bioactive potential osteoconductive bone formation [11,12,27]. These results have encouraged the use of GIC after reacted as materials for the graft and restoration of areas of bone defects for grafting without providing an adverse effect on the structure and adjacent bone as observed in the study of Eldridge et al [28].

Hydroxyapatite (HA) is a calcium phosphate dihydrate ($Ca_{10}(PO_4)_6(OH)_2$) major component (about 95%) of the mineral phase of human bones and teeth, the main

inorganic component of bone acting as a reserve calcium and phosphorus. Moreover, the HA may have biological ions in its structure as $Ca^{+2}$, $Mg^{+2}$, $Na^{+}$, $CO_3^{+2}$ .

Among its features, the HA is one of the most biocompatible materials known due to similarity with the mineral phase of bone tissue. Presents osteoconductivity, favoring bone growth, establishing chemical bonds with bone tissue. Cells such as fibroblasts and osteoblasts do not distinguish between hydroxyapatite and bone surface [29].

The HA coating on other materials may enable an improvement in the biocompatibility properties of the substrate, accelerating the response of bone tissue due to its interaction with this structure. Techniques for HA coating materials are already widely used in the biomedical industry for making various articles in the medical and dental, as the various types orthopedic and prosthetic osseointegrated implant.

The binding capacity of a material to a bone structure, is usually evaluated by analyzing the ability to form apatite on its surface when placed in contact with simulated body fluid (SBF). By presenting ion concentrations similar to those of human blood plasma, these solutions enable the conduct of an in vitro assay to predict the bioactivity of seeking material with the bone structure [30,31].

In this study, by SEM and EDS was observed for the deposition of HA crystals on the surface of the samples tested after being subjected to immersion in simulated blood plasma.

In the samples containing the addition of calcium carbonate (Group 1 and Group 2 ), we observed a higher deposition of HA on their surface compared to those without addition of calcium carbonate (control group). The increased amount of HA should be observed in the formation of scaffolds in the structure of the sample, caused by the

addition of calcium carbonate to glass ionomer cement, as described in the study of Giacomelli *et al* [13].

In conclusion of this study, the results indicate that the addition of calcium carbonate powder to the glass ionomer cement provided changes that may contribute to the biocompatibility of glass ionomer cements conventional, but there is a need for new *in vitro* tests and *in vivo* to assess the feasibility of clinical use.

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**Table 1. Distribution of groups (n=5)**

| Group | Proportions of seashell powder(wt%) |
|---|---|
| 1 | 5 |
| 2 | 10 |
| 3 | |
| (Control) | 0 |

**Table 2. Composition of Hank's solution – pH = 7,4**

| | |
|---|---|
| NaCl | 0,137mM |
| KCl | 5,4 mM |
| $Na_2HOPO_4$ | 0,25 mM |
| $KH_2PO_4$ | 0,44 mM |
| $CaCl_2$ | 1,3 Mm |
| $MgSO_4$ | 1,0 mM |
| $C_6H_{12}O_6$ | 5,55 Mm |
| $NaHCO_3$ | 4,2 Mm |

