Brazilian Dental Journal (2019) 30(6): 599-606
http://dx.doi.org/10.1590/0103-6440201902619

ISSN 0103-6440

Tissue Response after Subcutaneous Implantation of Different Glass Ionomer-Based Cements

Carolina Maschietto Pucinelli[1], Raquel Assed Bezerra da Silva[1], Luã Lopes Borges[1,2], Alberto Tadeu do Nascimento Borges[1,2], Paulo Nelson-Filho[1], Alberto Consolaro[3], Patricia Gaton-Hernández[4], Lea Assed Bezerra da Silva[1]

[1]Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, USP – Universidade de São Paulo, Ribeirão Preto, SP, Brazil
[2]Faculty of Amazonas, IAES - Manaus, AM, Brazil
[3]Department of Oral Pathology, Bauru Dental School, USP – Universidade de São Paulo, Bauru, SP, Brazil
[4]Department of Integrated Pediatric Dentistry, School of Dentistry, UB – Universidade de Barcelona, Barcelona, Spain

Correspondence: Carolina Maschietto Pucinelli, Avenida do Café, s/n, 14040-904 Ribeirão Preto, SP, Brasil. Tel: +55-16-3315-4099. e-mail: carolinamaschietto@yahoo.com.br

The aim of this study was to evaluate the subcutaneous connective tissue response of isogenic mice after implantation of different glass ionomer-based cements (EQUIA® Forte Fil, EQUIA® Fil and Ketac™ Universal Aplicap™). Eighty-seven isogenic BALB/c mice were allocated in 12 groups, 9 were considered as experimental groups (Ketac, E. Fil and E. Forte at 7, 21 and 63 days) and 3 controls (empty polyethylene tubes at 7, 21 and 63 days). After the experimental periods, the subcutaneous connective tissue surrounding the implanted material was removed and subjected to histotechnical processing and staining with hematoxylin and eosin. A histopathological description of the tissue reaction surrounding each material and a semi-quantitative analysis of collagen fiber formation and inflammatory infiltrate were performed. Additionally, the thickness of the granulomatous tissue in contact with each material was measured. Data were analyzed statistically (α=0.05) by the Kruskal-Wallis test, followed by Dunn post-test. Initially, the collagen fiber formation was not different among all the tested materials ($p>0.05$) but was different at 21 days with the control group presenting the most advanced stage of collagen fiber formation. At 63 days, EQUIA® Forte Fil group showed the most advanced stage of collagen fiber formation, compared to EQUIA® Fil group ($p<0.05$). The inflammatory infiltrate was not different among the tested materials in any experimental period ($p>0.05$). The thickness of the granulomatous tissue was greater in the E. Forte group, compared to control in all periods. All glass ionomer-based cements showed tissue compatibility, according to the evaluated parameters.

Key Words: glass ionomer cement, isogenic mice, subcutaneous connective tissue, tissue response.

Introduction

Minimal Intervention Dentistry is a concept based on scientific evidence for the treatment of caries disease (1). It is characterized by a set of procedures that includes prevention, dental structure preservation and a less invasive approach with minimum removal of healthy tissue, when it is extremely necessary (2). Risk assessment, implementation of preventive procedures, detection and early treatment of non-cavitated lesions and the use of minimally invasive restorative techniques in cavitated lesions are basic conducts for this treatment philosophy (3).

Currently, numerous dental materials are available for this purpose (4). Despite the attempt to avoid restorative treatment, when a cavitated lesions is present it is necessary. Thereunto, the infected tissue is removed, followed by filling the cavity with suitable restorative materials (5). These materials should preferably be adhesives to prevent loss of healthy tissue (6). Among these materials, glass ionomer cement (GIC) is stands out. They presents adhesion to the dental structure and have good properties, such as liberation and absorption of fluoride ions (7), biocompatibility with oral tissues (8) and coefficient of thermal expansion similar to dentin (9).

Ketac™ Universal Aplicap™ (3M ESPE, Germany) is a conventional GIC. An In vitro study conducted by Alrahlah (10) showed that Ketac™ Universal Aplicap™ achieved the highest values of Vickers hardness compared to Tetric N-Ceram (Ivoclar Vivadent, Schaan, Liechtenstein), SDR Dentsply Caulk (Milford, DE, USA), ACTIVA bioactive (Pulpdent, Germany) and GC Fuji II (LC GC Corporation, Tokyo, Japan). However, there are no in vivo studies evaluating the performance of this cement, thus biological studies are necessary.

EQUIA® Fil (GC Corporation) is other conventional GIC that deserves attention. In a retrospective cohort study (11) EQUIA® Fil inserted in class I and II cavities was considered as clinically acceptable, after 2 years. In addition, EQUIA® Fil presented high fracture resistance in vitro and could be used in areas with high stress level (12) and presented good adaptation in class II restorations of primary teeth, with less steps for application and shorter operative time (13).

