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# Biocompatibility analysis of MTA, Portland cement and modified Portland cement on cultured fibroblast cells and subcutaneous tissue

## *Análise da biocompatibilidade do MTA, cimento de Portland e cimento de Portland modificado em cultura de fibroblastos e no tecido subcutaneo*

Kristianne Porta Santos Fernandes[1]; Elaine Marcílio Santos[2]; Salua Haidar Redá[3]; Lara Jansiski Motta[4]; Sandra Kalil Bussadori[5]; Victor Perez Teixeira[6]; Raquel Agnelli Mesquita-Ferrari[7]; Manoela Domingues Martins[8]

[1] PhD in Immunology – ICB -USP, Professor of Rehabilitation Science and Odontology – Uninove. São Paulo, SP – Brasil.
[2] PhD in Odontopediatrics- FOUSP, Professor of Odontology – Unicastelo. São Paulo, SP – Brasil.
[3] Specialist in Odontopediatrics, Professor of Odontology – UBC. Mogi das Cruzes, SP – Brasil.
[4] Master in Rehabilitation Science - Uninove, Professor of Odontology – Uninove. São Paulo, SP – Brasil.
[5] PhD in Odontopediatrics – FOUSP, Professor of Rehabilitation Science – Uninove. São Paulo, SP – Brasil.
[6] Specialist in Oral and Maxillofacial Surgery, Student of Postgraduation in Rehabilitation Science – Uninove. São Paulo, SP – Brasil.
[7] PhD in Physiology - UFSCAR, Professor of Rehabilitation Science – Uninove. São Paulo, SP – Brasil.
[8] PhD in Pathology – FOUSP, Professor of Rehabilitation Science – Uninove. São Paulo, SP – Brasil.

**Postal address**
Kristianne Porta Santos Fernandes
Curso de Mestrado em Ciências da Reabilitação
Av. Francisco Matarazzo, 612, Barra Funda
05001-100 São Paulo – SP [Brasil]
Telefax: +55-11- 3665-9325
kristianneporta@gmail.com

**Abstract**

Objectives: This study evaluated the biocompatibility in vivo and in vitro of MTA (Pro-Root®), Portland cement and modified Portland cement (gypsum added). Method: For the in vivo analysis, polyethylene tubes were implanted subcutaneously in rats. After 7, 14, 30 and 60 days the tissue specimens were prepared for histological examination. For cytotoxic analysis the materials were placed in contact with NIH-3T3 cells. After 1, 3, 5 and 7 days, the cells viability was analyzed. Results: The histological analysis showed moderate inflammatory response at 7 days in all groups. After 14 days the control group, MTA and Portland cement showed a mild inflammatory process while modified Portland group showed moderate inflammatory process. After 30 and 60 days all materials showed scarce inflammatory infiltrate and fibrosis. All the substances permitted the cell growth throughout the 7 days of experiment and presented similar cell viability. Conclusions: According to these experimental conditions, all the tested materials were biocompatible.

**Key words:** Biocompatibility; Cytotoxicity; MTA; Portland cement.

**Resumo**

Objetivos: Este estudo avaliou a biocompatibilidade in vivo e in vitro do MTA (Pro-Root®), do cimento de Portland e do cimento de Portland modificado (com adição de gesso). Método: Para a análise in vivo, tubos de polietileno foram implantados subcutaneamente em ratos. Após 7, 14, 30 e 60 dias os esécimes teciduais foram preparados para análise histológica. Para a análise da citotoxicidade os materiais foram colocados em contato com células NIH-3T3. Após 1, 3, 5 e 7 dias a viabilidade celular foi avaliada. Resultados: A análise histológica mostrou moderada resposta inflamatória após 7 dias em todos os grupos. Após 14 dias, o grupo controle, o grupo MTA e o grupo do cimento de Portland modificado exibiram uma resposta inflamatória suave enquanto que o grupo do cimento de Portland exibiu um processo inflamatório moderado. Após 30 e 60 dias todos os materiais exibiram um infiltrado inflamatório escasso e fibrose. Todas as substâncias testadas permitiram o crescimento celular durante os 7 dias do experimento e demostraram viabilidade celular similar. Conclusões: De acordo com estas condições experimentais, todos os materiais testados são biocompatíveis.

