In vitro Evaluation of the Cytotoxicity of Different Root Canal Filling Materials

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Abstract:

Objective: Aim of the present study was to evaluate the cytotoxicity of Real Seal 1 compared to other commercially available endodontic filling materials: Real Seal (SybronEndo, Orange, CA, USA) and Thermafil (Tulsa Dental, Tulsa, OK, USA).

Material and Methods: Periodontal ligament cells from healthy patients were cultured. The eluate of Real Seal 1™ (RS1), Real Seal (RS) and Thermafil (TF) samples was used for the cells viability tests, both diluted (50%) or undiluted (100%). Incubation of the specimens was performed in culture medium for 24 h, 48 h and 72 h at 37 °C under sterile conditions. The cellular mortality was evaluated by MTT test. Results were statistically analysed and the statistical significance was set at $p < 0.05$.

Results: None of the studied materials showed toxic effects during the period of observation (0 -72 h) when compared to the control group. Only RS induced a very modest increase in cell mortality (about 3% at both concentrations used, during the first 24 hours), when increasing the incubation time, however, only the lower concentration continued to show modest toxicity.

Conclusions: Results of the present study showed that all tested materials did not exhibit cytotoxic effects when compared to the control group.

Key Words: Cytotoxicity, root canal, filling materials.

INTRODUCTION

The biocompatibility of root canal filling materials is of importance because the components released from the latter can get in contact [1] –with the periradicular connective tissue producing irritation or degeneration even of the surrounding tissues [2]. Ideally, a root canal filling material, in addition to suitable chemical and physical properties, should be biologically compatible and well tolerated by the periapical tissues. This will avoid any possible modification and delay of the healing process.

In vitro tests – although not exhaustive for a conclusive clinical evaluation –are suitable for a careful evaluation of the interactions between the components of these materials, allowing a separate analysis of the different metabolic aspects not obtainable by *in vivo* trials [3]. *In vitro* tests, characterized by speed, sensitivity and reproducibility, can be performed both directly and through analysis of the eluate [4, 5] using cell culture [3, 6] such as permanent cell lines (i.e. 3T3 cells) and/or primary cells (oral fibroblasts). Human fibroblasts reproduce the *in vivo* behaviour of oral mucosa [3, 5, 8, 9] representing so a suitable model for preliminary studies regarding the possible cytotoxic effects of root filling materials [5,7].

Gutta-percha is the most common component used in root canal filling materials because it is well tolerated from host tissues [10] but other compounds such as zinc-oxide,eugenol are capable of inducing cytotoxic effects [11-13]. Recently, a new endodontic filling material based on a polyester thermoplastic-filled polymer (Resilon™; Resilon Research LLC, Madison, CT), which looks and performs like gutta-percha, has been developed and put on the market. Resilon™ cones (Real Seal™, SybronEndo, Orange, CA, USA) contain bioactive glass and radiopaque fillers. They have the same handling properties and, for retreatment purposes, can be softened with heat or dissolved with solvents like chloroform. Resilon™ is used in conjunction with a self-etching primer, which contains sulfonic acid terminated functional monomer, hydroxyethyl methacrylate (HEMA), water, and a polymerization initiator. Real Seal™ is a dual-cured resin-based root canal sealer, which forms a bond between the dentin walls and the Resilon core, commonly referred as "monoblock". More recently Real Seal

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1™ (SybronEndo, Orange, CA, USA), a new product derived by an improvement of Resilon™ technology, has become available: it is a carrier-based filling material, in which all components, sealer, thermoplastic filler and carrier, are resin-based. RealSeal 1™ also introduces a new self-etching, resin-based sealer, which eliminates the priming step, necessary using the original system.

As the purpose of the development of new endodontic filling materials is enhancing successful clinical applications, trials must be carried out to evaluate their cytotoxicity. Recent studies [13] showed satisfactory *in vitro* biocompatibility of both the new self-etching sealer Real Seal 1™ and Real Seal ™ filling materials. The first one showed a mild cytotoxic effect comparable with that of Pulp Canal Sealer (SybronEndo, Orange, CA, USA), a traditional zinc-oxide and eugenol based endodontic sealer currently used in endodontic practice [14]. Another study showed that both Real Seal ™ and gutta-percha points exhibited mild cytotoxic effects, with no statistically significant differences [15].