EQUIA® Fil was also compared with other materials with respect to radiopacity (14) and met the requirements of the "International Organization for Standardization" and

the "American National Standard Institute (ANSI/ADA)". However, in order to improve its physical and aesthetic characteristics, in 2015 was developed the EQUIA® Forte Fil. According to the manufacturer, this material consists of ultra-fine and highly reactive glass particles dispersed in the conventional structure of the GIC. In addition, the polyacrylic acid has a higher molecular weight, which leads to a high strength restorative system.

However, there are few published studies evaluating the EQUIA® Forte Fil. Van Duinen et al. (14) evaluated the increase in pulp temperature after the use of EQUIA® Forte Fil and showed that the temperature rise was safe, without risk of pathological damage to the pulp tissue. Additionally, there are no in vivo studies in the specific literature evaluating EQUIA® Forte Fil, thus biological studies are necessary in order to provide evidences for its clinical use.

The aim of this study was to evaluate the subcutaneous connective tissue response of BALB/c mice after implantation of polyethylene tubes filled with different GICs (EQUIA® Forte Fil, EQUIA® Fil e Ketac™ Universal Aplicap™). The null hypothesis was adopted: it is expected that these GICs exhibit no differences in tissue compatibility.

Material and Methods

The research project was approved by the Animal Care and Use Committee of School of Dentistry of Ribeirão Preto, University of São Paulo (FORP-USP), protocol #2015.1.824.58.1. The tests were conducted as suggested by the International Organization for Standardization, number 10993-6:2007, to evaluate the biocompatibility of biomaterials for use in medical devices.

The following GIC-based cements were used: EQUIA® Forte Fil (GC Corporation,), EQUIA® Fil (GC Corporation) e Ketac™ Universal Aplicap™ (3M ESPE). The cements were handled according to manufacturers' instructions in a laminar flow to maintain the aseptic conditions. Twenty-four specimens were prepared for each material using a Teflon matrix with 1.5 mm diameter and 5 mm length.

Eighty-seven 7-week-old male isogenic BALB/c mice, weighting 15 to 20 grams, were obtained from the central animal facility of the University of São Paulo, Ribeirão Preto, Brazil. The animals were randomly allocated in 12 groups (9 experimental and 3 controls). The control groups consisted of the subcutaneous implantation of empty polyethylene tubes. Each animal received one implant in the dorsal region with one of the tested materials or one empty polyethylene tube.

The animals were anesthetized by an intramuscular injection (10% ketamine; Agener União Química Farmacêutica Nacional S/A, Embu-Guaçu, SP, Brazil; and xylazine; Dopaser, Laboratórios Calier, SA, Barcelona, Spain), in the proportion of 0.20 mL/kg and 0.8 mL/kg, respectively, prior to surgery. Then, trichotomy of the surgical area was performed, and antisepsis with 1% chlorhexidine gluconate. After, was performed the incision (1cm) with sterilized surgical scissors in the dorsal region, followed by divulsion. The specimen was placed inside the subcutaneous connective tissue and the skin borders were closed with 4-0 silk sutures (Vicryl 4-0; Ethicon - Johnson &Johnson, São Paulo, SP, Brazil).

The animals were kept at the animal facility of the School of Dentistry of Ribeirão Preto - University of São Paulo during the experimental periods of 7, 21 or 63 days, with feed and water "*ad libittum*". They were periodically monitored in order to observe any local, systemic or behavioral abnormality. At the end of each period, the animals were euthanized by anesthetic overdose to remove the tested specimen with the surrounding subcutaneous connective tissue and skin.

Histotechnical Processing

The samples were fixed in 10% buffered formalin for 24 h at room temperature and washed for 3 h in running water. The materials or the polyethylene tubes were carefully removed from the samples. Then, the tissues were dehydrated in alcohol, cleared in xylol and embedded in paraffin. The blocks containing the tissues were cut longitudinally using a microtome (Leica RM2145, Leica Microsystems GmbH, Wetzlar, Germany). Five-micrometer-thick sections were obtained and stained with hematoxylin and eosin (HE).

Microscopic Evaluation

The tissue analysis was performed by an experienced pathologist blind with respect to the groups, using an Axio Imager.M1 microscope in 10x magnification (Zeiss, Göttingen, Germany) coupled to a camera Axiocam MRc5 (Zeiss, Göttingen, Germany).

Descriptive Analysis

The histopathological description of the reactionary tissue in contact with each material or polyethylene tube was performed in each experimental period.

Semi-Quantitative Analysis

In the surrounding tissues of each material or polyethylene tube, the following semi-quantitative analyses were performed based on Queiroz et al. (15):

Collagen Fiber Formation. The number and density of collagen fibers in the tissues surrounding the materials were considered. The collagen fiber formation was classified in 3 degrees of severity, according to the following scores: 1 (mild): individual collagen fibers as presented in a normal connective tissue, interspersed with negative spaces

indicative of non-fibrous extracellular matrix components; 2 (moderate): areas with presence of individual collagen fibers, and areas alternating eosinophilic extracellular matrix, without typical linear and undulate formation; 3 (intense): presence of collagen fibers in the middle of an eosinophilic extracellular matrix, without typical linear and undulate formations, not allowing an individual collagen fiber.

