Cytotoxicity, Histopathological, Microbiological and Clinical Aspects of an Endodontic Iodoform-Based Paste Used in Pediatric Dentistry: A Review

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This review aims at describing and comparing materials commonly used in root canal therapy, the cytotoxicity, histopathological, microbiological and clinical aspects of a iodoform-based paste (Guedes-Pinto Paste -GPP) used in endodontic treatment of primary teeth. GPP has shown excellent biocompatibility to pulp fibroblasts and mild inflammatory reactions, having been well-tolerated by the periapical and connective tissues. Moreover, GPP bactericidal and bacteriostatic effects against many oral microorganisms were also demonstrated. Regarding clinical trials, the GPP technique has achieved success rates when considering clinical and radiographic examinations. In the face of all the above mentioned results, this paper would like to propose the use of this endodontic material as a root canal filling for primary teeth.

Keywords*: review, pulpectomy, primary teeth, iodoform, camphorated parachlorophenol*
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INTRODUCTION

A number of inherent difficulties in pulp therapy in primary teeth, more specifically related to instrumentation, such as molar root curvature, the great number of collateral canals, the complexity of the apical delta, and physiological root resorption process, the possibility of damage to periodontium and to the permanent tooth buds, have led to look for research of a filling material which could overcome all instrumentation obstacles, leading to pulp therapy success.[1,2] Therefore, selecting an effective material for the endodontic treatment of primary teeth is important. In addition to knowledge regarding the potential toxicity of the material, histological reactions and clinical use, one must know the biological mechanisms by which the material induces repair.

An ideal root canal filling material for primary teeth must have several properties, such as resorption at a rate similar to that of the primary root, be harmless to periapical tissues and permanent tooth germs, readily resorb if extruded beyond the apex, and be a strong antiseptic. It should easily fill the root canal, adhere to the walls. It should not be susceptible to shrinkage and should be easily removed if needed. The ideal material should also be radiopaque and not discolor the tooth. In addition, it also should not set to a hard mass which could deflect the erupting succedaneous tooth.[1-5]

Based on that, Guedes-Pinto *et al.,* in 1981[6] proposed a root filling material for primary teeth named as Guedes-Pinto Paste (GPP), composed of Rifocort®, camphorated parachlorophenol, and Iodoform (Table 1). The paste is made up of one equal part of each component, mixed on a sterilized glass plate.

The GPP technique is described as follows: after anesthesia and rubber dam placement, the content from the pulp chamber is removed followed by irrigation with Tergentol-Furacin detergent. the detergent is also placed in the pulp chamber and activated with continuous dropping of Dakin liquid, thus initiating the chemical-surgical preparation (CSP). The canal CSP is performed by means of Kerr type-root files, in series, previously prepared according to work length carried out during the first radiographic examination. Two larger size files are used, and the CSP is completed when all instruments and chemical substances appear white, without turbidity. Final irrigation is done with Tergentol-Furacin, and then absorbent paper points are used to dry the

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Table 1. Components of Guedes-Pinto Paste proposed in 1981.

GPP Components	Composition (per gram)	Physical Aspect	Property
Rifocort ® Merrel Leppetit	* prednisolone acetate (corticosteroid). (5mg) – anti-inflammatory * rifamycin sodium salt - antibiotic * propilenglycol - vehicle * macrogol (polyethylene-glycol) - vehicle	ointment	Anti-inflammatory Antibiotic
Camphorated parachlorophenol (Biodinâmica)	(proportion 3:7) *30% parachlorophenol * 70% camphor	liquid	Antimicrobial Analgesic
Iodoform (K-Dent)	* iodine	powder	Antimicrobial

canals. The GPP is prepared as recommended and taken to the root canals with the aid of a lentulo or files. Guttapercha is placed as a temporary stopper and the tooth is restored. A final radiographic examination must be performed in order to evaluate the filling quality.

This study describes the cytotoxicity, histopathological, microbiological and clinical aspects of the GPP as a root filling material for primary teeth.

