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Original Article

Glass ionomer heated or not to identify bone defect created in rat calvaria

Ionômero de vidro aquecido ou não para identificação de defeitos ósseos criados em calvárias de ratos

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Resumo

Introdução: Alguns modelos experimentais têm sido usados para avaliar o uso de biomateriais na regeneração óssea. Entre eles estão os defeitos de tamanho crítico (DTC) criados em calvárias de ratos. Um modelo experimental foi descrito na literatura onde marcações em L são realizadas nas margens do defeito ósseo para auxiliar na identificação precisa desses defeitos durante o processamento laboratorial e análise dos resultados. No modelo experimental proposto, as marcações em "L" são preenchidas com amálgama. **Objetivo:** Avaliar a substituição do amálgama por ionômero de vidro aquecido ou não em um modelo experimental para identificação de defeito ósseo criado em calvária de ratos. **Material e método:** Foram utilizados 24 ratos. Um DTC de 5 mm de diâmetro foi criado na calvária de cada animal. Duas marcações em "L" foram realizadas a 2 mm das margens do defeito ósseo, preenchidas com amálgama (Grupo AM), ionômero de vidro aquecido (Grupo CIVaq) ou não (Grupo CIV). Os animais foram eutanasiados aos 15 dias pós-operatórios. A área do defeito cirúrgico e das marcações em "L" foram histomorfometricamente avaliadas e os dados estatisticamente analisados ($p<0,05$). **Resultado:** Não houve diferença estatisticamente significativa entre os grupos experimentais para as análises metodológicas, clínicas ou histomorfométrica realizadas. **Conclusão:** Dentro dos limites deste estudo, pode-se concluir que CIV pode substituir o AM no modelo experimental proposto e o aquecimento do CIV não promoveu benefícios adicionais.

Descritores: Cimentos de ionômeros de vidro; amálgama dentário; regeneração óssea; rats.

Abstract

Introduction: Some experimental models have been used to evaluate the use of biomaterials in bone regeneration. Among them are the critical size defects (CSD) created in rat calvaria. An experimental model has been described in the literature, in which "L" markings are performed on the margins of the bone defects in order to assist in the precise identification of these defects during laboratory processing and analysis of the results. In the proposed model, the "L" markings are filled with amalgam. **Objective:** The purpose of the present study was to evaluate the amalgam replacement of an experimental bony defect model in rat calvaria by heated or unheated glass ionomer. **Material and method:** 24 rats were used. A 5 mm CSD was created at each animal calvaria. Two "L" shaped markings were made 2 mm from the margins of the bone defect, filled with amalgam (Group AM), heated glass ionomer cement (Group GIh) or not (Group GI). The animals were euthanized 15 days postoperatively. The areas of the surgical defect and the L-shaped marking were histomorphometrically analyzed and the data were analyzed statistically ($p<0.05$). **Result:** There were no significant clinical, histological or methodological differences among the experimental groups. **Conclusion:** It can be concluded that GI can replace AM in the proposed experimental model and GI heating did not promote additional benefits.

Descriptors: Glass ionomer cements; dental amalgam; bone regeneration; rats.

INTRODUCTION

Bone is a structure that contains connective tissue with a mineralized matrix. Bone regeneration is divided into 3 parts: the first part is characterized by an inflammatory process, the appearance of a blood clot, the removal of cell debris and collagen matrix and the formation of tissue granulation[1]. The second part consists of the deposition of mineral matrix and the last phase of the bone repair process is prioritized by osteoclasts and corresponds to the remodeling of bone tissue in its original configuration[1]. In addition to the excellent mechanical properties, bone tissue has a unique potential for repair[1].

The bone can restore fractures or local defects through the regeneration process, with the formation of a new formed tissue and with the same organization as the previous tissue[2]. Thus, it is possible to return the morpho functional characteristics of the injured organ/ tissue[3]. Animal models have been a better option for evaluating the use and effectiveness of new substances and materials used in bone repair[4]. The biggest challenge is to find a material that is biocompatible with tissues, so, in order to be successful, new biomaterials with various characteristics have become necessary[5-7].

Some experimental models have been used to evaluate the use of these biomaterials in bone regeneration. Among them, the critical-size defects (CSD) created in rat calvaria have presented a reliable model since they do not regenerate spontaneously[8]. Messora et al.[9] proposed an experimental model in which "L" markings are performed on the margins of the bone defect created in the calvaria of rats, in order to assist in the identification of these defects during laboratory processing and analysis of results.