Inflammatory Infiltrate. The concentration of neutrophils in the reactionary tissue was also classified in 3 degrees of severity: 1 (mild): presence of 1 to 10 inflammatory cells in the reactionary tissue; 2 (moderate): presence of 11 to 20 inflammatory cells in the reactionary tissues; 3 (intense): presence of more than 21 inflammatory cells in the reactionary tissue.

Quantitative Analysis

The thickness of the granulomatous reactionary tissue in contact with the materials or polyethylene tube was measured (Fig. 1) at 10× magnification. The images were analyzed in the microscope software (AxioVision Rel, v 4.8, Carl Zeiss MicroImaging GmbH) using the "measures" tool.

Statistical Analysis

The results of the semi-quantitative and quantitative evaluations were analyzed with the Kruskal-Wallis test and Dunn post-test. All analyses were performed using the Graph Pad Prism 4.0 software (Graph Pad Software Inc., San Diego, CA, USA), with a significance level of 5%.

Results

Descriptive Analysis

7 days

Representative images of the 7-day period are presented in Figure 2. After 7 days the reactional tissue of Control group samples presented uniformly collagen fiber formation for both parameters thickness and severity. In 100% of the specimens the collagen fiber was discrete and thin. The inflammatory infiltrate, represented by neutrophils, was predominantly moderate in 75% of the specimens. Macrophages permeated the fibroblasts.

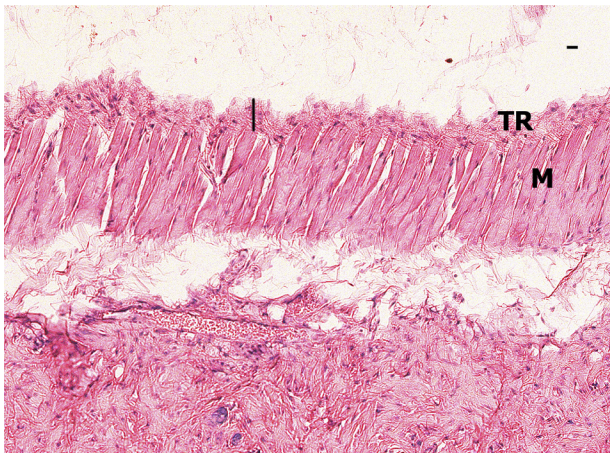

Figure 1. Representative image that illustrate where the measurement of the reaction tissue (TR) was performed, from the muscle layer (M) to the gap left by the tested material (HE, Zeiss, 10×).

The experimental groups presented a reactionary tissue disorganized by edema. The collagen fiber formation was discrete in 75% of Ketac group specimens, 62.5% Equia Fil and minimum degree of collagen fiber formation (100%) was observed in Equia Forte samples. The neutrophilic infiltrate was moderate and casually distributed for Ketac and Equia Fil groups. However, for Equia Forte the samples were intensely (50%) infiltrated by neutrophils. Ketac and Equia Fil presented irregular and birefringent fragments, compatible with particles of crystallized structure material.

21 days

Representative images of the 21-day period are presented in Figure 3. After 21 days, the collagen fiber formation of Control group ranged from moderate (40%) to intense (60%). The peripheral capsule was still permeated by neutrophils. The inflammatory infiltrate ranged from discrete (40%) to moderate (60%). Macrophages were observed at the interface between the reactionary tissue and the polyethylene tube.

The majority of samples of Ketac (62.5%) and Equia Fil groups (86.7%) presented moderate collagen fiber formation. However, Ketac presented uniform and thin reactionary tissue while Equia Fil presented a very disorganized tissue. Equia Forte samples ranged between discrete (50%) and moderate (50%) collagen fiber formation. Ketac (87.5%) and Equia Forte (83,3%) samples presented discrete neutrophil infiltration while Equia Fil presented moderate (57.1%) inflammatory infiltration. Eventually, birefringent particles of material were observed in Ketac and EQUIA Fil groups.

63 days

Representative images of the 63-day period are presented in Figure 4. After 63 days, the samples of Control group presented moderate collagen fiber formation (50%) and discrete neutrophilic infiltrate. No macrophages were observed.

Was observed thin and disorganized reactionary tissue with discrete collagen fiber for Ketac (71.4%) and Equia Fil (75%) groups. The fibrous connective tissue of Equia Forte group was moderate (100%). The neutrophilic infiltrate was discrete for Ketac (71.4%), Equia Fil (75%) and Equia Forte (87.5%) groups.