CYTOTOXICITY STUDIES

Santos *et al*.[7] evaluated the GPP cytotoxicity using *in vitro* assays comparing it with formocresol, glutaradehyde and phosphoric acid. NIH 3T3 cell line (ATCC CRL 1658) and pulp fibroblasts (FP1) were used for cell viability test. The paste was directly applied to coverslips placed in contact with the cultured cells. The control group cultures received plain coverslips. In the short-term assay (cell viability), the experimental periods were 0, 4, 8, and 12 hrs, and in a long-term assay (cell survival), the periods were 1, 3, 5, and 7 days. Cell number and cell viability (%) were determined by counting the cells in a hemocytometer, using the Trypan blue dye exclusion assay. The data obtained in these experiments showed that groups treated with the GPP had higher viable cell numbers and percentages than the groups treated with the other drugs; therefore, the authors concluded that the GPP cytotoxicity was lower in fibroblast culture tests. Using the same method, Lopes-Marotti[8] evaluated the cytotoxicity with stored GPP in different storage periods (1, 2 and 3 months) under refrigeration and ambient temperature. The cytotoxicity increased significantly when stored in both storage conditions; however, refrigeration gave more stability to the paste.

HISTOPATHOLOGICAL ASPECTS

The histopathological reaction of rat subcutaneous tissue to the GPP was first evaluated by Michel in 1985.[9] A mild inflammatory reaction was observed in 7 days. Twenty-eight days later, the paste was no longer observed in the tissue; and at the end of the experiment (90 days), the subcutaneous tissue presented normal characteristics, demonstrating paste compatibility with connective tissue.

The histopathological reaction of rat dental pulp after pulpotomies with zinc oxide added to the GPP was also investigated.[10] A mild inflammatory reaction and, the beginning of a dentinal bridge formation, a normal-aspect of remnant pulp tissue were observed on the seventh day. Moreover, the addition of zinc oxide to the GPP did not cause any histopathological reaction on rat dental pulp.

Chedid *et al*.[11] compared, on a histological basis, the effect of two pulp capping agents (formocresol and GPP) in rat molar pulp after pulpotomy procedures. In the final period of the experiment (90 days), teeth treated with the GPP paste presented complete wound healing and the formation of a dentinal bridge, while those treated with formocresol presented a large necrotic area, close to the exposed coronal pulp and extending to the radicular pulp. Whaterhouse *et al*.[12] performed a histological evaluation on human primary molars failing to formocresol (5% of group-F) and calcium hydroxide (CH) pulpotomies (11% of failure-CH). All teeth exhibited reactionary dentin deposition, but only teeth from group-C showed dentinal bridge formation. Purulent exudate was identified in all specimens.

When Faraco-Junior and Percinoto[13] compared the histological effects of two pulpectomy techniques (using the GPP paste and another paste composed of calcium hydroxide, iodoform and propilenoglycol) on dog's teeth, the results after 30 days demonstrated that both techniques were well-tolerated by the periapical tissues; however, the technique with the GPP displayed higher levels of inflammation and bone resorption only at the apical region.

Sousa *et al*.[14] observed the biocompatibility of the GPP, Calcium hydroxide paste (PA), and CTZ paste placed on "guinea pigs" bone implants. The morpho-histological analyses were classified based on the FDI/ADA criteria for inflammation. The results for 30 days showed severe inflammatory reaction for PA and CTZ paste, while none, or a mild reaction, was observed for the GPP. After 90 days, the reactions to PA paste were absent or mild; the CTZ paste, perpetuation of the inflammatory process showed, ranging from moderate to severe. Conversely, the GPP was replaced by neoformed bone tissue. Santos *et al.*[15] have also evaluated and compared the subcutaneous reactions of mice to the application of the GPP and calcium hydroxide. The analysis of exposed subcutaneous tissue reaction from mice has

demonstrated that, a less intense inflammatory reaction was observed for the GPP group.

