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Novel Experimental Cements for Use on the Dentin-Pulp Complex

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This aim of this study was to evaluate the physicochemical and biological properties of novel experimental cements (Hybrid, Paste and Resin) based on synergistic combinations of existing materials, including pH, diametral tensile strength (DTS) and cytotoxicity comparing them with mineral trioxide aggregate (MTA - Angelus®) and a glass ionomer cement (GIC) developed at our laboratory. For the physicochemical and biological tests, specimens with standard dimensions were produced. pH measurements were performed with digital pH meter at the following time intervals: 3, 24, 48 and 72 h. For the DTS test, cylindrical specimens were subjected to compressive load until fracture. The MTT assay was performed for cytotoxicity evaluation. Data were analyzed by ANOVA and Tukey's test (α=0.05). Paste group showed pH values similar to MTA, and Hybrid group presented pH values similar to GIC ($p>0.05$). The tested materials showed pH values ranging from alkaline to near neutrality at the evaluated times. MTA and GIC showed similar DTS values. The lowest and highest DTS values were seen in the Paste and Resin groups, respectively ($p<0.05$). Cell viability for MTA and experimental Hybrid, Paste and Resin groups was 49%, 93%, 90% and 86%, respectively, when compared with the control group. The photo-cured experimental resin cement showed similar or superior performance compared with the current commercial or other tested experimental materials.

Key Words: dental pulp, dental cements, pulp capping, biocompatibility, mechanical properties.

INTRODUCTION

Direct pulp capping relies on the application of a material directly on the exposed pulp to promote its vitality, while indirect pulp capping is conducted in teeth with deep carious lesions and presenting reversible pulp pathosis, when the protective material is placed over a remaining layer of dentin. Both treatments aim to maintain the vitality of pulp tissue and its biological homeostasis (1).

Several materials are commercially available for use as capping agents, and among them calcium hydroxide (CH) has been one of the most widely used due to its ability to stimulate pulp repair and dentin bridge formation and its antimicrobial activity arising from the high pH generated by the material (2). However, CH-based cements show several limitations, including high solubility, short-term effects, rapidly buffering by the fluid contents of dentinal tubules, low mechanical resistance and lack of adhesion to dental structures (3).

In an attempt to improve the behavior of CH-based cements, a light-cured material (Prisma VLC Dycal; Dentsply De Trey, Konstanz, Germany) was formulated using urethane dimethacrylate resin (UDMA), which improved the mechanical properties of chemically cured calcium hydroxide cements, while also enhancing their working times (2). However, a significant limitation of this photo-cured cement was the possible risk of pulpal injury from release of uncured monomers from the resin (4).

Mineral trioxide aggregate (MTA) has also found wide use as a pulp-capping agent (5). Other

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investigations have demonstrated improved mechanical and biological properties for MTA when compared with calcium hydroxide cements (5,6). The superior biological responses of MTA have been attributed to the higher solubilization of growth factors from dentin with MTA, although other mechanisms may also contribute to its performance. Despite the promising results observed with this material, its handling characteristics and slow setting reaction are considered limitations of current MTA formulations (7).

Conventional glass ionomer cements (GICs) have been indicated for protection of the pulp-dentin complex, presenting valuable properties, such as fluoride release, coefficients of thermal expansion and elasticity modes similar to dentin, and adhesion to dental structures. However, some limitations are related to these materials, including their high solubility, relatively poor mechanical properties and long setting times (8). The addition of resinous compounds, such as 2-hydroxyethyl methacrylate (HEMA), to conventional GICs has improved their mechanical properties and resistance to wear (9), although accompanied by a reduction in biocompatibility due to release of uncured monomers causing pulpal irritation (4).

To improve the biocompatibility of resinous materials, new monomers have been proposed. Ethoxylated bisphenolA glycol dimethacrylate (Bis-EMA 30) has a high molecular weight, thereby reducing opportunities for monomer diffusion through dentin. Since the diffusion coefficient is inversely proportional to the molecular weight, the replacement of HEMA (MW=130 g/mol) by Bis-EMA 30 (MW=1,686 g/mol) should result in toxicity reduction (10).

While several materials have been indicated for capping procedures, there is still no ideal material that incorporates all the properties required for optimal performance. A combination of the properties of choice from the different current materials may allow an interesting approach to synergistically develop new dental cements with optimal properties, which could improve the outcomes of current pulp therapeutic strategies (11).

The purpose of this study was to develop new experimental dental cements for pulp capping, combining components of different currently available materials, including MTA. The tested hypothesis was that the experimental cements should exhibit better physicochemical and biological properties, when compared with commonly used current commercial materials (MTA and GIC).