In the model proposed by Messora et al.[9], the "L" markings were filled with amalgam. Dental amalgam is an alloy composed of silver (Ag), tin (Sn), mercury (Hg) and other metals in which the percentage of mercury varies from 43 to 54%[10,11]. One of the disadvantages presented by this material is the high toxicity content of mercury in its metallic form, its restrictive or prohibited use in some countries and its unfavorable aesthetics[10,11]. Besides the disadvantages over mentioned, amalgam can negatively interfere in the laboratorial processing during histological slices obtaining because of its traces remains even after processing of the specimens when the substance is removed.

To avoid these problems during the execution of this methodology, the use of other material could be suggested to identify the L-shaped marking, such as glass ionomer cement (GI). GI has the correct biocompatibility to be used for various purposes in Dentistry. Glass ionomer cement is a material consisting of a hydrogel matrix and inorganic glass particles[12,13]. In order to improve its properties, ease of use and resistance, a new modality of this cement appeared on the dental market in the prefabricated capsule system. This system allowed for a more satisfactory agglutination reaction, contributing to a decrease in porosity, thus increasing its mechanical properties as well as its cohesive resistance[4,14].

Experimental models that involve the evaluation of bone regeneration are of great relevance within Dentistry and other areas of health. The materials used in these types of studies are constantly studied to improve their biophysical, chemical and mechanical properties. Studies have also evaluated other ways to further improve the properties and longevity of dental materials. In this context, the heating of some materials used in restorations has been showing promising results in terms of decreasing its viscosity with a drop in the long-term degradation process[15-21]. Thus, within the limits of each study, experimental models can follow these changes, starting to use materials with better properties and more used today that bring more benefits to the technique study.

The purpose of the present study was to evaluate the amalgam replacement of an experimental bony defect model in rat calvaria by heated or unheated glass ionomer.

MATERIAL AND METHOD

Ethical Considerations

The research was carried out respecting the ethical principles of animal experimentation established by the Brazilian College of Animal Experimentation, and the ARRIVE guide (Animal Research: Reporting of in vivo Experiments). The animals were kept in shared ventilated cages with 3-4 animals/cage under a controlled environment with 12-hour cycles of light per day and temperature between 22-24 ºC. Food and water were offered *ad libitum*.

Experimental Model

It was used 24 male rats (*Rattus norvegicus, albinus*, Wistar), weighing between 250 and 300 g. The animals were randomly assigned to 3 experimental groups (n = 8): Group Amalgam (AM): defect of 5 mm and identification L-shaped marking filled with amalgam (Figure 1); Group Glass Ionomer (GI): defect of 5 mm and identification L-shaped marking filled with glass ionomer cement; Group heated glass ionomer (GIh): defect of 5 mm and identification L-shaped marking filled with heated glass ionomer cement. The animals were submitted to euthanasia at 15 days postoperative.

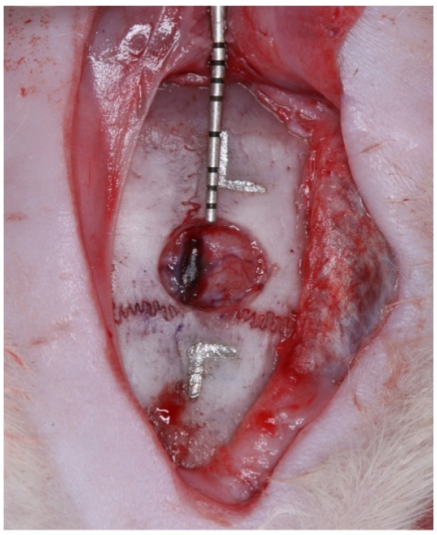

Figure 1. Image illustrating the defect created surgically and the “L” markings filled with amalgam.

Surgical Procedure

The bone defect was created in the animals' calvaria, following the methodology proposed by Messora et al.[9] The animals were anesthetized by intramuscular injection of xylazine (6 mg / kg of body weight) (Coopazine, Coopers, São Paulo, Brazil) and ketamine (70 mg / kg of body weight) (Dopalen, Agribands Purina do Brasil Ltda., Paulinia, Brazil). After aseptic preparation, a semi-lunar incision was made in the anterior region of the calvaria, allowing a full-thickness flap to be folded in the posterior direction. A CSD of 5 mm in diameter was created with a trephine (Neodent, JJGC Indústria e Comércio de Materiais Dentários S.A., São Paulo, Brazil) attached to a low rotation hand piece (Kavo Dental, São Paulo, Brazil) under constant irrigation with sterile saline.