The histological effect of stored GPP in periods of 1, 2 and 3 months, under refrigeration and ambient temperature showed a mild inflammatory reaction after 7 days and at the end of the experiment (90 days), subcutaneous tissue displayed normal characteristics, demonstrating paste compatibility with connective tissue.[16]

In addition to knowledge of the potential material toxicity and of the reactions it may cause under histological aspects, one must know the biological mechanisms by which the material induces repair. Therefore, Santos *et al*[15] have evaluated the potential GPP and calcium hydroxide (CH) chemotaxis for macrophage migration in culture. After obtaining macrophages for culture from a subcutaneous insert in mice, adherence macrophage capacity to the plastic surface, through the use of Eppendorf's, and an invasive test with Transwell, were performed. The tests developed demonstrated a greater adherence capacity for macrophages with the GPP vis-à-vis the calcium hydroxide paste, moreover, the GPP had a greater potential for macrophage chemotaxis compared with the control group.

Regarding the *in vivo* biocompatibility and *in vitro* repairing capacity of stored GPP placed on polyethylene implants on rats, the different storage periods and conditions did not influence subcutaneous tissue histopathological reaction. However, *in vitro* results have demonstrated that the GPP storage for 90 days had slightly increased macrophage chemotaxis.[16]

Ranly and Garcia-Godoy,[17] in a review of current and potential pulp therapies for primary and young permanent teeth, also pointed unfavorable histopathological reactions to formocresol, calcium hydroxide, ferric sulfate, laser therapy and electrocautery as pulpotomy modes. Studies with glutaradehyde, commercial preparations with PA, and, especially, with biological growth factors are promising, as these are deemed to have better biocompatibility. Regarding pulpectomy therapy, the authors stated that obturation with zinc oxide and eugenol is a bioincompatible material.

MICROBIOLOGICAL ASPECTS

In 1996, Bonow *et al*[18] evaluated the immediate and mediate antimicrobial effectiveness of seven drugs used in pulp therapy of primary teeth: (1- GPP, 2- GPP + zinc oxide, 3- GPP + CH, 4- GPP + CH + zinc oxide, 5- formocresol, 6- diluted formocresol and 7- glutaradehyde 2%) against *S. aureus, S. epidermidis, S. mutans, S. sanguis, E. faecalis and E. coli*, microorganisms that can be detected in infected root canals, and also against *B. subtilis*, a spore-forming bacteria displaying acknowledged resistance to many chemical agents. Samples of the antimicrobial agents were assayed just after their preparation, and 7, 14, 28, 60 and 90 days later. The antimicrobial effect decreased with time, depending on the drug used and on the bacterial strain tested. The data indicates that the zinc oxide paste had the strongest, and that 1/5 formocresol, the weakest antimicrobial activity. The differences were all statistically significant except for 2% glutaradehyde and formocresol, found to be similar to each other.

Gomes *et al*[19] evaluated microbiologically the biomechanical preparation and the GPP as a root filling material of 25 primary teeth during necropulpectomy procedures. Samples were obtained by placing a sterilized paper point inside the root for one minute. Positive cultures were observed before and after biomechanical preparation; turbidity intensity was strong and moderate, respectively. One week later, 48% of the samples presented negative cultures and 52% positive cultures with mild turbidity.

The bactericidal and bacteriostatic effect against *S. mutans, S. aureus, E. faecalis* and *C. albicans* of 1 (GPP), 2 (iodoform 0.08g, CMPC 0.06g, CH 0.09g, propilenoglycol 0.08g), 3 (CH 0.06g, propilenglycol 0.08g), 4 (CMPC 0.06g, CH 0.09g, propilenglycol 0.08g); 5-(iodoform 0.08g, CA 0.09g, propilenglycol 0,08 g), and each component separately were also researched.[20] The authors concluded that the GPP was the only paste displaying bactericidal and bacteriostatic effect against all tested microorganisms. Similar results were observed by Wen-Shiun *et al*,[21] when CMCP was mixed with CH or ZOE, as they showed the strongest antibacterial effect ($p<0.05$) against microbial specimens obtained from 13 infected primary teeth. The materials with no or minimum antibacterial effect included Vitapex™ and $CH + H_2O$.