MATERIAL AND METHODS

Experimental materials (hybrid, resinous, paste) based on MTA and GIC were developed and their compositions as well as those of the MTA (White MTA: Angelus, Londrina, PR, Brazil) and GIC used in the study are presented in Table 1.

Post-Setting pH Changes

For pH measurements, 15 standard disks (h=1 mm; Ø=6 mm) were prepared for each material. They were either photoactivated by light-emitting diode source (LED - Radii-cal; SDI, São Paulo, SP, Brazil) with 1,200 mW/cm^2 light intensity for 40 s (dual-cure mode) or not photoactivated (self-curing mode). After preparation, the disks were placed in Eppendorf micro-tubes with 1 mL of culture medium (Dulbecco's Modified Eagle's Medium-DMEM, Sigma Chemical Co., St Louis, MO, USA) supplemented with 10% bovine fetal serum (FBS) (Cultilab, Campinas, SP, Brazil) and stored at 37ºC. The pH values were measured at the following intervals: 3, 24, 48 and 72 h after preparation, using a digital pH meter (Quimis Q400A, Diadema, SP, Brazil). In the measurement intervals, the disks were carefully removed and placed in a new micro-tube with fresh medium (12).

Diametral Tensile Strength (DTS) Test

Standard disks (h=2±0.1 mm; Ø=4±0.1 mm) were prepared for each evaluated material (n=8). The photocured materials were light-cured as described above. The borders were gently polished with 600-grit abrasive paper (Norton Abrasivos Brasil, São Paulo, SP, Brazil) and the samples were stored at 37ºC and 100% humidity for 24 h before the test. Then, the disks were measured with a digital caliper (Mitutoyo 500-144B;Suzano, SP, Brazil). The DTS test was performed in a universal testing machine (EMIC 2000; Equipamentos e Sistemas de Ensaio Ltda., São José dos Pinhais, PR, Brazil) under a 100 kgf load at 0.5 mm/min. The resistance values were expressed in MPa.

Cell Viability Evaluation

An immortalized cell line, 3T3/NIH mouse fibroblasts, was grown in culture medium (Dulbecco's

Modified Eagle's Medium - DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics (10,000 IU/mL of penicillin G and 10,000 mg/mL of streptomycin; Gibco Laboratories Inc., Grand Island, NY, USA). The cells were seeded in culture dishes and maintained in an incubator (37ºC, 5% of CO_2) until getting subconfluent.

Standard disks (h=1 mm, Ø=4 mm) were prepared for each material (n=3) and individually placed in Eppendorf micro-tubes containing 1 mL of DMEM and maintained in an incubator (37ºC, 5% of CO_2) for 24 h. The samples were then removed from the micro-tubes and the eluates from each material were added to 96-well plates (n=8 per sample) where $2x10^4$ cells were previously seeded. The 96-well plates were incubated (37ºC, 5% of CO_2) for 24 h. Cell viability was assessed using the MTT assay. A control group was included containing only cells without addition of eluates.

Absorbance readings were measured using a universal ELISA reader (ELX 800; BIO-TEK Instruments, Winooski, VT, USA), with a 570 nm wavelength filter, where absorbance values were considered the indicator of cell viability. The inhibitory effect of the different tested materials on mitochondrial activity of cells was calculated and expressed as proportional rates compared with the control group.

Statistical Analysis

Statistical analyses were carried out using the SigmaStat® software package (Version 3.5 for Windows®; Systat Software Corporation, San Jose, CA, USA) and analyzed descriptively. In addition, differences between groups were tested by ANOVA and Tukey's test with a significance level of 5%.

RESULTS

Post-Setting pH Change

Changes in pH of the tested materials were observed along the various assessed time points (Table 2). The experimental Paste group showed changes in pH values similar to MTA for all the tested time intervals. The experimental Hybrid group exhibited a profile of pH change similar to the GIC ($p>0.05$). After 72 h, most materials showed a similar reduction in pH values, with the exception of the experimental resin group. This latter group showed significantly higher pH values after 72 h ($p<0.05$) compared with the other materials and its pH was relatively constant throughout the experiment. All the evaluated materials showed pH values ranging from

Table 1. Composition of experimental and commercial materials used in the study.