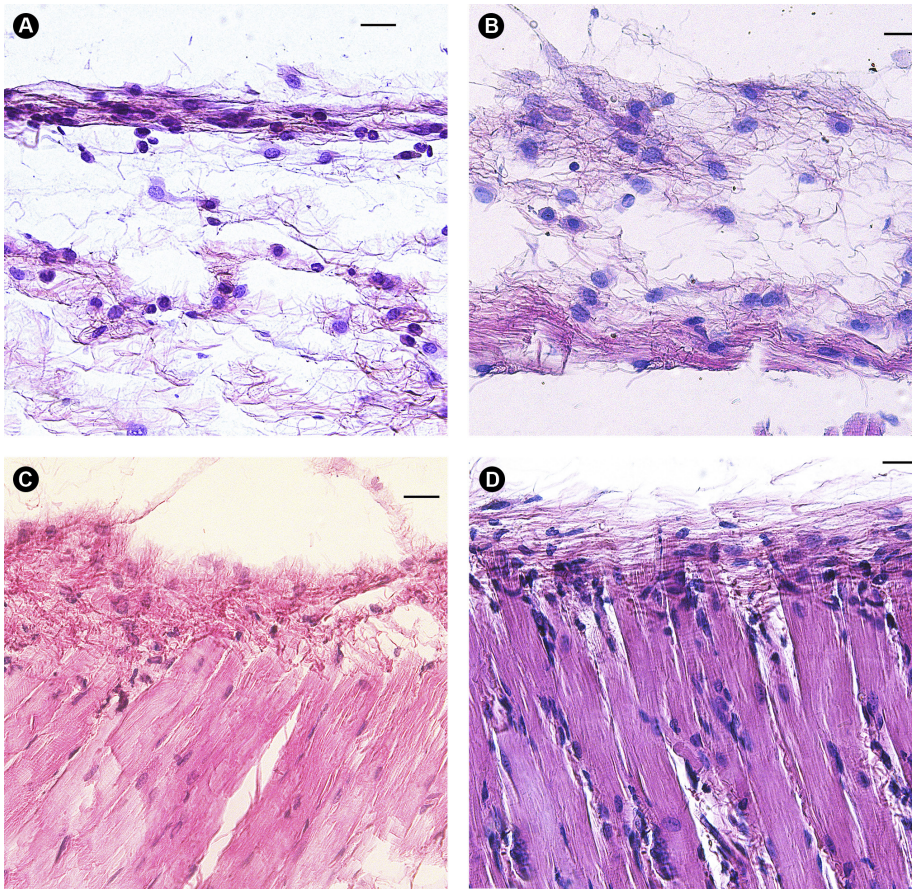

Figure 2. Microscopic analysis at 7 days. A: Control: reactionary tissue with discrete collagen fiber formation infiltrated by occasional neutrophils. B: Equia Forte: delicate collagen fibers, presence of macrophages and early fibroblasts. C: Equia Fil: reactionary tissue with moderate collagen fiber formation and occasional macrophages. Skeletal muscle tissue in the subjacent area. D: Ketac: reactionary tissue with thin-thickness and delicate collagen fibers supported by the subjacent skeletal muscle tissue (HE, Zeiss, 40×).

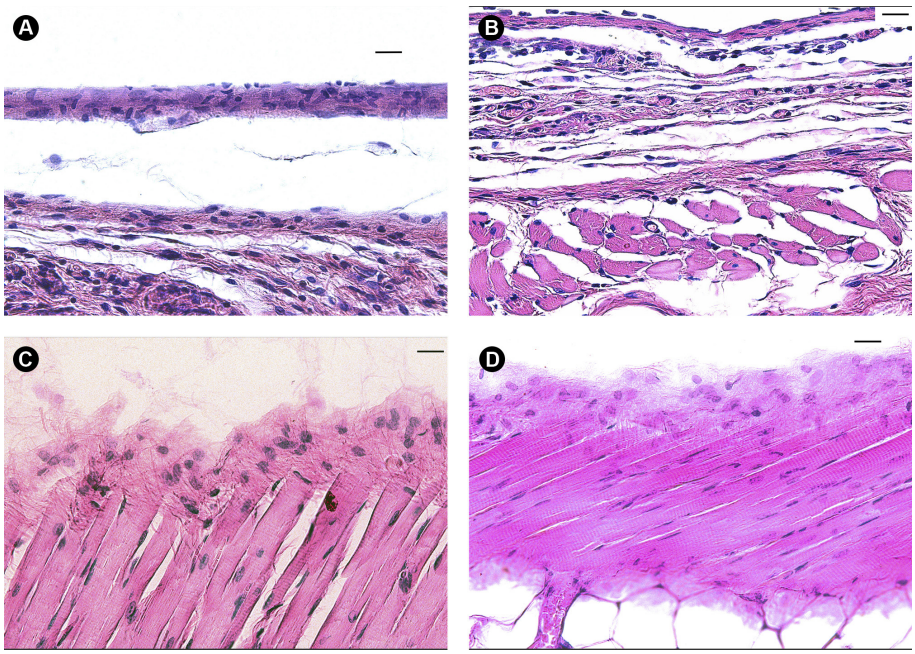

Figure 3. Microscopic analysis at 21 days. Well-established and organized reactionary tissue. A: Control: neutrophil infiltration and discrete collagen fiber formation. B: Equia Forte: vascular proliferation and edema among the collagen fibers and well-organized reactionary tissue. C - Equia Fil: prominent fibroblasts and macrophages among the collagen fibers supported by skeletal muscle fibers. D: Ketac: moderate collagen fiber formation, macrophages and early fibroblasts on the skeletal muscle layer (HE, Zeiss, 40×).

Semi-Quantitative Analysis

Collagen Fiber Formation. Table 1 shows the median of collagen fiber formation obtained after the analysis of the granulomatous reactionary tissue in contact with the materials or polyethylene tube.

