https://dx.doi.org/10.22161/ijaers.712.41 *ISSN: 2349-6495(P) | 2456-1908(O)*

Evaluation of the cytotoxicity of two endodontic cements based on calcium silicate and Pulp Canal Sealer cement in human fibroblasts

Leonardo Lemos Andrade, Carlos Eduardo da Silveira Bueno, Claudia Fernandes de Magalhães Silveira, Eduardo Fernandes Marques, Camila Paiva Macedo, Diana Leão Rodrigues Frota, Alexandre Sigrist De Martin

Received: 01 Oct 2020; Received in revised form: 27 Oct 2020; Accepted: 03 Nov 2020; Available online: 29 Dec 2020

Abstract**—** *During the gutta-percha filling stage, the endodontic cement can extend beyond the apex, interacting directly with the periapical tissues. Thus, endodontic cements should exhibit several positive biological characteristics, such as cytocompatibility. The objective of this study was to compare the cytotoxicity of two bioceramic endodontic cements, Bio-C Sealer and TotalFill BC Sealer, and of the cement and Pulp Canal Sealer based on zinc oxide and eugenol. For this purpose, human gingival fibroblasts (FG11 and FG15) were submitted to the cell culture medium conditioned by the cements (TFBCS Group: TotalFill BC Sealer Cement; BCS Group: Bio-C Sealer Cement; PCS Group: Pulp Canal Sealer Cement), and cell viability was evaluated using the MTT assay. The test was performed 48 and 72 hours after contact with the cement extracts (1:5 dilution). Cells cultivated in DMEM medium served as control. Descriptive analysis and data submitted to the ANOVA and Tukey's test (P <0.05) were performed using the SOSS 23 program (SPSS INC., CHICAGO, IL, USA). The results showed that the cultures submitted to the two endodontic bioceramic cements presented greater viability in relation to Pulp Canal Sealer cement (P <0.05), for both evaluation times. Within the limitations of the methodology, it could be concluded that bioceramic cements Bio-C Sealer and TotalFill BC Sealer presented higher cytocompatibility compared to Pulp Canal Sealer, for the times of 48 and 72h.*

Keywords**—** ***Bioceramic endodontic cement, Cell culture, Human gingival fibroblasts, Cytocompatibility*.**

I. INTRODUCTION

The success of the endodontic treatment is achieved by eliminating microorganisms from the root canal system, followed by an appropriate sealing with the obturator materials [1]. Endodontic cement is essential to seal the space between the dental wall and the obturator material, also representing the lubricating agent for filling the root canal system. During the filling stage, the perirradicular tissues may come into contact with the endodontic cements, mainly by extrusion besides the apical foramen [2]. Thus, such cements should be biocompatible and non-cytotoxic to perirradicular tissues [3].

Currently, different types of endodontic cements are available: zinc oxide eugenol, resin based, containing calcium hydroxide, MTA and bioceramic based cements [4-7]. Eugenol zinc oxide based cements have a long history of successful use due to their widely demonstrated positive qualities; however, they have been shown to be cytotoxic, which has been attributed to the eugenol present in different formulations [8,9].

Recently, dicalcium silicate and tricalcium-based cements have received significant attention due to their favorable physicochemical and biological properties [10-13]. These cements present high pH, allow the release of Calcium ions and are able to form hydroxyapatite during the setting process, interacting with the dentin (infiltration of the mineral content of the cement based on bioceramics in the intertubular dentin) and forming the so-called zone of mineral infiltration [15,16].

TotalFill BC Sealer (FKG Dentaire SA, La-Chaux-de-fonds, Switzerland) is another cement based on calcium silicate that has demonstrated good physical and biological

https://dx.doi.org/10.22161/ijaers.712.41 ISSN: 2349-6495(P) | 2456-1908(O)

properties, with the capacity to release calcium ions [2]. It is composed of dicalcium silicate, tricalcium silicate, calcium hydroxide, monobasic calcium phosphate, zirconium oxide, tantalum oxide, fillers and thickeners [12-14].