Material	Composition	Curing mode	Proportion
Hybrid	Powder: GIC + MTA; Liquid: distilled water and GIC liquid	Chemical	1:1
Resinous	Powder: MTA, yterbium fluoride, DHEPT, EDAB; Liquid: PEGUDMA 400, TEGDMA, H_2O, GDMAP, UDMA, camphorquinone, benzoylperoxide	Light activation	3:2
Paste	Paste 1: MTA, Bis-EMA 10, Bis-EMA 30, camphorquinone, DHEPT, EDAB; Paste 2: yterbium fluoride, Bis-EMA 10, Bis-EMA 30, benzoyl peroxide	Light activation	1:1
MTA (Angelus®)	Powder: BI_2O_3, CaO, MgO, K_2O, Na_2O, Fe_2O_3, SO_3, SiO_2, Al_2O_3; Liquid: distilled water	Chemical	3:1
GIC	Powder: radiopaque fluoroaluminum silicate crystals, polycarboxylic acid and pigments; Liquid: copolymers of polycarboxylic acids, maleic, itaconic and tartaric acids and purified water	Chemical	1:1

GIC: glass ionomer cement (developed at the author's laboratory). MTA: mineral trioxide aggregate. PEGUDMA 400: 400 polietilienoglicoldimethacrylate. TEGDMA: triethyleneglycoldimethacrylate. GDMA-P: glycoldimethacrylatephosphide. UDMA: urethanedimethacrylate. DHEPT-N- N: dihidroxietil-p-toluidine. EDAB: ethyl-4-dimethylamino benzoate. Bis-EMA: dieterdimethacrylate. Bi_2O: bismuth dioxide. CaO: calcium oxide. SiO_2: silicon oxide. Al_2O_3: aluminum oxide.

near neutrality to alkaline throughout the experiment (Fig. 1A).

DTS Test

Differences in DTS values were observed for the different groups (Fig. 1B). Data from the experimental hybrid group are not shown due to complete solubilization of the material during storage in distilled water for 24 h.

MTA and GIC showed similar values of resistance and the lowest resistance was observed for the experimental paste group ($p<0.05$). The experimental resin group exhibited the highest resistance values ($p<0.05$).

Cell Viability Assay

Differences in cell viability for the experimental Hybrid, Paste and Resin groups and MTA, when compared with control group, were observed (values of 93, 90, 86 and 49% respectively) (Fig. 1C and Table 3). Total loss of cell viability was observed for the GIC group ($p<0.05$). While the cell viability for the experimental Paste and Resin groups was significantly lower than that for the control group, the values were still 86% or greater than those of the control group. For the experimental Hybrid group, cell viability was very similar to that of the control group, not differing significantly. Considerable loss of cell viability was seen for the MTA group (49% of control), which was significantly different from the other groups (Fig. 1C).

DISCUSSION

The present study demonstrated that the experimental materials generally had similar or superior properties to those of MTA or GIC, confirming in part the study hypothesis. In this study were evaluated key physical properties (pH, diametral strength) and biological properties (viability/cytotoxicity), which are fundamental to the initial screening of new materials (7).

MTA showed high pH values (alkaline) that initially were higher than those obtained for GIC and the experimental cements, except for the experimental Paste group. A high pH favors the antimicrobial effects of capping cements (13). While the mean pH values for MTA were similar to those found in another study (13) for the first 24 h, there was a significant decrease over time, which may relate to the specimen size. To evaluate pH from endodontic materials, circular specimens with dimensions of 10 mm x 1 mm are often used (13), but different sample dimensions (1 x 6 mm) were used in the present study in order to simulate those used clinically as closely as possible.

The initial alkaline pH of MTA and CH may be important for growth factor release from dentin, which has been implicated in signaling events for pulp repair (9). After 72 h, the majority of the evaluated materials showed a reduction in pH values, tending to neutrality, thus indicating an initial release of ions followed by stabilization of the materials. Notably, the experimental resin material showed a stable pH value throughout the entire observation period, reflecting the higher

Table 2. Mean and standard deviations (SD) of pH for the different materials stored in culture medium supplemented with fetal bovine serum at the time intervals of 3, 24, 48 and 72 h.