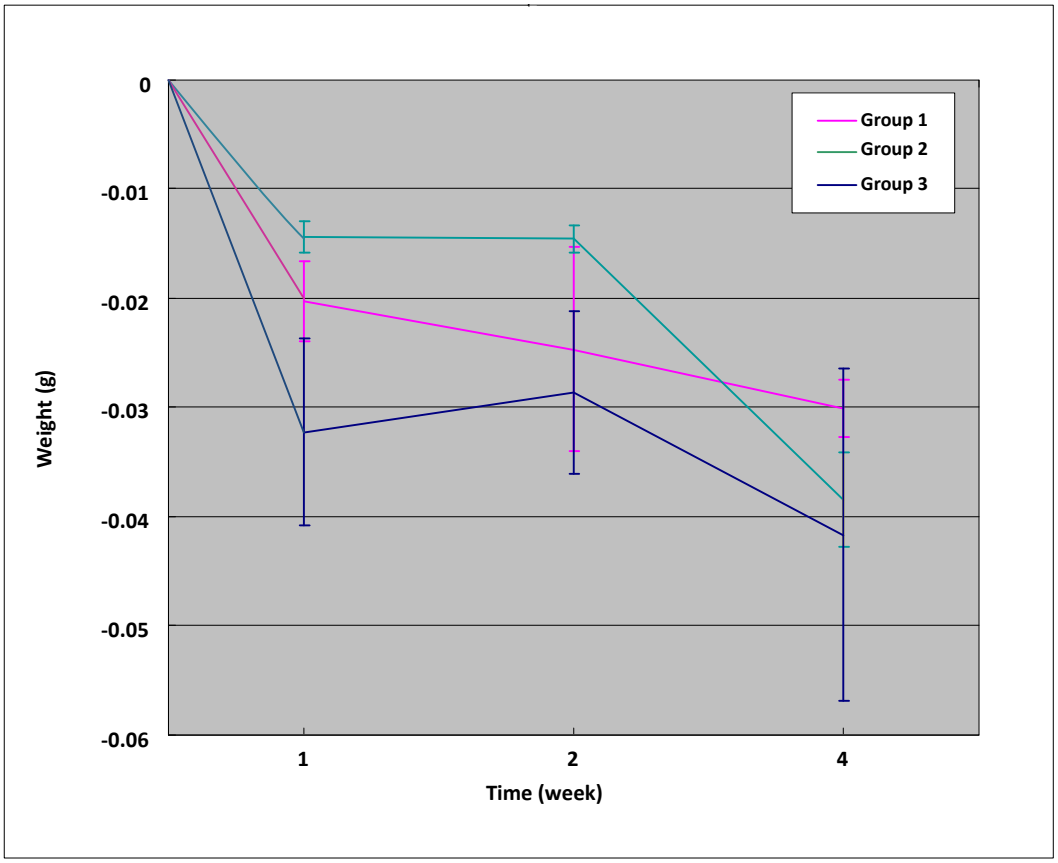


**Fig 1. Graph of weight change of group 1, 2 and 3 after immmersion in Hanks' solution for 1, 2 and 4 weeks.**

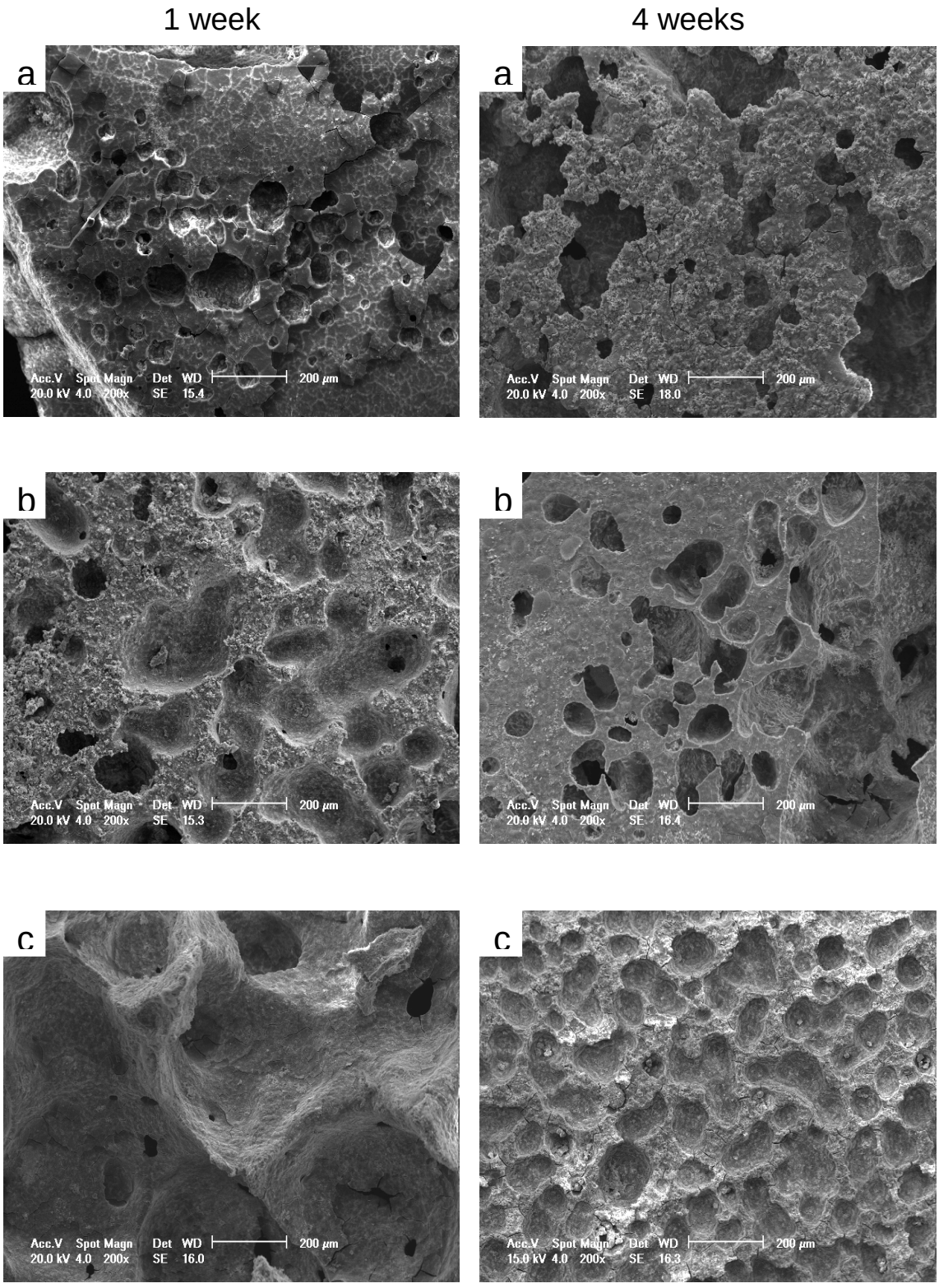


**Fig 2. SEM photomicrographs of group 1 (a), 2 (b) and 3 (c) after immersion in Hanks′ solution for 1 and 4 weeks.**

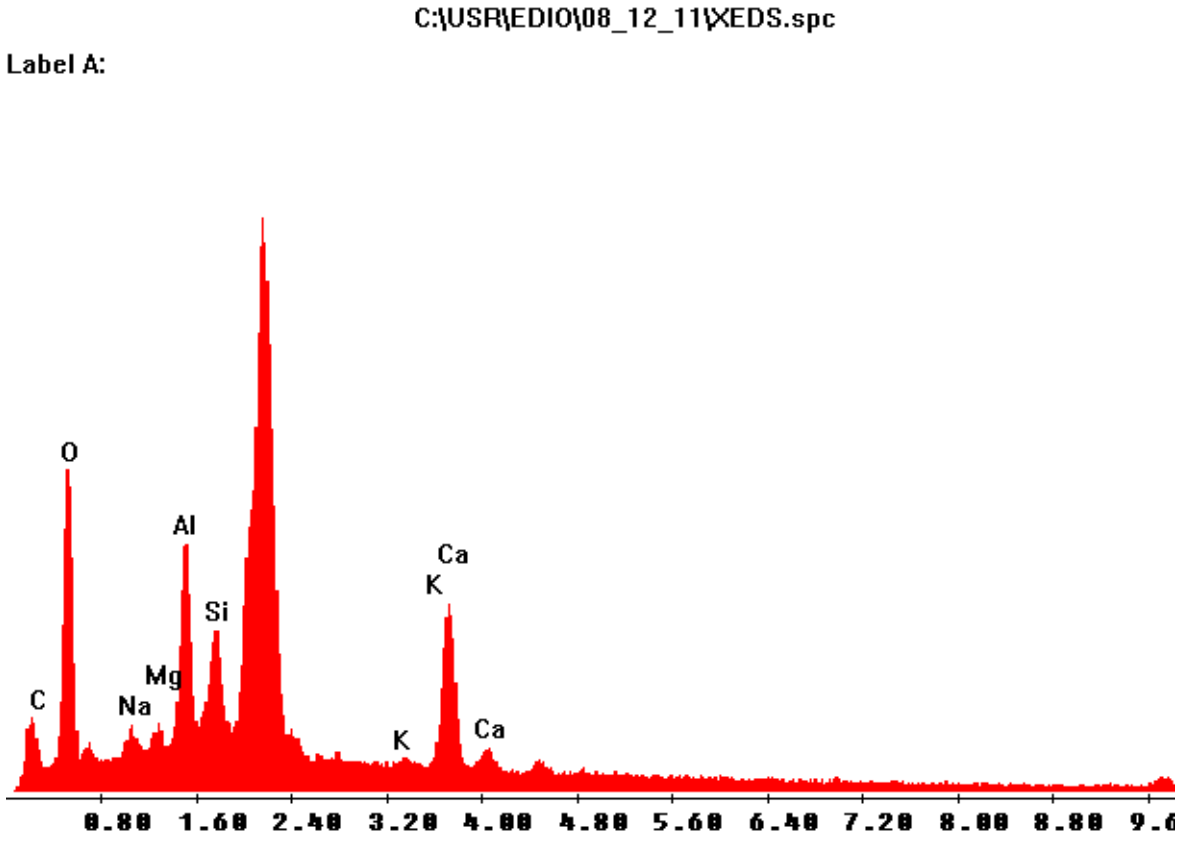


**Fig 3. EDS sample of group 2 after 4 weeks of immersion in SBF.**

## 5. DISCUSSÃO GERAL

A avalição *in vitro* ou mesmo *in vivo* é de grande importância uma vez que esses mecanismos permitem uma simulação do comportamento dos materiais para uma aplicação clínica. Dessa forma, a literatura nos mostra que ao longo do tempo foram desenvolvidas inúmeras metodologias a fim de avaliar *in vitro* o comportamento dos materiais testado.

Nesse sentindo, a utilização da levedura S. Cerevisiae como modelo experimental em análises de toxicidade celular vem despertando o interesse entre as ciências biomédicas. Este microorganismo apresenta uma série de características favoráveis