Bio-C Sealer cement (Angelus, Londrina, PR, Brazil) is a ready-to-use bioceramic cement containing calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide and dispersing agent in its composition. According to the manufacturer, its bioactivity is attributed to the release of calcium ions that stimulate the formation of mineralized tissue [7]. However, until now, there are few studies evaluating its effects on periapical tissues and related cells [17].

Obturator cements should be tested for their biological properties, comprehensively and independently, by *in vitro* and *in vivo* tests, before their unlimited clinical use, in order to minimize the incidence of local and/or systemic adverse effects [2,7,10,]. From the clinical point of view, there are clear limitations in the correlation between in vitro data and clinical behavior. However, in vitro cytotoxicity tests are important to understand the biological risks of these materials [10,11,20]. This is the first study that evaluated the cytocompatibility of Bio-C Sealer cement by means of cell proliferation and viability tests in human fibroblasts.

The objective of this study was to investigate the cytocompatibility of Bio-C Sealer and TotalFill BC Sealer bioceramic cements compared with Pulp Canal Sealer cement. The null hypothesis was that there would be no difference in cytocompatibility between the tested cements.

II. MATERIALS AND METHODS

The study protocol (No. 3141789) was approved by the Research Ethics Committee of the São Leopoldo Mandic School of Dentistry, Campinas, São Paulo, Brazil (CAAE 99349218.0.0000.5374).

2.1 Cell culture

Two cell lines from FG11 and FG15 human fibroblasts cultures were obtained from the cell bank. These cells were thawed and transferred to centrifuge tubes containing 10 mL of DMEM (Sigma, St. Louis, MO, USA) and centrifuged at 336 g (grams) for 3 minutes. The supernatant was discarded and the cells were cultivated in 75 cm2 (square centimeters) culture vials (Corning Incorporated, Costar, Corning, New York, NY, USA) containing DMEM (Sigma) supplemented with 15% bovine fetal serum (Gibco), Invitrogen, Grand Island, New York, NY, USA), 100 IU/mL (international unit/ milliliters) penicillin (Invitrogen) and 50 µg/mL (microgram/militers) streptomycin (Invitrogen). In the subfluence, the culture medium was removed and 0.25% trypsin solution (Gibco) and 1 mM EDTA (millimeters) (Gibco) were added to obtain cell suspension. Next, 110 cells/mm2 (milliliter squared) were plated (in 24-well polystyrene plates (Corning Incorporated) and cultivated at McCoy's 5A (Sigma) supplemented with 10% bovine fetal serum (Gibco), 7 mM of β-glycerophosphate (Sigma), 5 µg/mL of ascorbic acid (Gibco), 100 IU/mL of penicillin (Invitrogen) and 50 µg/mL streptomycin (Invitrogen). The cultures were maintained for periods of up to 14 days and their progression was evaluated under an inverted phase microscope (Nikon, Eclipse TS100). The culture medium was changed every 3 days. During the whole culture time the cells were kept at 37ºC in a humidified atmosphere containing 5% of CO2 (carbonic gas) and 95% of atmospheric air.

2.2 Conditioned medium

The endodontic cement Pulp Canal Sealer was handled at room temperature (25°C), following the instructions of its manufacturer. Samples of the handled cement and ready-to-use cements were obtained using silicone devices of 6 mm in diameter and 2 mm in height. After the prey reaction, the specimens were weighed, sterilized in ethylene oxide and kept in basal culture medium (DMEM, 15% fetal bovine serum and 1% antibiotic-antimycotic), for 24 h in an oven at 37oC, obtaining the conditioned medium.

2.3 Experimental groups and control group

Four groups were outlined, as follows:

- Control group (GC): cells cultivated in fresh medium (DMEM);
- TotalFill BC Sealer Cement Group (TFBCS): cells cultivated in environment conditioned by TotalFill BC Sealer Cement;
- Bio-C Sealer Cement Group (BCS): cells cultivated in a conditioned medium by the Bio-C Sealer;
- Pulp Canal Sealer Cement Group (PCS): cells cultivated in a conditioned medium by the Pulp Canal Sealer cement.