**Descritores:** Biocompatibilidade; Citotoxicidade; MTA; Cimento de Portland.

# Introduction

MTA has been indicated in many clinical situations as: apexification, pulpotomy, pulp-capping, root-end fillings and repairing of root perforations[1,2,3,4,5,6].

The main components of MTA are tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide. Beyond these components, small portions of substances can be added in order to assist the physical and chemical properties of the aggregate (as bismuth). The essential ions in this material are calcium and phosphorous, those are also the components of dental mineralized tissues[7,8].

As MTA and Portland cement are almost identical macroscopically, microscopically and chemically[9,10] and behave in very similar way in rat subcutaneous, osseous tissue of guinea pigs, pulpal tissue, and in cell culture[11,12,13,14,15].

The main disadvantage when using MTA as a restorative material is primarily due to its long setting time. So, the improvement of setting time is a significant step in the development of Portland cement as a restorative material. Accelerated Portland cements have been created and evaluated[16,17].

Recently, it was reported that gypsum addition reduced the setting time of Portland cement from 101,26 ± 0,02 to 6,6 ± 2,07 minutes[18]. However, with the addition of gypsum to Portland cement, there is a possibility that the biocompatibility might be adversely affected.

The aim of this study was to compare the biocompatibility and the cytotoxicity of ProRoot MTA® (MTA) with Portland cement (PC) and gypsum added Portland cement (PCG).

# Materials and methods

## Experimental groups

The materials were prepared as follows:

Group I (GI): Portland cement (Cimento Votoran®, São Paulo - Brazil) refined (710 mm/ μm), sterilized and mixed with distilled water (0,20g).

Group II (GII): ProRoot MTA® (Dentisply Tulsa Dental, Oklahoma – USA) mixed according to the manufacturer' instructions.

Group III (GIII): gypsum (Marquart e Cia Ltda, São Paulo – Brazil) added Portland cement (0,0599g /1,0001g) mixed with distilled water (0,20g) as previously described (18).

Group IV (GIV): control – empty tube *(in vivo*) or plain round coverslips (*in vitro*)

## In *vivo* assay methods

Twenty rats (wistar lineage) weighing 250 to 300g were used in this study. There was one group of five animals each for an experimental period of 7, 14, 30 and 60 days.

Polyethylene tubes (Sondaplast®, São Paulo- Brazil), 10 mm in length with an inner diameter of 1.5 mm, were washed with ethanol and distilled water and autoclaved before being filled with the cements. After ether inhalation, the animals were anesthetized by the administration of ketamine HCL and xylazine (40-80 mg/kg) intraperitoneally. The back of the animals was shaved and disinfected with 5% iodine in ethanol. Incisions (5 mm) were made in the dorsum, and four subcutaneous pockets were prepared by a blunt dissection.

A tube containing freshly mixed cement was placed into each pocket. Empty polyethylene tubes were used as the control. Finally, the incisions were closed with surgical nylon sutures. At the end of each period (7, 14, 30 and 60 days), the animals were sacrificed by overdose anesthesia and the tubes were removed along with the surrounding tissue and immersed in 10% buffered formalin. After fixing for 48 hours, the tissue was processed for paraffin embedding and serials sections were cut to a thickness of 3μm. The sections were stained with hematoxylin and eosin.

The tissue responses were graded as being mild, moderate, and severe according to the criteria already published[19, 20, 21].

The criteria for scoring the inflammatory tissue response are as follows: Grade 1 (no/slight inflammation): the thickness of the reaction zone is similar to or only slightly wider than the thickness along the side tube, no or a few inflammatory cells.