The aim of the present study was to investigate the cytotoxicity of Real Seal 1™ in comparison with some other commercially available endodontic filling materials eg Thermafil ™ (Tulsa Dental, Tulsa, OK, USA).

MATERIALS AND METHODS

All chemicals and reagents (cell culture grade) were supplied by Sigma-Aldrich Srl (Milan, Italy) unless otherwise indicated.

Cell Culture of Human Periodontal Ligament Fibroblasts

Periodontal ligament cells from healthy patients (obtained with informed consent and with approval from the Ethics Committee) were scraped from third molars extracted only for orthodontic reasons, and were enzymatically digested for 1 h at 37 °C in a solution of collagenase type I (3 mg/mL) and dispase (4 mg/mL). The cells were plated in tissue culture flasks (25 cm^2) with Dulbecco's Modificated Eagles' Medium (DMEM), supplemented with 10% foetal calf serum (FCS), L-glutamine (2 mmol/L), streptomycin (100.0 μg/mL) and penicillin (1000 units/mL), at 37 °C in humidified atmosphere (95% air, 5% CO_2). The medium was replaced before cells have formed the monolayer. Cells at sub-confluence, obtained with no more than 5 passages, were used in all experiments.

Canal Filling Materials

- Real Seal 1™(SybronEndo, Orange, CA, USA) is composed by a polysulfone based carrier with difunctional methacrylate resin, bioactive glass, radiopaque fillers and coloring agent.
- Real Seal™ (SybronEndo, Orange, CA, USA) is a mixture of UDMA (Urethane Dimethacrylate), PEGDMA (polyethyleneglycol dimethacrylate) and Bis-GMA(bisphenol A glycidyl methacrylate) resins, silane-treated barium borosilicate glasses, barium sulfate, silica, calcium hydroxide, bismuth oxychloride with amines, peroxide, photo initiator, stabilizers and pigments.
- Thermafil™ (Tulsa Dental, Tulsa, OK, USA) consists of a flexible central carrier coated with a layer of α-phase gutta-percha.

The eluate of Real Seal 1™ (RS1), Real Seal ™ (RS) and Thermafil ™ (TF) samples was used for the cell viability tests. The incubation of specimens was performed in culture medium without FCS (24 h, 48 h and 72 h, 37 °C, atmospheric pressure) under sterile conditions. The ratio between the sample surface and volume medium (0.5 cm^2/mL) was selected according to International Organization for Standardization (ISO) standards [16]. The incubation in absence of FCS was performed to avoid possible interaction between compounds released by the tested materials and serum components. After the incubation, 10% FCS was added to all extracts; the latter, diluted (50%) or undiluted, were then added to cell monolayers by medium change and similar volumes of DMEM were added also to the control wells (untreated cells). As positive control UDMA (10 μmol/L) treated cells were used. After 24 h of incubation (at 37 °C in humidified atmosphere), the cellular vitality was evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [17]. This is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The MTT enters into the cells and passes into the mitochondria where it is reduced to an insoluble, formazan product. Since reduction of MTT can only occur in metabolically active cells, the activity level represents a measure of their viability.

MTT Test

The MTT test was performed according to Wataha *et al.* [18]. A solution (20 μL) of MTT in PBS (phosphate buffer, 5 mg/mL) was added to the medium (200 μL) and, after incubation (4 h, 37°C) the intracellular formazan crystals produced were dissolved in a solution of HCl in isopropanol ($4x10^{-2}$ N, 200 μL). The optical density (OD) of the solution contained in each well was determined using an automatic microplate photometer (Packard Spectracount™, Packard BioScience Company, Meriden, USA) at a wavelength of 570 nm. Each experiment was performed in sextuplicate and the cell cytotoxicity was calculated according to the following equation [19]:

$$\text{Percentage of cell mortality} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100$$

Statistical Analysis

All results are expressed as Mean ± Standard Deviation. The group means were compared by analysis of variance (ANOVA) followed by a multiple comparison of means by Student-Newman-Keuls; if necessary, comparison of means by *t*-Student test was used. The statistical significance was set at $p < 0.05$.