Table 1. Median, and p-value regarding Collagen Fiber Formation in the periods of 7, 21 and 63 days

Period	Group	Median	p-value
7 days	Control	1	0.18
	Ketac	1	
	E. Fil	1	
	E. Forte	1	
21 days	Control	3**/++	0.02
	Ketac	2**	
	E. Fil	2	
	E. Forte	1.5++	
63 days	Control	2	0.009
	Ketac	1	
	E. Fil	1**	
	E. Forte	2**	

Same symbols indicate a statistically significant difference (p>0.05).

At 7 days, it was not possible to detect statistically significant difference among groups (p>0.05).

At 21 days, there was statistically significant difference between Control and Ketac groups (p=0.04) and between Control and E. Forte groups (p=0.02). Control group presented the highest scores for collagen fiber formation in both cases, i.e., more advanced stage in the repair process.

At 63 days, there was statistically significant difference between the groups E. Fil and E. Forte (p=0.03). E. Forte presented the highest scores for fibrosis, i.e., more advanced stage in the repair, compared to E. Fil group.

Inflammatory Infiltrate. Table 2 shows the median of inflammatory infiltrate obtained after the analysis of the granulomatous reactionary tissue in contact with the materials or polyethylene tube.

Regarding the inflammatory infiltrate, in all experimental periods it was not possible to detect statistically significant difference among groups (p>0.05).

Figure 5 shows the results obtained after the semi-quantitative evaluation of the tissues in contact with each cement or empty polyethylene tube, in the periods of 7, 21 and 63 days.

Table 3 shows the data obtained after the measurement

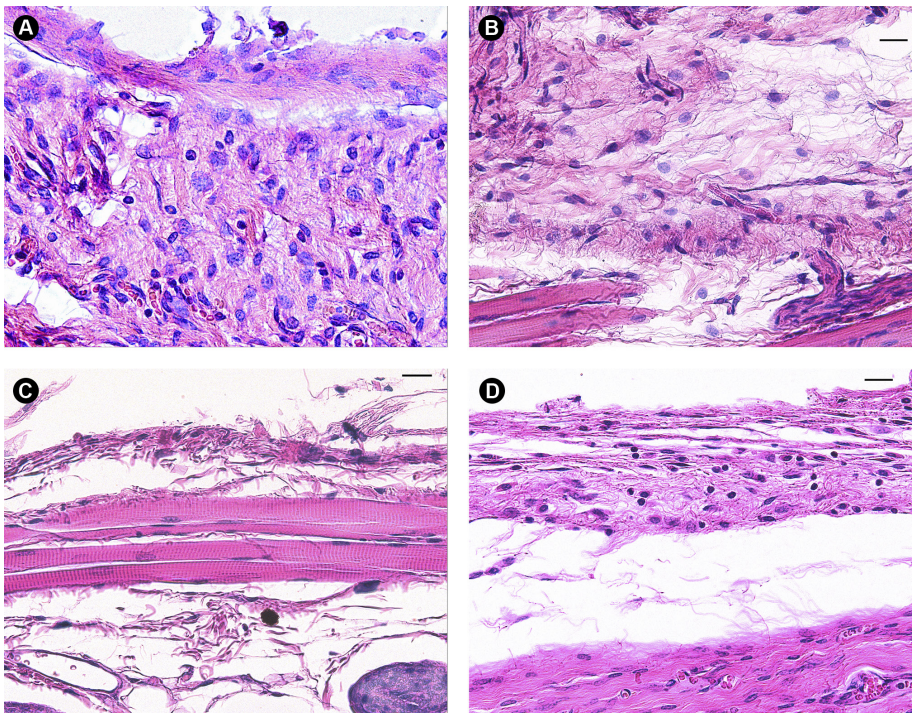

Figure 4. Microscopic analysis at 63 days. A: Control: reactionary tissue infiltrated by neutrophils with disorganized collagen fibers and prominent macrophages and early fibroblasts. B: Equia Forte: reactionary tissue with individualized but discreetly disorganized collagen fibers, occasional neutrophils, and some skeletal muscle fibers. C: Equia Fil: thin-thickness reactionary tissue with moderate collagen fiber formation and occasional macrophages on skeletal muscle layer. Occasional subjacent hair follicles. D: Ketac: well-organized reactionary tissue with dense collagen fibers and occasionally infiltrated by neutrophils, macrophages and fibroblasts (HE, Zeiss, 40×).

of the granulomatous reactionary tissue in contact with the materials or polyethylene tube.

At 7 days, there was statistically significant difference between Control and E. Forte groups (p=0.001). The thickness of the granulomatous reactionary tissue was greater in the E. Forte group.

At 21 days, there was significant difference between Control and Ketac groups. Ketac group presented the specimens with the greatest thickness of the reaction tissue. There was also significant difference (p=0.008) between Control and E. Forte groups, with E. Forte presenting the highest values.

At 63 days, it was possible detect significant difference between Control and E. Forte groups (p=0.02). The specimens in the E. Forte group presented the highest values of thickness of the reactionary tissue.

