G It Endo - vol. 21 - n. 1, pp. 11-14
Gennaio-Marzo 2007

Gilberto Debellian
Gianluca Gambarini[1]

Associate Professor, Department of Endodontics, University of Oslo, Norway
[1] Professor of Endodontics, University of Rome "La Sapienza", Dental School,

Correspondence:
Dott. Gilberto Debellian
e-mail: gildeb@mac. com

Pervenuto in Redazione il 29 settembre 2006
Accettato per la pubblicazione il 5 dicembre 2006

In vitro cytotoxicity of a soft resin core filling material for canal obturation

ABSTRACT

Aim: a new endodontic obturation system (RealSeal, SybronEndo, Orange CA) has been recently advocated for successful filling of root canal. RealSeal (RS) consists of filling materials made of soft resin that look and handle like gutta-percha. The present investigation was designed to test and compare the cellular toxicity of the new soft resin core filling material (RS) *versus* traditional gutta-percha (GP) points (Roeko, Langenau, Germany), using NRU test.
Methodology: mouse 3T3 fibroblasts were seeded and cultured and subsequently dilution of RS and GP points were added. After 24h of incubation, the cellular vitality of fibroblasts was evaluated by neutral red uptake test (NRU) which measures the membrane permeability. Data were collected and statistically analyzed (t-Student test).
Results: mean cell mortality % was 26, 584 for RS (SD 3,295) and 25,101 for GP (SD 3,060).
Results showed that both tested materials exhibited mild cytoxic effects, but no statistically significant difference was noted between them.
Conclusions: taking into consideration the limitations of an *in vitro* experiment, both RS and GP points showed the same biological properties. Since GP is known to exhibit very low toxicity, RS points seem to be recommendable products for clinical use in endodontics.

Key words:
Gutta-percha, biocompatibility, endodontic obturation.

INTRODUCTION

Many materials have been advocated over decades for successful root canal obturation. Altough not the ideal solid core filling material, gutta-percha still is the material of choice (2), showing minimal toxicity and tissue irritability (13, 15), and satisfying the majority of requirements for an ideal root canal filling material described by Grossmann (4). It has been stated that gutta-percha (GP) is the most widely used root canal filling material because of its well-known low toxicity (9). In cases of inadvertent moderate overextension of guttapercha into periradicular tissues, it is considered to be clinically well tolerated as long the canal is adequately clean and sealed (3).
Among disadvantages of gutta-percha the lack of adhesiveness to canal walls and sealer is probably the most important. A hermetic seal cannot be obtained without the use of a sealer, because gutta-percha does not spontaneously bond to dentin walls. An ideal endodontic sealer should adhere firmly both to dentin and gutta-percha. Such adhesion would also be desiderable in stabilizing the apical seal during post-space preparation without didslodgement or loss of seal. However, recent studies showed how adhesion of endodontic sealers to gutta-percha can be poor (6). Some sealers exhibited very low bond strength (approxim. 0,20 Mpa) to gutta-percha, lower than sealer bond strengths to dentin (approx 0,30-0,80 Mpa). It has been demonstrated that despite the use of gutta-percha in conjunction with a sealer, materials and techniques currently used do not routinely provide an hermetic seal of the root canal system. All obturated canals leak to a greater or lesser extent over time (5). Torabinejad et al. (14) showed than when obturated canals where challanged by bacteria, 50% allowed penetration through the entire length of the canal within 30 days.
Recently, due to the increased amount of root canal treatments being performed by general practitioners and endodontic specialists, there have been renewed efforts to develop better sealer and core obturation materials and techniques. Research has been focused on new innovative materials that bind intimately together (sealer and points) and with canal walls, allowing a fluid-impervious, bacteria tigth -seal both apically and coronal. Among these new innovative materials dentin-bonded composite resins have shown very promising properties, in an attempt to fill root canals with a system that more resembles the typical coronal restoration (10).
Following these premises, a new endodontic obturation system (RealSeal, SybronEndo, Orange CA) has been recently advocated for successful filling of root canals. The RealSeal (RS) system can be used with present root canal filling techniques only substituting the RS materials for gutta-percha and sealer. It consists of core filling materials made of soft resin that look and handle like gutta-percha. They are supplied in points with a variety of tapers, as well as pellets, for heated filling methods. The RS filling materials can be thermoplasticized, like gutta-percha, but at lower tem-