bem como um fácil manuseio, possibilita uma análise quantitativa com grande numero amostral em um curto período de tempo (GRALLA *et al*, 1991). Além disso, possui propriedades metabólicas únicas, como as chamada células "petite", que pode ser utilizadas de forma adequada para uma análise de toxicidade respiratória. É também um microorganismo eucariótico com características genéticas e bioquímicas semelhantes aos de organismos animais (AUCOTT *et al*, 1990 e CASALONE *et al*, 2005).

Os testes realizados neste estudo mostraram que os valores de UFC/ml de todos os grupos induziram uma ligeira redução da viabilidade celular de S. cerevisiae em relação ao controle negativo. Embora o controle positivo e o grupo 2 mostram uma maior perda de viabilidade celular entre as amostras testadas, as suas diferenças em relação ao controle não foram significativas.

O comportamento do cimento de ionômero de vidro modificado por carbonato de cálcio observado neste estudo se mostrou semelhante aos resultados dos testes de citotoxicidade *in vitro* do CIV convencional, utilizando diferentes culturas de células

conforme descrito na literatura (BROOK *et al* e NICHOLSON *et al* 1991, OLIVA *et al* 1996, COSTA *et al* 2003 e HATTON *et al* 2006).

Métodos *in vitro* são também utilizados para elucidar os mecanismos celulares que levam a efeitos tóxicos observados. Neste contexto, especial atenção foi recentemente voltada à verificação da ruptura de equilíbrio do redox celular, como resultado do aumento da geração de espécies reativas ao oxigênio (ROS) (SCHWEIKL *et al* 2006). Situações em que o equilíbrio entre oxidantes e antioxidantes é perturbado, uma condição conhecida como estresse oxidativo ocorre. O estresse oxidativo quando pode acarretar uma série de eventos às células bem como danos oxidativos a lipídios, proteínas e ácidos nucléicos acumula e, eventualmente, resultar em efeitos biológicos que vão desde a alteração de vias de transdução de sinal e os níveis de expressão de genes para transformação celular ou até mutagênese (SCHWEIK *et al* 2006).