Table 2. Median, and p-value regarding Inflammatory infiltrate in the periods of 7, 21 and 63 days

Period	Group	Median	p-value
7 days	Control	2	0.29
	Ketac	2	
	E. Fil	2	
	E. Forte	2.5	
21 days	Control	2	0.052
	Ketac	1	
	E. Fil	2	
	E. Forte	1	
63 days	Control	1.5	0.59
	Ketac	1	
	E. Fil	1	
	E. Forte	1	

Table 3. Median, and p-value regarding reaction tissue thickness in the periods of 7, 21 and 63 days

Period	Group	Median	p-value
7 days	Control	0.020[a]	0.001
	Ketac	0.070[ab]	
	E. Fil	0.055[ab]	
	E. Forte	0.220[b]	
21 days	Control	0.030[a]	0.008
	Ketac	0.060[b]	
	E. Fil	0.050[ab]	
	E. Forte	0.065[b]	
63 days	Control	0.030[a]	0.02
	Ketac	0.070[ab]	
	E. Fil	0.070[ab]	
	E. Forte	0.095[b]	

*Different superscript letters indicate statistically significant difference in the comparison among groups (p<0.05).

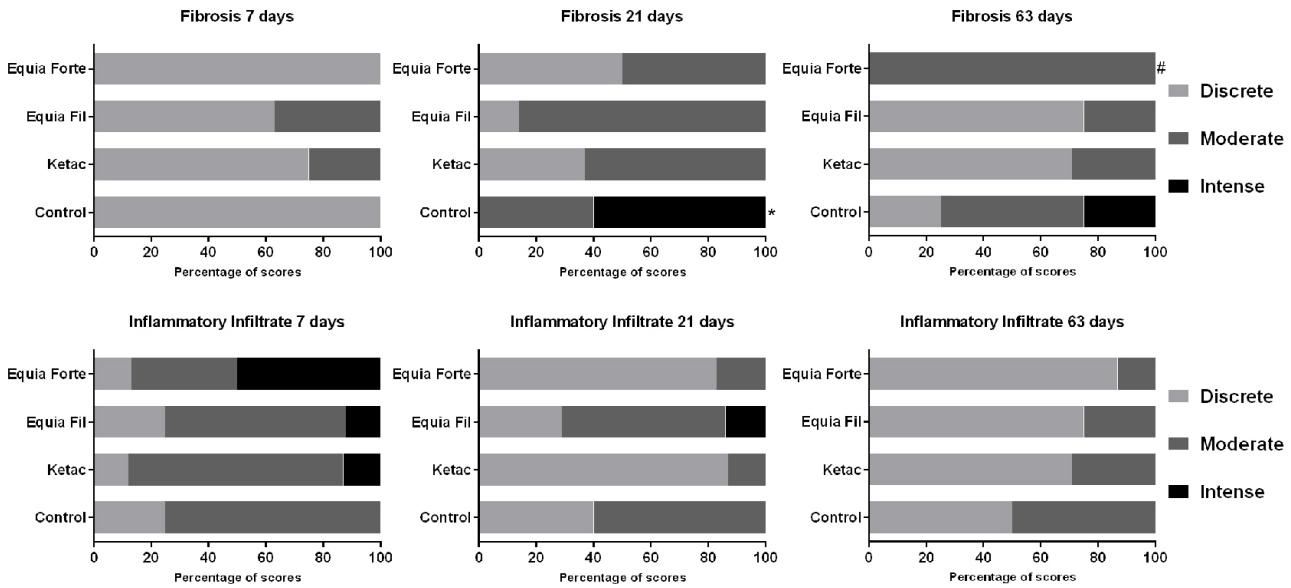

Figure 5. Percentage of scores and results obtained after the semi-quantitative evaluation of the tissue reaction in contact with each material at 7, 21 and 63 days. * = Statistically significant difference between control and Ketac groups, and between control and Equia Forte groups (p<0.05). # = Statistically significant difference between Equia Forte and Equia Fil groups (p<0.05).

Discussion

The null hypothesis was accepted. This study showed that the tissue response varied according to the parameters evaluated, the materials tested and the different experimental periods. However, all them presented tissue compatibility. Initially, the collagen fiber formation was not different among the tested materials. Nevertheless, it was different at 21 days, being the Control the highest severity. At the end of the experiment the E. Forte group showed greater collagen fiber formation compared to E. Fil group. On the other hand, the inflammatory infiltrate showed no difference among the materials in any tested period. The thickness of the granulomatous reactionary tissue was greater for E. Forte group compared to control, in all periods.

GIC-based cements are extremely versatile materials, since can be used for crowns, prostheses and orthodontic bands cementation, in restorations, pulp capping, and also as sealant of fissures (16). Additionally, GIC is one of the main materials of choice when the minimal intervention philosophy is employed (17). In this philosophy, GIC is mainly used due to many beneficial properties inherent to this material. In the oral cavity, the release of fluoride ions benefits the remineralization, making GIC a good material for clinical use (18). In general, GICs present good tissue biocompatibility and adhesion to dental tissues, which enhances its indications (8).