Grade 2 (moderate inflammation): an increased reaction zone in which macrophages, plasm cells, or both are present.

Grade 3 (severe inflammation): an increased reaction zone in which macrophages and plasm cells and occasional foci of neutrophil granulocytes, lymphocytes, or both are present.

Histopathological examination of the specimens was performed by two investigators jointly in a blind manner.

The results were analyzed statistically by Kruskal-Wallis test. The interpretations of the results were based on statistical analysis of the data to determine whether the material should be accepted or rejected as indicated by Federation Dentaire Internationale[22].

This study was conducted under approval of the Ethical Committee of Universidade Metropolitana de Santos (CEP-UNIMES n 030/2006).

## Cell survival assay

The toxicity of PCG, PC and MTA was measured *in vitro*. NIH-3T3 fibroblasts (ATCC CRL 1658), obtained from American Type Culture Collection (Rockville, MD, USA) were grown at 37°C in Dulbecco's Modified Eagle Medium (DMEM, Sigma Chemical Company, St Louis MO, USA) supplemented by 10% fetal bovine serum (Cultilab Ltda, Campinas, Brazil) and 1% antibiotic/antimycotic solution (Sigma Chemical Company, St Louis MO, USA) in a humid 5% $CO_2$ atmosphere. Cultures were supplied with fresh medium every other day. Cells between the fifth and 10th passages were used in all experimental procedures.

Fibroblasts ($1X10^4$) were plated on 60mm diameter culture dishes as previously described[23]. After 4h treated cultures received cover slips coated by freshly mixed cements (0,06g of material). Control cultures received plain cover slips. One, 3, 5 and 7 days after seeding the cells were counted and growth curves were plotted.

Growth curves were carried out as described elsewhere[23,24]. Briefly, cell counts were determined by counting the viable cells in a hemocytometer using the Trypan blue dye exclusion assay. For each time period, three dishes of each group were counted. The number of viable cells harvested from each Petri dish was obtained by the following mathematical equation: UC x D x $10^4$/nSQ, where UC = unstained cell count (viable cells), D = the dilution of cell suspension, and nSQ = number of squares of the hemocytometer counted.

Each data point corresponded to the mean ± SEM (standard error of the mean) of viable cell count from 3 dishes. The data were compared by test Kruskal–Wallis test. The level of significance was 5% ($p<0.05$).

# Results

The histopathological score means are summarized in table 1. There were no statisti-

**Table 1**: Grade 1: no/slight inflammation, Grade 2: moderate inflammation, Grade 3: severe inflammation

| Cement | Mean ± SD | | n = 5/wk | | Interpretation |
|---|---|---|---|---|---|
| | 7 days | 14 days | 30 days | 60 days | |
| Portland cement | 2.20 ± .71 | 1.80 ± .45 | 1.40 ± .55 | 1.20 ± .45 | Acceptable |
| ProRoot MTA® | 2.20 ± .45 | 1.40 ± .55 | 1.20 ± .45 | 1.20 ± .45 | |
| Gypsum + Portland cement | 2.20 ± .84 | 2.20 ± .45 | 1.40 ± .55 | 1.20 ± .45 | |
| control | 2.20 ± .45 | 1.60 ± .45 | 1.20 ± .45 | 1.10 ± .00 | |

cally significant differences among the groups ($p > 0.05$).

A slide from histological photomicrographs is showed on figure 1:

7 days - GI exhibited moderate inflammatory process and presence of neutrophil granulocytes (a). Slight inflammatory process with the predominance of lymphocytes associated to moderate fibrosis and angiogenesis on GII (b). GIII presented intense inflammatory process, focus of neutrophilic infiltrate associated to few fibroblasts and angiogenesis (c). Scarce inflammatory process, lymphocyte on GIV(d).