Other studies[22-25] have all shown the poor bacterial effect of iodoform. As the GPP is an iodoform-based paste, such behavior could also be expected; however, other GPP components as Rifocort® (which contains rifamycin sodium salt, an antibiotic) and CMPC also have an antimicrobial effect on root canal microorganisms.[20]

Immediate GPP antimicrobial activity and following its storage under refrigeration and ambient temperature, in different periods, were evaluated against *S. mutans; S. oralis; S. aureus; S. epidermidis; E. coli; E. faecalis and B. subtilis*.[26] Both storage conditions showed, in all the experimental periods, a bacteriostatic potential against all tested microorganisms. Massa *et al*[27] have also ascertained similar results of the GPP immediate activity, and following its storage for 7, 14, and 21 days against *S. epidermidis, E. coli, E. faecallis* and *B. subtillis* ($p>0.05$). The results demonstrated that the GPP storage did not cause any change to its bacteriological property.

CLINICAL TRIALS

Pulpectomy treatment for primary teeth has been recommended by many authors[1, 2, 28, 29]; and its goals are eliminating infection and maintaining the tooth in a functional state until its normal exfoliation, without endangering succedaneous permanent dentition.

The most common materials used for filling primary canals are ZOE, iodoform-based pastes and CH,[12, 17] Zinc oxide and eugenol (ZOE) paste was the first root canal filling material to be recommended for primary teeth and reported by several authors to have moderate to high success rates in preserving chronically-infected teeth. Until recently, ZOE had been the material of choice, but concerns have

been expressed regarding the difference between its rate of resorption, and that of the tooth and its slow absorption when pushed into the apical tissues [5]. In addition to its bioincompatibility, ZOE is not particularly antibacterial once it has set.[30] Calcium hydroxide presents favorable antibacterial effects, is easily resorbed and causes no foreign body reaction.[31] It also presents high success rates; however, some studies have described it as causing internal root resorption.[32]

Iodoform-based pastes have been advocated as root filling, such as KRI 1, Walkhoff, Vitapex and Maisto's pastes.[2, 3, 28, 29, 33-5] They fulfill most of the requirements of an ideal filling material for primary teeth as they are more easily resorbed from the periapical area, cause no foreign body reaction, and display potent germicidal properties. Moreover, many resorb in synchrony with primary roots, can be easily forced into the pulp canals and accessory canals, and have no undesirable effect on succedaneous teeth.

A retrospective analysis of pulp therapy using the GPP paste is described on Table 2, depicting a high success rate in clinical and radiographic aspects.

Ortega *et al*[41] demonstrated another important clinical use of the GPP paste. In a random-controlled clinical trial, the authors assessed the efficacy of a medicated dressing (GPP) in healing sockets following dental extractions in 40 HIV positive patients, with CD4 counts below 200 cells/mm,[3] who needed to have molar teeth extracted. Patients were randomly allocated to have their sockets dressed with GPP and sutured (treated group), or merely sutured (control group). The results showed that the sockets in the treated group healed more quickly than the control group.

The American Academy of Pediatric Dentistry,[42] in its Guideline on Pulp Therapy for Primary and Young Permanent Teeth, proposes that root canals should be filled with a resorbable material such as nonreinforced zinc oxide-eugenol. The UK National Clinical Guidelines of Pulp therapy for primary molars also recommends the use of resorbable paste, e.g. slow-setting pure zinc oxide eugenol, non-setting calcium hydroxide paste or calcium hydroxide and iodoform paste (Vitapex™ or Endoflas™).[43]

In Brazilian Dental Schools, the GPP is the most common material used in pulpectomy procedures for primary teeth. When Kramer *et al*.[44] analyzed the current status of pulp therapy at 27 Universities found that 48% used the GPP as a root canal filling material, followed by ZOE (19%), ZOE with iodoform (7%) and GPP with ZOE (7%). Brusco *et al*.[45] also observed at 48 Brazilian Dental Schools that the GPP was selected as the preferable filling material (65%) followed by ZOE (23%).