Several methodologies have been applied to evaluate the biological compatibility of dental materials (19), including the response of subcutaneous connective tissue after implantation in animal models. This methodology is based mainly on the evaluation of the tissue reaction, emphasizing the characteristics of collagen fibers and inflammatory infiltrate by using qualitative and quantitative analyses (15,20). The scores levels for collagen fiber formation and inflammatory infiltrate were based on Queiroz et al. (15). Originally, the paper presented 4 different levels for both analysis: 0 absent, 1 discrete, 2 moderate and 3 intense. However, after the results analysis, was observed that all the experimental materials promoted some tissue reaction. In order to be more faithful to these results, the level 0, absent of collagen fiber formation or inflammatory infiltrate, were removed from our analysis.

The histopathological analysis of the subcutaneous tissue response after different experimental periods includes the tissue description after contact with a potential irritant and also the duration of its effect on the tissue (21). Although this methodology does not faithfully reproduce the characteristics of dental and oral tissues, it offers important preliminary data regarding the biological properties of dental materials and the characteristics of the tissue reaction (22). The tissue reaction observed is a consequence of the host's response to the materials implanted in the subcutaneous tissues of isogenic mice. Inflammatory and resident cells produce and release many cytokines and growth factors, which result in a complex cascade involved in the tissue response (21).

According to the microscopic results showed in this study, initially the fibrosis was not different among the tested materials. However, there was difference in the thickness of the reaction tissue between the control and E. Forte groups, the latter with the highest values. At 21 days, the fibrosis was different and the control group presented the most advanced stage of fibrosis compared to Ketac and E. Forte groups. These results are in agreement with the data of the thickness of the reaction tissue, where the difference between control and Ketac and Control and E. Forte groups was observed. The control group showed the lowest values. This means that the reaction tissue in the control group was more organized with respect to collagen fibers formation, with better biological results and more advanced repair process. At the end of the experiment, E. Forte group presented the more advanced degree of fibrosis compared to E. Fil group. With respect to the thickness of the reaction tissue, there was difference between the control and E. Forte groups, the latter showing the highest values. On the other hand, the inflammatory infiltrate showed no difference among the tested materials.

Study the tissue response after placed any material in dental cavity is extremely important, specially because the teeth are interconnected by the pulp-dentin complex. Although the significant number of dentinal tubules (approximately 75,000 tubules/cm^2) being closer to the pulp, the superficial dentin also presented 20,000 tubules/cm^2 (23). All the tested GICs presented at the end tissue compatibly. The direct comparison of our results with the literature becomes impossible due to the absence of studies evaluating the tissue response after implantation of the evaluated materials. However, Gurgan et al. (24) observed that Equia Fil showed acceptable clinical performance, including postoperative sensitivity, when used in Class 1 and Class 2 cavities over the course of four years.

Thus, additional studies are necessary, including with other experimental models, to evaluate the response of pulp and periapical tissues after the use of these materials, providing evidences for their safe clinical indication.

Based on the results obtained, it was concluded that the GIC-based cements presented tissue compatibility, according to the different evaluated parameters.

Resumo

O objetivo deste estudo foi avaliar a resposta subcutânea do tecido conjuntivo de camundongos isogênicos após o implante de diferentes cimentos à base de ionômero de vidro (EQUIA® Forte Fil, EQUIA® Fil e Ketac

™ Universal Aplicap ™). Oitenta e sete camundongos isogênicos BALB/c foram alocados em 12 grupos, 9 como grupos experimentais (Ketac, E. Fil e E. Forte aos 7, 21 e 63 dias) e 3 controles (tubos de polietileno vazios aos 7, 21 e 63 dias). Após os períodos experimentais, o tecido conjuntivo subcutâneo ao redor do material implantado foi removido e submetido ao processamento histotécnico e coloração com hematoxilina e eosina. Uma descrição histopatológica da reação tecidual envolvendo cada material e uma análise semi-quantitativa da fibrose e infiltrado inflamatório foram realizadas. Além disso, foi realizada a mensuração da espessura do tecido granulomatoso em contato com cada material. Os dados foram analisados estatisticamente (α=0,05) pelo teste de Kruskal-Wallis, seguido do pós-teste de Dunn. Inicialmente, a fibrose não foi diferente entre todos os materiais testados (p>0,05), mas foi diferente aos 21 dias, com o grupo controle apresentando o estágio mais avançado de fibrose. Aos 63 dias, o grupo EQUIA® Forte Fil apresentou o estágio mais avançado de fibrose, comparado ao grupo EQUIA® Fil (p<0,05). O infiltrado inflamatório não foi diferente entre os materiais testados em nenhum período experimental (p>0,05). A espessura do tecido granulomatoso foi maior no grupo E. Forte, comparado ao controle em todos os períodos. Todos os cimentos à base de ionômero de vidro apresentaram compatibilidade tecidual, de acordo com os parâmetros avaliados.

Acknowledgments

The authors deny any conflicts of interest related to this study.