An L-shaped marking was performed 2 mm anterior and another 2 mm posterior to the margin of the surgical defect using a conical carbide bur (#3195, KG Sorensen, Cotia, Brazil) and a surgical guide. The longest axis of each L was located on an imaginary cranio-caudal

longitudinal line that divides the surgical defect in half. The markings were filled with amalgam (GS-80, SDI Holdings Pty Ltd, São Paulo, Brazil) (Group AM), glass ionomer (Group GI) or heated glass ionomer (Group GIh). These markings were made to allow identification of the medium of the original surgical defect during laboratory processing. The soft tissues were then repositioned and sutured to obtain a primary wound closure.

Each animal received an intramuscular injection of 24,000 IU of Penicillin G-benzathine (Pentabiotic* Small Veterinary, Fort Dodge® Saúde Animal Ltda., Campinas, Brazil) and analgesic (Tramadol 3-5 mg / Kg) (Tramadol hydrochloride, União Química, São Paulo, Brazil) after surgery.

Heating of Glass Ionomer Cement

For this study, glass ionomer-based restorative cement reinforced with resin and light cured (Riva Light Cure®, SDI, Victoria, Australia) was used. After the homogenization of the capsules in an amalgamator, GI was deposited on a glass plate and, with the aid of a hair dryer (Taiff Red Ion 1900W, Brazil) was heated to 54° C and 10 cm distance between the nozzle of the hair dryer and the GI, for 10 seconds. A digital thermometer (Digital thermometer TH150, G-Tech Center Medical, São Paulo, Brazil) was used to control the temperature. The GI was heated prior to its application in the L-shaped marking[20].

Tissue Processing

All animals were euthanized at 15 days postoperative with an overdose of general anesthetic (Tiopental 100-150 mg/Kg) (Thiopentax®, Cristália Produtos Químicos Farmacêuticos Ltda, Itapira, Brazil). The area of the original surgical defect and the surrounding tissues were removed *en bloc*. The blocks were fixed in a 10% neutral formaldehyde solution, washed in running water and decalcified in a 10% ethylenediaminetetraacetic acid (EDTA) solution. After an initial descaling, each specimen was divided longitudinally into two blocks, exactly along the center line of the original surgical defect using the long axis of both L-markings as references. Transverse cuts were also made that touch the minor axis of each L-shaped mark, so that each specimen is 9 mm long in the longitudinal direction. Thus, it becomes possible to precisely determine the limits of the original surgical defect during histological and histometric analyzes (Figure 2). After further descaling, the parts were processed and embedded in paraffin. Serial longitudinal cuts, 5 µm thick, were performed, starting from the center of the original surgical defect. The sections were stained using Hematoxylin and Eosin techniques for analysis with light microscopy.

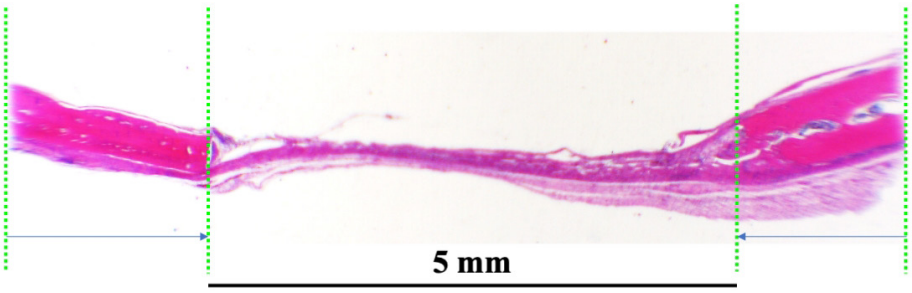

Figure 2. Scheme illustrating the performance of the histomorphometric analysis. The blue arrows indicate how the linear measurements of the margins of the surgical defect were determined.

Histomorphometric Analysis

Two histological sections, representing the center of the original surgical defect, were selected for histological and histometric analysis to increase the reliability of the data used in

the statistical analysis. Analyzes were performed by calibrated examiners who were blind to the treatment performed.

Images of the histological sections were captured with a digital camera connected to a polarized light microscope at 160x magnification (Leica ICC50 HD, Wetzlar, Germany). Histomorphometric analysis was performed with the aid of software for image analysis (Image J - National Institutes of Health, Washington, DC, USA).