Time interval	Paste	Hybrid	Resinous	MTA	GIC
3 h	$8.50^{AB}(\pm0.05)^{ab}$	$8.20^{CD}(\pm0.14)^{cd}$	$8.46^{AB}(\pm0.10)^{bc}$	$9.52^{A}(0.16)^{a}$	$8.02^{A}(0.11)^{d}$
24 h	$8.96^{AB}(0.13)^{ab}$	$8.50^{AB}(0.17)^{c}$	$8.65^{AB}(0.16)^{bc}$	$9.26^{AB}(0.24)^{a}$	$8.13^{A}(0.40)^{c}$
48 h	$8.27^{B}(0.86)^{bc}$	$8.47^{BC}(0.27)^{bc}$	$8.70^{AB}(0.21)^{ab}$	$8.88^{AB}(0.34)^{a}$	$7.94^{A}(0.68)^{c}$
72 h	$7.50^{BC}(0.45)^{b}$	$7.57^{D}(0.68)^{b}$	$8.89^{BC}(0.25)^{a}$	$7.57^{B}(0.52)^{b}$	$7.38^{B}(0.43)^{b}$

Different uppercase letters in columns and lowercase letters in rows indicate statistically significant difference ($p<0.05$).

Table 3. Mean and standard deviation (SD) for cell viability in the different groups after 24 h in contact with cement eluates.

Time interval	Paste	Hybrid	Resinous	Control	MTA
24 h	$2.242\ (0.180)^{b}$	$2.427\ (0.239)^{ab}$	$2.325\ (0.191)^{b}$	$2.598\ (0.081)^{a}$	$1.283\ (0.158)^{c}$

Different letters indicate statistically significant difference ($p<0.05$).

dimensional stability of resinous materials (9). This may favor maintenance of the potential antimicrobial effects for longer periods (not tested in the study) and allow a sustained low intensity stimulation of growth factor release from dentin. Furthermore, repair is favored in an alkaline environment (14), especially where acids from cariogenic microorganisms may provide an unfavorable environment for pulp repair (4).

The biocompatibility of dental materials is fundamental to their effectiveness (15). The relatively poor viability seen with MTA compared with the control (49%) is suggestive of at least an initial toxic effect of MTA. While some of the previously published studies have reported higher cell viability in the presence of MTA (16,17), the data obtained during the first 24 h post-setting perhaps reflected the high pH generated by the material during this period (11). The pH values for MTA of around 12.0 have been reported in other studies (18,19). The complete loss of viability in the presence of GIC corroborates previous studies (16), highlighting the need for the apposition of another protective material under GICs in deeper cavities. Acid release from GICs during setting represents a possible source of injury to the pulp cells following tubular diffusion and emphasizing the need for a barrier to such diffusion (16). Despite the presence of GIC in the experimental hybrid material, excellent cell viability (93%) was observed, which may reflect incorporation of the resin component (Bis-EMA 30), with its lower diffusion characteristics (10). Good cell viability was also observed with the experimental resin material, which may in part be a consequence of its high dimensional stability and stable pH post-setting. The combination of the experimental resin material's stability relative to MTA and the use of a high-molecular-weight monomer for its resin component with consequent impact on diffusion behavior (10) highlights the potential biological benefits of this new material.

Determining the resistance of a material is important for its development and subsequent clinical application. The present study evaluated DTS, which is used to test fragile materials with low or no plastic deformation. In this type of test, the specimen is submitted to a compressive load in the diametral plane perpendicular to the longitudinal axis (20). The diametral resistance can be an important parameter for capping materials in cases where a restorative material requiring condensation is placed over the capping material. The low diametral resistance of CH cement is one of its major limitations, due to the inability to place a condensable restorative material without interposition of another more resistant protective material. In terms of diametral resistance, conventional GIC and MTA showed similar values and the resistance of these materials after setting is sufficient to allow the condensation of restorative materials (21). Nevertheless, the relatively long setting time of both the conventional GIC and the MTA used in this study, represents an important clinical limitation due to the increase of the chair-side time for using either of these materials (22).

In the case of conventional GICs, there is a time delay of at least 8 min before other restorative procedures can be applied (8), and for MTA the waiting time wait is similar or longer (7). The DTS testing of the experimental hybrid material could not proceed