Estudos realizados por CHANG *et al*, (2005) e SCHWEIKL *et al* (2007) mostraram que um estado de desequilíbrio redox da célula causados por monômeros de resinas compostas foi associado com atraso do ciclo celular e apoptose. Ainda, LEE e colaboradores (2007), concluíram que uma conseqüência de desequilíbrio do redox celular induzido por materiais podem causar ser efeitos genotóxicos e mutagênicos.

Para este estudo, os testes aplicados para verificar a perda da capacidade respiratória das células de *S. cerevisiae*, os resultados revelaram a ausência de lesão mitocondrial quando exposta diretamente para as amostras. Esta análise sugere que o CIV modificado não foi capaz de induzir o estresse oxidativo em células de levedura.

Em um ambiente fisiológico *in vivo* ou *in vitro*, um material que esteja em contato com tecidos ou fluídos corporais, pode apresentar uma degradação ou dissolução. Segundo WILLIANS (1987), isso, no entanto, não afetará seu caráter

biocompatível desde que o próprio material ou os produtos da degradação sejam o tolerados pelos tecidos envoltórios e não venham a causar danos ao organismo em qualquer tempo.

Neste estudo, foi observado que as amostras com cimento de ionômero de vidro modificado por carbonato de cálcio apresentaram uma sensível diminuição do peso médio após a imersão na solução de Hanks′. Essa redução de peso das amostras está associada a dissolução do CIV.

Assim, de acordo com o que foi observado neste estudo, os resultados confirmam a tendência observada nas pesquisas relacionadas a testes de degradação do CIV, coforme descrito por NICHOLSON *et al* (1991) e ÖILO (1992).

Segundo NICHOLSON e colaboradores (1991) a dissolução do CIV promove a lixiviação de substâncias inorgânicas tais como a sílica, fosfato de cálcio, flúor e alumínio. No entanto, os elementos liberados pela dissolução do CIV podem não representar um comprometimento nas biocompatibilidade do material.

Entre os produtos da dissolução, a sílica não tem sido associada com problemas de biocompatibilidade. O fosfato de cálcio é o principal constituinte inorgânicos dos ossos e dentes, contudo, sua liberação em ambiente biológico poderia representar um efeito benéfico mesmo em uma pequena concentração (NICHOLSON *et al*, 1991, ÖILO, 1992).

Quando relacionado como material restaurador, a liberação de flúor promovida pelo CIV é uma característica de grande valia, porém no ponto de vista biológico pode gerar uma preocupação sobre seu potencial efeito tóxico. No entanto diversos estudos sugerem que o flúor pode exercer um efeito benéfico sobre a nova formação óssea

(KAWAHARA, 1979, LUNDY *et al*, 1986, HANSSON *et al*, 1987, MAMELLE *et al*, 1988, JONCK *et al*, 1989 E HATTON *et al*, 1992). Ainda, estudos *in vivo* e *in vitro* demonstram que o fluoreto de sódio (NaF) é capaz de estimular a proliferação osteoblástica (MARIE, 1992 e KASSEN *et al*, 1994).

Do mesmo modo, alumínio por ser comprovado biologicamente prejudicial em certas concentrações poderia afetar a biocompatiibilidade dos CIV's. No entanto, estudos de MEYER (1993) avaliando o comportamento de culturas de osteoblatos *in vitro*, observou que a quantidade de alumínio liberada a partir do CIV poderia ser biologicamente aceitável sob tais circunstâncias.