14 days - GI and GII exhibited slight inflammatory process, presence of lymphocytes, fibroblasts and angiogenesis (e, f). GIII showed moderate lymphocytes inflammatory (g). GIV (control group) revealed absence of inflammatory process, with well organization of fibrosis and angiogenesis (h).

30 days - Absence of inflammatory reaction and intense reparative process characterized by the angiogenesis and fibrosis on GI, GII and GIV (i, j and l). GIII exhibited a scanty inflammatory infiltrate and intense reparative process (k).

60 days - All groups were similar at and revealed only fibrosis at the tube extremity (m, n, o, p).

## Cell survival assay

There was a progressive cell growth in all groups cultures from day 1 to 7 (figure 2). However, all the cements evaluated presented significantly fewer cells than control groups after the 3rd day.

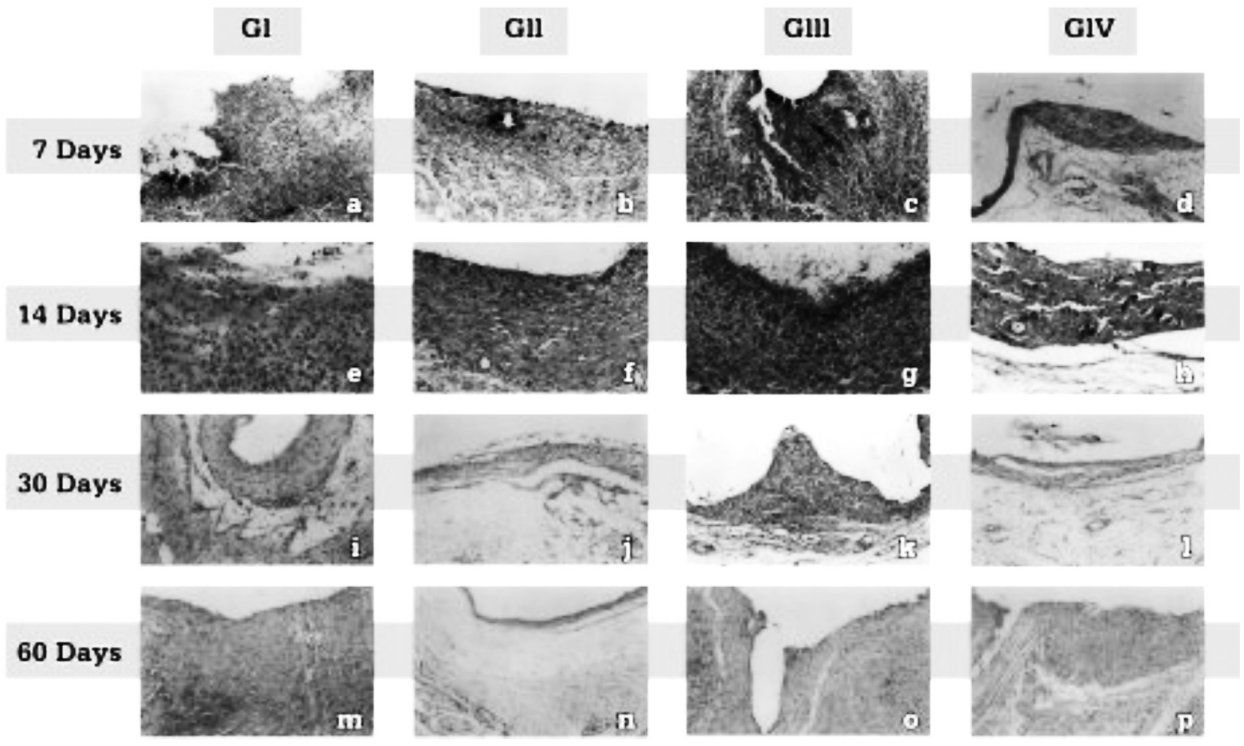


**Figure 1:** Photomicrographs of histological of rat subcutaneous tissue. (H&E stain, original magnification, x10)

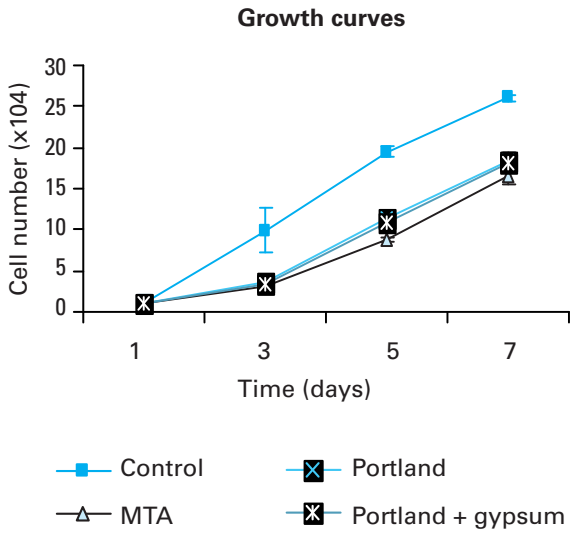


**Figure 2:** Growth curves of NIH-3T3 cells

There was no statistical difference between Portland cement and Portland cement plus gypsum treated cultures throughout the experimental time.

Fibroblast cultures treated with PC (GI and GIII) and MTA presented no significantly difference in the 1st and 7th days of the experimental period. However, cultures from the MTA group presented a decrease in cell numbers starting from the 3rd day with statistically different values comparing to PCG in 5 days and in 3rd and 5th days when comparing with PC.

## Discussion

MTA and PC have similar chemical composition, behavior in culture of cells and also in subcutaneous, osseous and pulpal tissues[9,10,11,12,13,14,15].

The main disadvantage when using MTA or PC is their long setting time. An ideal material should have biocompatibility and a relatively short setting time.

For this purpose we evaluated the biocompatibility of PC modified by the gypsum addition, as, this substance speeds up its setting time from 101,26 to 6,6 minutes[18].