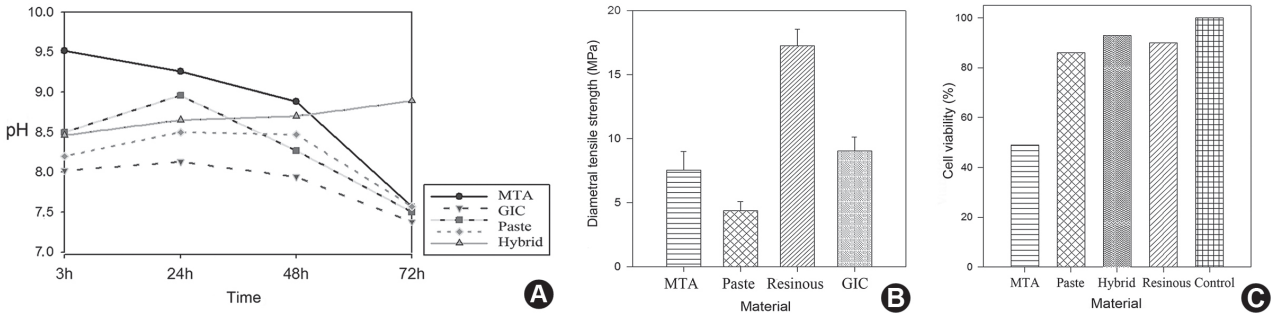

Figure 1. A: Results from pH values over time, demonstrating that all of them were alkaline with values near to neutrality. There was a decrease in pH values overtime for all materials, except for the experimental Resinous group. B: Diametral tensile strength of tested cements demonstrating that the highest value observed was related to the experimental Resinous group. The experimental Hybrid group suffered complete dissolution and could not be tested. C: Cell viability after exposure to materials' eluates revealing that MTA produced the highest reduction in absorbance levels, while cell viability values higher than 80% were observed for the experimental materials. GIC showed total cell depletion and zero value of absorbance readings.

due to the dissolution of specimens after 24 h in water. While the material's lack of resistance clearly limits its use clinically, it is interesting to speculate whether the material's dissolution characteristics might have application in the release of bioactive molecules from dentin for novel pulp regenerative therapies (11).

Poor resistance is a limitation for traditional CH cements (19) and resistance values for the experimental paste material were lower than those from MTA and GIC, suggesting a possible clinical limitation for this material. This contrasts with the experimental Resin group where higher values (17.2 MPa) were obtained compared with all the other groups. The high resistance of this material reflects the inclusion of resin components in its formulation together with the efficiency of light curing. Both the high resistance and curing efficiency are positive characteristics for a pulp capping material ensuring a longer clinical life, especially in the context of the deflections occurring in the material under masticatory load and also reducing the clinical treatment time. In this study, the resinous experimental cement was the material that presented, in general, the best performance among all materials for all conducted tests.

The intention in the present study was to gather positive characteristics of the current materials to develop new cements. Recently, one study questioned the presence of materials/tissues of lower resistance under composite restoration in posterior teeth, because this could contribute to restoration fracture (23). It is also noteworthy that if the capping material obtains its highest mechanical values soon after photoactivation, this material would not suffer the degradation caused by acid conditioning or by application of acidic primers (24). Furthermore, a resinous capping material may have the additional advantage of chemical bonding with the composite resin used for restoration, minimizing the occurrence of failures at the capping material/restorative material interface (25).

In conclusion, on the basis of the analyses conducted in the present study, the experimental MTA-based capping material showed superior performance to the other experimental materials and the currently used commercial materials. Such performance includes the possibility to significantly improving the clinical outcomes obtained with pulp capping materials, but requires further investigation. These results could warrant for the material a potentially better clinical behavior.

RESUMO

O objetivo deste estudo foi avaliar propriedades físico-químicas e biológicas de novos cimentos experimentais (Híbrido, Pasta e Resinoso) baseado na combinação sinérgica de materiais existentes, incluindo pH, resistência à tração diametral (RTD) e citotoxidade, comparando-os ao MTA (Angelus®) e a um cimento de ionômero de vidro (CIV) desenvolvido em nosso laboratório. Para a realização dos testes físico-mecânico e biológico, foram confeccionados espécimes com dimensões padrão. O teste de pH foi realizado por meio de pH-metro digital nos tempos: 3, 24, 48 e 72 h. Para o teste de RTD, espécimes cilíndricos foram submetidos a carga compressiva até sua fratura. Para avaliação da citotoxidade, utilizou-se o teste MTT. Os dados foram analisados utilizando ANOVA e teste de Tukey (α=0,05). O grupo Pasta apresentou valores de pH semelhantes ao MTA, assim como o grupo Híbrido seguiu os parâmetros do CIV ($p>0,05$). Todos os materiais apresentaram valores de pH alcalinos ou próximos à neutralidade nos tempos avaliados. MTA e CIV apresentaram valores de RTD similares. Os menores e maiores valores observados foram do grupo Pasta e Resinoso, respectivamente ($p<0,05$). A viabilidade celular para os grupos MTA, Híbrido, Pasta, Resinoso, quando comparados ao grupo controle foi de: 49, 93, 90 e 86%, respectivamente. O cimento experimental Resinoso apresentou desempenho similar ou superior aos materiais comerciais e experimentais avaliados.

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