After this period, the plated cells (density of 110 cells/mm2) were supplemented with the conditioned medium in the proportion of 0.2 g/mL (ISO 10993), for the experiments described below.

https://dx.doi.org/10.22161/ijaers.712.41 *ISSN: 2349-6495(P) | 2456-1908(O)*

2.4 Cytocompatibility evaluation

Cell viability analysis was performed by colorimetric assays with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay). 110 cells per mm² were used in each well of the 96 thermometer wells, incubated with the tested substances for 48 and 72 hours, at 37ºC. Immediately after, 10 µl of MTT solution (5 mg/mL - SIGMA) diluted in DMEM culture medium without serum was placed, adding the treated cultures and these incubated for a period of 4 hours at 37 ºC. After this incubation period 100µl of 10% sodium dodecyl sulfate (SDS) and 0.01N hydrochloric acid solution were added and the experiment maintained for 1 hour at 37ºC. The mitochondrial activity of the cells indicates their viability by means of an optical analysis (Optical Density - OD) [41]. For this study, this quantification was performed by a multiplate reader ELX800 (Epoch biotek instruments, inc.) at 570 nm.

2.5 Statistical analysis

Shapiro-Wilk's normality test showed a sample of normal distribution. Thus, descriptive analysis and data submitted to Tukey's ANOVA and test (P <0.05) were performed using the SPSS 23 program (SPSS INC., CHICAGO, IL, USA).

III. RESULTS

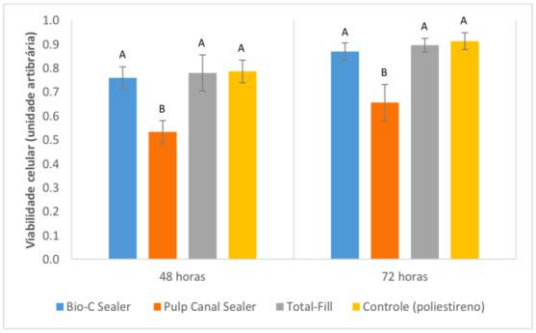
Regarding cell viability, the cultures submitted to the two endodontic bioceramic cements presented higher viability in relation to the PCS group, the times 48 hours (p = 0.001) and 72 hours (p < 0.001), not differing significantly from the GC (Table 1 and Graph 1).

Table.1: Average values and standard deviations of cellular viability according to group and time interval

Group	Time	
	48 hours	72 hours
Bio-C Sealer	0,76 A (0,05)	0,87 A (0,04)
Pulp Canal Sealer	0,53 B (0,05)	0,66 B (0,05)
Total-Fill	0,78 A (0,08)	0,90 A (0,03)
Control (polystyrene)	0,79 A (0,05)	0,91 A (0,04)

Note: Standard deviation in brackets. Averages followed by equal letters indicate no statistically significant difference between groups within each time interval.

Graph.1: Line diagram of cell viability according to group and time interval



IV. DISCUSSION

The recognition of the need to use endodontic cements is a fact recognized in the literature [1]. However, it is valid to emphasize that these materials can be extruded to the perirradicular tissues through their communication with the root canal system, delaying the healing of these areas [21,22]. With this knowledge, it becomes evident the necessity of studies that analyze the cytocompatibility of these obturator cements by means of methodologies that can evaluate their cytotoxic behaviour, thus observing the viability of their use [23,24].

For the evaluation of the biological behavior of cements, there is a need for the use of in vitro cell culture [3]. Thus, the relevance of the use of human fibroblasts, which have the ability to simulate an in vivo tissue response, is observed [25,26].

Moreover, the moment of these evaluations becomes somewhat significant, since, in clinical practice, endodontic cements are inserted in the root canal soon after their manipulation, or even, for cements in ready-to-use form, they take some time for their final prey, when they present a higher degree of cytotoxicity [27]. However, evaluations in other periods after manipulation become pertinent for monitoring possible changes in their biological behavior [7,17].