Criteria based on the work of Messora et al.[9] were used to standardize the histomorphometric analysis of digital images. Briefly, the identification of the external and internal surfaces of the original calvaria on the right and left margins of the surgical defect was first determined. Then, linear measurements from the right and left ends of the specimen towards the center of the surgical defect were performed. Thus, the length of the margins of the original surgical defect was determined (Figure 2).

Statistical Analysis

Analysis of variance (ANOVA) was performed, followed by the Tukey post-test, considering a significance level of 5% (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp.).

RESULT

No differences were observed in the execution of the methodology, clinically or histologically among the different experimental groups.

In the clinical analysis, no cracks or porosities were observed on the surface of the AM, GI or GIh. All evaluated materials also remained inside the L-shaped marking during the healing period.

In the histological analysis, all groups did show the neo formed bone tissue confined to the margins of the surgical defect presented normal characteristics, rich in osteoblasts. The connective tissue that fills the center of the surgical defect had only a few inflammatory cells, consistent with the healing period. Figure 3 shows histological panoramic images and a high magnification of specimens from each experimental group.

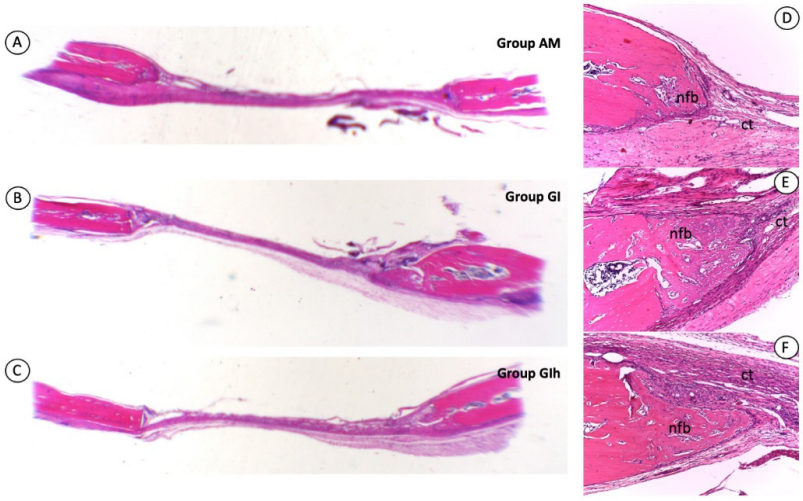

Figure 3. Panoramic histological images (A-C) and higher magnification (D-F) of specimens from each experimental group. Abbreviations: AM, amalgam; GI, glass ionomer cement; GIh, heated glass ionomer cement; on, newly formed bone; tc, connective tissue. Hematoxylin and eosin staining, 40x (A-C), 100x (D-F).

In the statistical analysis, there were no statistically significant differences in the length of the defect margins between the experimental groups. Figure 4 shows the graph of the means and standard deviations of the linear measurements of the surgical defect margins, for each experimental group.

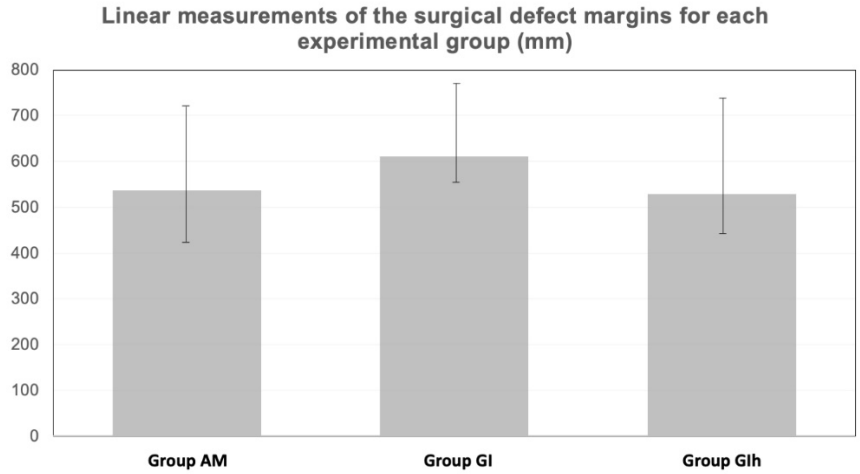

Figure 4. Graph of the means and standard deviations of the linear measurements of the surgical defect margins for each experimental group.