peratures. It is highly radiopaque and retrievable with conventional solvents and files. Because the filling material is made of soft resin, it is bondable, after conditioning root canal walls with a primer. The primer will bond to the walls of the canal, the sealer will bond to the primer and the soft resin filling material will bond to the sealer. According to the manufacturer the bond both to the core resin material and the canal walls results in the creation of a monoblock, which resists leakage and strengthens the root. Some preliminar researches have been shown the new obturating system to seal the root canal significantly better than traditional filling materials (10).

Being a new endodontic material, very few researches have been published yet on biological properties of RS points. Where the aim of the development of new solid core filling materials is enhancing successful clinical applications and biocompatibility, trials must be conducted to evaluate the toxicity of the new products (1). Therefore the aim of the present study was to investigate the *in vitro* cytotoxicity of the new (RS) points and compare it with traditional GP points.

MATERIALS AND METHODS

The following standardized RS and GP points of greater tapers (size 04-15) were selected for the present study and used to test for possible effects on cell growth:

1. **RS** = RealSeal (SybronEndo, Orange CA). Composition of the new points sealer is a mixture made from polymers of polyester, with fillers and radiopacifiers in a soft resin matrix.
2. **GP =** Gutta-percha points of greater taper (Roeko, Langenau, Germany), Composition is approximately 20% gutta-percha, 50% zinc-oxide and various waxes, coloring agents, antioxidants and metallic salts.

Three millimeters from the tip were cut and weighed, in order to allow the same amount of tested materials to be put in contact with cells. Both materials were then UV sterilized and tested.

Each sample was immersed in DMEM (0. 4 mL) and left on site for 24h at 37°C; simultaneously mouse 3T3-Swiss fibroblasts (10. 000) were seeded on each well of a 96 wells plate and cultured to sub-confluent monolayer for 24 hours. After this period, DMEM extracts obtained from each sample were added to the monolayer and the mortality of cells, incubated for 24h, was then evaluated through NRU test.

A 0.4% water solution of neutral red was added to each well medium in a 1:80 ratio to obtain a neutral red concentration of 50 µg/mL. After incubation for 4h at 37°C, the supernatant was removed and the intracellular neutral red were solubilized with 200 µl of a water of solution of 50% ethanol with 1% acetic acid. The absorbance of each 96-well plate was determined using an automatic microplate photometer (Packard Spectracount™, Packard BioScience Company, Meriden U.S.A.) at 540 nm. The cell cytotoxicity for each experiment, performed in sestuplicate, was calculated according to the equation:

Statistical analysis was performed as follows**:** each value represents the mean of three experiments, using three replicates of each material per experiment. All results are expressed as mean SEM; the group means were compared by t-Student test and $p < 0.05$ was considered significant.

$$\% \text{ cell mortality} = \frac{\text{Control OD} - \text{sample OD}}{\text{Control OD}} \times 100$$

RESULTS

Results are shown in Table 1 and Figure 1, which summarize cell toxicity of the different core filling materials. The higher the percentage of cell mortality, the higher the toxicity of the points. Mean cell mortality % was 26,584 for RS (SD 3,295) and 25,101 for GP (SD 3,060). All tested materials showed mild cytotoxic effects, but no statistically significant difference was noted between them when compared by t-Student.