References

1. Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ. Minimal intervention dentistry-a review. FDI Commission Project 1-97. Int Dent J 2000;50:1-12.
2. Dalli M, Colak H, Mustafa Hamidi M. Minimal intervention concept: a new paradigm for operative dentistry. J Investig Clin Dent 2012;3:167-175.
3. Featherstone JD, Domejean S. Minimal intervention dentistry: part 1. From 'compulsive' restorative dentistry to rational therapeutic strategies. Br Dent J 2012;213:441-445.
4. Wang Y, Mei L, Gong L, Li J, He S, Ji Y, et al. Remineralization of early enamel caries lesions using different bioactive elements containing toothpastes: An in vitro study. Technol Health Care 2016;24:701-711.
5. Gao W, Smales RJ, Yip HK. Demineralisation and remineralisation of dentine caries, and the role of glass-ionomer cements. Int Dent J 2000;50:51-56.
6. Moraschini V, Fai CK, Alto RM, Dos Santos GO. Amalgam and resin composite longevity of posterior restorations: A systematic review and meta-analysis. J Dent 2015;43:1043-1050.
7. Dionysopoulos D, Koliniotou-Koumpia E, Helvatzoglou-Antoniades M, Kotsanos N. Fluoride release and recharge abilities of contemporary fluoride-containing restorative materials and dental adhesives. Dent Mater J 2013;32:296-304.
8. Tamilselvam S, Divyanand MJ, Neelakantan P. Biocompatibility of a conventional glass ionomer, ceramic reinforced glass ionomer, giomer and resin composite to fibroblasts: in vitro study. J Clin Pediatr Dent 2013;37:403-406.
9. Gorseta K, Glavina D. Thermo-cured glass ionomer cements in restorative dentistry. J Istanb Univ Fac Dent 2017;51:122-127.
10. Alrahlah A. Diametral Tensile Strength, Flexural Strength, and Surface Microhardness of Bioactive Bulk Fill Restorative. J Contemp Dent Pract 2018;19:13-19.
11. Friedl K, Hiller KA, Friedl KH. Clinical performance of a new glass ionomer based restoration system: a retrospective cohort study. Dent Mater 2011;27:1031-1037.
12. Al-Angari SS, Hara AT, Chu TM, Platt J, Eckert G, Cook NB. Physicomechanical properties of a zinc-reinforced glass ionomer restorative material. J Oral Sci 2014;56:11-16.
13. Gaintantzopoulou MD, Gopinath VK, Zinelis S. Evaluation of cavity wall adaptation of bulk esthetic materials to restore class II cavities in primary molars. Clin Oral Investig 2017;21:1063-1070.
14. van Duinen RN, Shahid S, Hill R, Glavina D. In-vitro Study on Temperature Changes in the Pulp Chamber Due to Thermo-Cure Glass Ionomer Cements. Acta Stomatol Croat 2016;50:287-291.
15. Queiroz AM, Assed S, Consolaro A, Nelson-Filho P, Leonardo MR, Silva RA, et al. Subcutaneous connective tissue response to primary root canal filling materials. Braz Dent J 2011;22:203-211.
16. Tay WM, Lynch E. Glass-ionomer (Polyalkenoate) cements. Part 1. Development, setting reaction, structure and types. J Ir Dent Assoc 1989;35:53-57.
17. Sidhu SK, Nicholson JW. A review of glass-ionomer cements for clinical dentistry. J Funct Biomater 2016;7:pii:E16.
18. ten Cate JM, van Duinen RN. Hypermineralization of dentinal lesions adjacent to glass-ionomer cement restorations. J Dent Res 1995;74:1266-1271.
19. Thomas B, Gupta K. In vitro biocompatibility of hydroxyapatite-added GIC: An SEM study using human periodontal ligament fibroblasts. J Esthet Restor Dent 2017;29:435-441.
20. Boaventura JM, Bertolini MJ, Padovani GC, de Oliveira MR, Zaghete MA, de Oliveira Junior OB, et al. Tissue response to experimental dental cements prepared from a modified powder glass composition. Dent Mater J 2012;31:583-592.
21. Andrade AS, Silva GF, Camilleri J, Cerri ES, Guerreiro-Tanomaru JM, Cerri PS, et al. Tissue Response and Immunoexpression of Interleukin 6 Promoted by Tricalcium Silicate-based Repair Materials after Subcutaneous Implantation in Rats. J Endod 2018;44:458-463.
22. Viola NV, Guerreiro-Tanomaru JM, da Silva GF, Sasso-Cerri E, Tanomaru-Filho M, Cerri PS. Biocompatibility of an experimental MTA sealer implanted in the rat subcutaneous: quantitative and immunohistochemical evaluation. J Biomed Mater Res B Appl Biomater 2012;100:1773-1781.
23. Costa CA, Hebling J, Hanks CT. Current status of pulp capping with dentin adhesive systems: a review. Dent Mater 2000;16:188-197.
24. Gurgan S, Kutuk ZB, Ergin E, Oztas SS, Cakir FY. Four-year randomized clinical trial to evaluate the clinical performance of a glass ionomer restorative system. Oper Dent 2015;40:134-143.

Received February 19, 2019
Accepted June 29, 2019