DISCUSSION

In Dentistry field, silver amalgam is still used to perform posterior tooth restorations[11]. Even the numerous disadvantages, amalgam has some advantages such as relatively low cost, high wear resistance, greater durability and ease of technical execution[14]. Several studies successfully followed the methodology proposed by Messora et al.[9] where amalgam was used to identify the L-shaped marking[5-7,22-24]. However, considering that the amalgam alloy is subject to corrosion mercury is a substance that is considerably toxic to both the environment and humans[10,11] and to follow the advances related to dental materials it was proposed here to replace amalgam with glass ionomer. In addition, presence of mercury in the amalgam alloy corrosion has high toxicity and can promote damage at the cellular and organic level of the human organism[10,11].

On the other hand, GI popularity was evidenced due to its biologically favorable properties, as this material releases fluoride when exposed to the oral environment, has an excellent chemical adhesion, is biocompatible and presents more and more resistance in its formulations modified by resin[12,13]. In addition to being used as the material of choice in atraumatic restorative technique (ART), it is also used in medicine and speech therapy in the treatment of bone regions[12,13].

Tissue repair occurs to establish the morpho functional characteristics of an injured organ or tissue, restoring the continuity of the margins of this tissue. Research related to bone repair has been carried out for years, with the intention of acquiring new knowledge that can assist in reconstructive surgeries, guided bone repairs and injured implants[3].

The use of synthetic biomaterials in the regeneration of bone tissue as an option for bone grafts is significant, as they do not harm bone tissue and do not increase the possibility of bacterial and viral contamination[25]. Some materials cause stimulation of bone formation. The biomaterials chosen must be biocompatible, biodegradable and osteoconductive and have an adequate structure that will support bone formation and must also function immediately after implantation and integration with the organism[25]. There are some properties that are indispensable for the effectiveness of a good biomaterial, such as: the material cannot trigger an inflammatory or toxic

response in its implantation in vitro, the degradation time of the material must allow the tissue regeneration process to occur, the material must have mechanical properties suitable for the application for which it was indicated and, finally, its degradation cannot generate toxic products and its products must be easily metabolized and released from the body[25].

In the present study, no differences were observed in the execution of the methodology, clinically or histologically between the different experimental groups.

Regarding the execution of the methodology and the clinical characteristics, difficulties could have occurred in relation to the identification of the GI to perform the reduction of the pieces during the laboratory processing, since its coloration is very similar to the color of the rats' calvaria. However, the difficulties encountered for both AM and heated or unheated GI were the same. GI and GIh also did not show differences between them. Another important factor that must be considered is the permanence of both the AM and the GI within the L-shaped marking, allowing the methodology to be executed in an equal way regardless of the material used.

Groups AM, GI and GIh had the same histological characteristics. The specimens of all groups showed inflammatory infiltrate consistent with the healing period. Considering that the first phase that occurs in the bone repair process is the inflammatory phase[1], it can be inferred that all experimental groups were in the same phase of the healing period and that none of the materials implied the delay in this process. These results are further confirmed by the presence of an abundance of osteoblasts in the newly formed bone tissue, which reflect the phase of bone mineral deposition in all groups[1] and by the organization of the newly formed bone tissue similar to the original bone tissue[2].

Also, it can be suggested that AM and GI (heated or not) showed biological compatibility and, corroborating previous studies on their characteristics fully compatible with their wide use in Dentistry[10-13]. The results of the present study did show that these materials have desirable characteristics for biomaterials: they did not promote an exacerbated inflammatory response (only compatible with the healing phase), the time of degradation of the materials allowed their maintenance in the L-shaped marking and they showed adequate mechanical properties for their application and, finally, its degradation did not generate toxic products[25].

Despite the presence of amalgam mercury in animals in the Group AM, its presence does not appear to have triggered any unwanted tissue response in the analyzed period. Although some studies have shown that the presence of mercury can promote toxicity[10,11], which may pose health risks, this characteristic was not observed. Thus, it can be suggested that the benefits of its use can still be used in the evaluated experimental period (15 days).

Regarding the GI present in groups GI and GIh, the same beneficial characteristics of biocompatibility and mechanical properties were observed. These results corroborate with data from the literature on its indication for use for different purposes in Medicine, Dentistry and other areas of health[12,13].

Heating of GI was suggested here in order to verify if the decrease in its viscosity due to the increase in temperature resulted in a change in its mechanical properties, as suggested in previous studies in the area of Dentistry with other materials used in restorative procedures[15-21]. However, in this study, there were no clinical, histological and methodological differences between groups GI and GIh.

CONCLUSION

Within the limits of the present study, it can be concluded that GI can replace AM in the proposed experimental model and GI heating did not promote additional benefits.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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