Our *in vitro* results showed that the presence of MTA, PC or PCG allows the proliferation of NIH3T3 cells (fibroblasts) even after 7 days what shows that these cements present a smaller cytotoxic potential, and are in according to others studies[13,16,17]. Saidon *et al.*[13] demonstrated that MTA and PC allow L929 (fibroblasts) proliferation after 3-day incubation period and Abdullah *et al.*[16] also showed that a modified PC (calcium chloride added) supported osteosarcoma cells proliferation.

In our *in vivo* results, tissue reactions associated with MTA and PC and PCG implants were comparable, suggesting that all materials are equally biocompatible. Others authors have already verified that MTA and PC have similar biocompatibility[11,12,13].

The group of the PCG presented more acute and intense initial reaction, however after 30 days it had similar evolution to the MTA and PC. We believe that gypsum although speed up the setting time of the material, can be a little more aggressive initially, but biocompatible after a long stated period.

Costa *et al.*[25] in a study of biocompatibility of different cements (MTA, PC, zinc oxide and eugenol cement and calcium hydroxide) had got similar results to our study in relation to the MTA and the PC behavior in rats subcutaneous. Our results concerning to the PCG were similar to the gotten for the authors when evaluating the zinc oxide and eugenol cement.

The results of our work had disclosed that the three evaluated materials had presented biocompatibility *in vitro* and in rat subcutaneous tissue. The gypsum added modified Portland cement could be an excellent alternative for the development of a new material that unites biocompatibility, effectiveness, low cost and an acceptable setting time.

Independently of all these positive results in studies *in vitro* and in relation to the applicability of the PC in living tissues, is important to stand out that the safe use of the PC, depends on the standardization of its components and the sterility of its presentation for dentistry.

## Conclusion

The results of this study confirmed that MTA, Portland cement and gypsum added Portland cement have similar biocompatibility.

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