RESULTS

The cytotoxic effects of the Real Seal 1™, Real Seal ™ and Thermafil ™ are shown in Table **1**. None of the examined materials showed statistically significant toxic effects during the period of observation (0 -72 h) when compared with the control group (Table **1**, $p > 0.05$, using ANOVA

Table 1. Percentage of Cell Mortality ± Standard Deviation Determined by MTT Test

Canal Filling Materials	24h	48h	72h
Real Seal 1™	0.05 ± 0.001	0.0	0.0
Real Seal™	2.5 ± 0.2	0.0	0.0
Thermafil™	0.06 ± 0.0015	0.0	0.0
Real Seal 1™ (50%)	0.0	0.0	0.0
Real Seal™ (50%)	3.3 ± 0.020	3.8 ± 0.025	1.5 ± 0.020
Thermafil™ (50%)	0.0	0.0	0.0

followed by a multiple comparison of means by Student-Newman-Keuls test). No differences due to the used experimental conditions (undiluted or 50 % diluted eluate) were detectable ($p > 0.05$ using *t*-Student test). Only Real Seal ™ induced a very small increase of cell mortality (about 3% in both reported experimental conditions, during the first 24 hours). When the the incubation time was increased, however, only the lower concentration continued to show a very mild toxicity. UDMA (used as positive control) induced a high toxicity in human periodontal ligament fibroblasts (data not shown).

DISCUSSION

In vitro cell cultures have been widely used to evaluate cytotoxicity of root canal filling materials. Since *in vitro* toxicity tests should be performed using the most appropriate cells [20,21], human primary periodontal ligament fibroblasts were used in this study.

Here the cytotoxicity of three different types of root canal filling materials (Real Seal ™, Real Seal 1™ and Thermafil ™) was examined using extracts of the specimens because this approach exhibits some advantages The choice of Thermafil ™ and Real Seal ™ cones was suggested because the former is the most common gutta-percha material using a carrier-based technique. There are some differences in composition and performance between RS and RS1 because the latter has been slightly modified to improve thermoplasticity, flow and adhesion to the carrier. None of these characteristics has been so far investigated in the dental literature. Although Thermafil ™ and Real Seal ™ are on the market for many years, only a few studies investigating their biocompatibility are reported [22, 23]. In a histopathologic study, Bodrumlu *et al.* [23] showed high tolerance of tissues to Resilon and gutta-percha after 60 days and that Resilon may serve as an alternative to gutta-percha in terms of biocompatibility. Resilon showed also an acceptable *in vivo* biocompatibility [24].

Donadio *et al.* reported, in two separate studies [25,26], showed that Resilon cones are more biocompatibles than regular GP and Activ GP cones [25]. Susini *et al.* [27] also reported that the cytotoxicity of Resilon + Epiphany sealer was due mainly to Epiphany and that this effect decreased after 2 days reaching a level comparable with that of the commonly used root canal sealers. In a recent study, Epiphany/Resilon root canal filling system showed satisfactory tissue reaction and therefore a good biocompatibility when tested in connective tissue of rats [28]. Cytotoxicity of Epiphany sealer and Resilon set has been reported as comparable with that of AH-Plus and gutta-percha [29].

The Results of the present study showed that all tested root canal filling materials did not exhibit cytotoxic effects when compared with the control group. The results concerning Real Seal ™ confirm the cytotoxicity data reported in literature [13,14]. A previous study showed that metal and plastic carriers of Thermafil ™ are not cytotoxic to fibroblasts [30]. This material was chosen in this study to obtain a direct comparison with carrier-based Real Seal 1™.

The results of the present study confirmed that plastic carriers of Thermafil™ are not cytotoxic. Since very little is present in the literature about the biocompatibility of Thermafil™, the results here reported are able to confirm the good biological properties of a material which has been successfully used in clinical practice for over 20 years. Furthermore, in the present study also RS1 carrier and filling material were shown to be not cytotoxic. Biocompatibility of the new RS1 filling material is similar to that of products which have been clinically used for many years; RS1 should be therefore used with the same precautions (i,e. avoiding overfilling and extrusion in the periradicular tissues) commonly adopted in routine endodontic practice. Furthermore, the choice among the above reported root canal filling materials should be based on other factors like user friendliness, simplicity of use, leakage over time, easiness of retreatment and post preparation procedures.

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