A hidroxiapatita (HA) é composta por um fosfato de cálcio hidratado ($Ca_{10}(PO_4)_6(OH)_2$) que compõe quase a totalidade da fase mineral de dentes e ossos humanos, servindo também como uma fonte de reserva de cálcio e fósforo. É um dos materiais mais biocompatíveis conhecidos devido a similaridade com a fase mineral dos tecidos ósseos. Apresenta osteocondutividade, favorecendo o crescimento ósseo e estabelecendo ligações químicas com o tecido ósseo e alem disso as células como fibroblastos e osteoblastos não distinguem a hidroxiapatita e a superfície óssea (FRANCIS *et al*, 2007).

A capacidade de ligação de um material a uma estrutura óssea, geralmente é avaliada através da análise da capacidade de formar apatita em sua superfície quando colocado em contato com soluçao simuladora do plasma sangüíneo (SBF). Por apresentar concentrações de íons semelhantes aos do plasma de sangue humano, essas soluções permitem realizar um ensaio *in vitro* buscando prever a bioatividade do material com uma estrutura óssea (KOKUBO, 1991 e KOKUBO *et al*, 2006).

Neste estudo, através da MEV e EDS observou-se deposição de cristais de HA na superfície das amostras testadas após serem submetidas à imersão em solução simuladora de plasma sangüíneo.

A deposição de hidroxiapatita mostrou-se mais acentuada nas amostras contendo a adição de carbonato de cálcio (Grupo 1 e Grupo 2), quando comparadas ao grupo sem adição de carbonato de cálcio (Grupo Controle). O aumento da quantidade de HA observado se deve pela formação de arcabouços na estrutura da amostra, causado pela adição de carbonato de cálcio ao cimento de ionômero de vidro, conforme descrito no estudo de GIACOMELLI *et al,* 2011. Esse resultado pode sugerir um aumento do potencial bioativo do CIV quando modificado por carbonato de cálcio.

Ainda, os testes *in vitro* do cimento de ionômero de vidro modificado por carbonato de cálcio não foi capaz de promover de toxicidade celular nem mesmo estresse oxidativo nas células da S. cerevisiae.

Estes resultados indicam que os materiais testados apresentam uma biocompatibilidade adequada, porém existe a necessidade de novos testes *in vitro* e *in vivo* para verificar a viabilidade de uso clínico.

## 6. CONCLUSÕES

A partir deste estudo, pôde-se concluir que:

1. O cimento de ionômero de vidro modificado por carbonato de cálcio de conchas marinhas não foi capaz de induzir um estresse oxidativo nas células de S. Cerevisiae. O mesmo também foi observado nos testes de sobrevivência celular, nos quais não se observou uma indução significativa de toxicidade celular do material testado.

2. A adição de carbonato de cálcio ao pó do cimento de ionômero de vidro proporcionou uma maior deposição de HA em sua superfície e isso pode contribuir para melhorar as características de biocompatibilidade dos cimentos de ionômero de vidro convencionais.

3. Estes resultados indicam que os materiais testados apresentam uma biocompatibilidade adequada, mas há uma necessidade de novos testes *in vitro* e *in vivo* para verificar a viabilidade de uso clínico.

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# 8. ANEXOS

*Comissão Científica e de Ética*
*Faculdade da Odontologia da PUCRS*

***Porto Alegre 27 de Abril de 2011***

***O Projeto de: Dissertação***

| | |
|---|---|
| **Protocolado sob nº:** | 0032/11 |
| **Intitulado:** | Avaliação da citotoxicidade celular "in vitro" do cimento de ionômero de vidro modificado por carbonato de cálcio de conchas marinhas |
| **Pesquisador Responsável:** | Profa. Dra. Luciana Mayumi Hirakata |
| **Pesquisadores Associados** | Édio Giacomelli; Renata Medina |
| **Nível:** | Dissertação / Mestrado |

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *27 de Abril de 2011.*

**Profa. Dra. Ana Maria Spohr**
Presidente da Comissão Científica e de Ética da
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# 9. APÊNDICES





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| Authors: | Giacomelli, Edio<br>Vilches, Patricia<br>Hirakata, Luciana |
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