In the face of all the cytotoxicity, microbiological, histopathogical aspects and *in vivo* successful results using the GPP, consolidated from many studies, this paper would

Table 2. A retrospective analysis of clinical trials using GPP paste in pulp therapy of primary and young permanent teeth.

Study	Sample	Inclusion Criteria	Irrigation solution	Endodontic drug	Clinical Results	Radiographic Results	Conclusions
Guedes-Pinto et al (1981) [6]	45 primary molars	Necropulpectomy, teeth with at least 2/3 of remnant root. Excluded: carious lesion on molar furcation.	- Endo-PTC, Dakin - Initial/ final: tergentol-furacin	GPP	- Pain: absent after treatment - Mobility: when present, disappeared in 7 to 15 days. - Fistula: spontaneous regression one week later.	- GPP extravasated: reabsorbed in 30 days, followed by bone neoformation concluded in 6 months postoperative	- High success rate in 2 years: 98%, due mainly to chemical cleaning agent and filling paste.
Bengston et al (1992) [36]	59 primary molars	Pulpotomy: caries lesions close to pulp. Clinical and radiographic absence of pulp infection	Tergentol-furacin	GPP	Group A: 1st evaluation period (12-17 months), 43 teeth- 4 failure Group B: 2nd period (18-24 months), 16 teeth- no failure		Overall, the success rate was 93.22%
Bengston et al (1994) [37]	90 primary molars	Pulpotomy: caries lesions close to pulp. Clinical and radiographic absence of pulp infection	Tergentol-furacin	GPP	Six-month new assessment from Bengston et al, 1992 Group A: (18-23 months), 33 teeth revaluated, no failure Group B: (24-30 months), 12 teeth revaluated, no failure Group C: (5-14 months), 31 new treatments, 2 failures		Favorable results, indicating the technique to pulpotomy
Puppin-Rontani et al (1994) [38]	23 primary teeth	Necropulpectomy in one section. Seventeen teeth with bone rarefaction on furcation.	- Endo-PTC, Dakin -Initial and final: tergen-tol-furacin	GPP	-Success rate at 6 months was 82% and 64% in 12 months. - Non-fistuled teeth at baseline: 100% of success	-Bone neoformation in 6/ 12 months, respectively: partial (41% - 47%) and complete (47% - 65%). - Increased bifurcation lesions: 6% in 1 year.	
Araújo et al (1995) [39]	80 primary molars	Pulpotomy: teeth with at least 2/3 of remnant root. Clinical and radiographic absence of pulp infection		G1: formocresol 1/5 (n=40) G2: GPP (n=40)	G1: success/failure 6 months: 37 teeth (92.5%) 12 months: 31 (77.5%) 24 months: 27 (67.5%)	G2: success/failure 6 months: 40 teeth (100%) 12 months: 36 (90%) 24 months: 34 (85%)	Pulpotomy with GPP was superior to formocresol, and failure cases were less severe.
Castro et al (1998) [40]	09 permanent molars	Pulpotomy: caries lesions close to pulp, incomplete root formation, absence of ext./internal resorption and lesion on furcation	- Dakin - Final: tergentol-furacin	GPP	Results in 12 months: - Absence of necrosis. - Periodontal integrity obtained - Absence of painful symptom	Results in 12 months: Absence of root resorption, periapical and furcation lesion. Root formation completed in all cases.	Absence of clinical and radiographic signs and symptoms of infection, GPP was well tolerated by remnant pulp tissue.

like to propose the use of this endodontic material as a root filling material for primary teeth.

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