Several methodologies were recommended to evaluate in vitro the biological effects of sealing cements [2,10,16-19]. The MTT assay is capable of measuring the metabolic activity of living cells, representing a simple, reproducible and precise technique [2,7,12]. However, it is important that the majority of cells are in exponential growth phase, being pertinent to validate and complement the results of the MTT assay with other methods that can evaluate cellular structural viability, apoptosis and/or cellular necrosis [28].

https://dx.doi.org/10.22161/ijaers.712.41 ISSN: 2349-6495(P) | 2456-1908(O)

There are publications regarding the in vitro cytotoxicity of several endodontic obturator cements [30-32]. Some so-called conventional cements demonstrated inadequate biological activity and high cytotoxicity in the culture, especially soon after its manipulation [33,34]. Bioceramic materials have been considered promising materials for the repair of mineralized tissues due to their excellent physical-chemical properties and biocompatibility [3,7,11,18]. The favorable biological activity of bioceramic cements may be associated with alkaline pH, higher release of Ca2+ ions and hydroxyapatite formation, as demonstrated in previous studies [10,11,20].

This study aimed to evaluate the cytocompatibility of two bioceramic cements by means of a cell viability test, in two distinct times. For these evaluations, an endodontic cement based on zinc oxide and eugenol was used for comparison. Based on the results obtained, the null hypothesis was partially rejected, since there was a difference in cytocompatibility between the tested cements, for the times of 48 and 72 hours.

From the cytocompatibility perspective evaluated by the MTT colorimetric assay, both bioceramic cements presented higher cellular viability in comparison to Pulp Canal Sealer cement, for 48 and 72h times. López-Garcia *et al.* [17] evaluated the cytocompatibility of TotalFill BC Sealer, Bio-C Sealer and AH Plus cements against human periodontal ligament stem cells, using the MTT assay, at three distinct times (24, 48 and 72 h). TotalFill BC Sealer and Bio-C Sealer were significantly less cytotoxic than AH Plus at all dilutions and for all times tested. TotalFill BC Sealer also demonstrated cytocompatibility in human periodontal ligament cells [2] and fibroblasts [35], revealing the biocompatibility of calcium silicate based cements with zirconium oxide [16,36-38].

The PCS group presented statistically inferior cellular viability in relation to all the other groups in the times of 48 and 72 hours. The Pulp Canal Sealer EWT cement demonstrated to be very cytotoxic after 24 hours in cell culture studies [10]. However, it was reported that the same cement produces a better tissue organization after subcutaneous implantation in rat connective tissues [39]. The difference may be related to the manner in which the extracts were presented to the cells and dilutions of the cements. Moreover, the severe in vitro cytotoxicity associated with zinc oxide based cements [24] was not apparent in a recent clinical study [40]. Thus, the results of in vitro cytocompatibility studies should be interpreted with caution. Da Silva *et al.* [35], using a three-dimensional (3D) cell culture model and the MTT assay, found that EndoSequence BC Cement (Brasseler, Savannah, GA, USA) presented lower cytotoxicity compared to Pulp Canal Sealer cement. Poggio *et al.* [12] compared the cytotoxic effects of eight obturator cements (BioRoot RCS, TotalFill BC Sealer, MTA Fillapex, Sealapex, AH Plus, EasySeal, Pulp Canal Sealer, N2) in immortalized human gingival fibroblasts for a period of 24, 48 and 72 hours. The authors verified that the TotalFill BC Sealer cement, in 24h, did not present cytotoxic effect, presenting only mild cytotoxicity in 48 and 72h; Pulp Canal Sealer cement presented moderate cytotoxic activity in all tested times.

Further investigations are required using different in vitro and in vivo models to validate possible biological responses to calcium silicate endodontic cements.

V. CONCLUSION

Within the limitations of the present study, it was possible to conclude that bioceramic cements Bio-C Sealer and TotalFill BC Sealer presented higher cytocompatibility compared to Pulp Canal Sealer, for the times of 48 and 72h.

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