DISCUSSION

Gutta-percha (and sealer) has historically proven to be the material of choice for the successful three-dimensional filling of the entire canal (8), eliminating avenues for leakage from the oral cavity or the periradicular tissues into endodontic space. As a consequence gutta-percha has been the most widely used endodontic core filling material, also because of its well-known low toxicity. Therefore if new obturation techniques and materials were to be improved over the standard ones used today, the new materials should increase the ability to seal the canal without affecting the currently available good biological properties. Following these premises, the result of this study showed that in the present test conditions both tested materials (GP and RS) showed cytotoxic effects, but no statistically significant difference was noted between them. Since GP is known to be well tolerated by periradicular tissues (7), these preliminar findings support the hypothesis of safe clinical use of the new RS material, even if more *in vivo* and clinical studies must be performed to assess its biological properties.

Biocompatibility of gutta-percha and endodontic sealers has been extensively evaluated in the last decades. Pascon

Cell mortality	%
RealSeal	Guttapercha
MEAN	MEAN
26,584°	25,101°
SD	SD
3,295	3,060
°NS	°NS

Tab. 1 - *Results of the NRU test.*
Risultati del Test NRU (mortalità cellulare %).

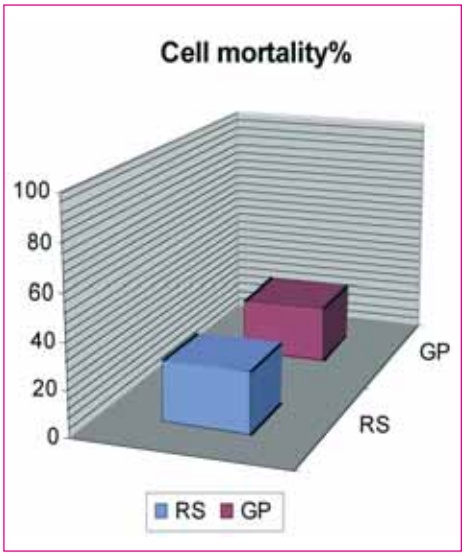

Fig. 1 - *Graphical results of the NRU test.*
Grafico dei risultati del Test NRU (mortalità cellulare).

and Spanberg (9) evaluated the toxicity of marketed endodontic GP using the radiochromium release test. Fourteen commercially available and three experimental GP brands were tested, plus raw GP, zinc oxide, and barium sulfate, which were considered major components of GP points. Results showed that the raw materials and barium sulfate were not toxic, whereas zinc oxide and zinc ions showed marked toxicity. All GP points tested showed good biocompatibility at 24 hours, but were toxic at long observation periods. This toxicity was attributed to leakage of zinc ions into the fluids.
These results were confirmed by Szep et al. (12), who tested the cellular toxicity of two medicated and four non-medicated brands of gutta-percha points, including the one selected for the present study. Results showed that cytotoxicity of the points containing calcium hydroxide was not significantly different from all other points tested, with the exception of those containing chlorhexidine, and that all tested gutta-percha materials caused cytotoxic reactions in varying extents. The data of the present study confirm these findings, showing cytotoxic effects in both tested materials.
Zmener et al. (15) evaluated the effects of two gutta-percha formulations and one zinc oxide-eugenol and Canada balsam-based endodontic material on the behavior of a mixed cell population of human monocytes and lymphocytes. Results showed that Ultrafil and the standard gutta-percha cones showed little or no adverse effects, whereas the inhibitory effects of Endoseal appeared to be severe. Moreover, differences between all tested materials and their respective controls were also statistically significant, showing that *in vitro* testing can be an useful tool for preliminar screening of biological properties of new root canal filling materials.
Sjogren et al. (11) evaluated tissue reaction to gutta-percha using subcutaneously implanted teflon cages in guinea pigs. GP was tested in three forms: (i) as large particles prepared by dividing gutta-percha cones into pieces, (ii) as fine particles prepared by ball-milling of gutta-percha, and (iii) as particles produced by dissolving gutta-percha in rosin-chloroform. Results showed that GP evoked two distinct types of tissue response. The large pieces were well encapsulated and the surrounding tissue was free of inflammation. The fine particles evoked an intense, localized tissue response, characterized by the presence of macrophages and multinucleated giant cells. The rosin-chloroform treated gutta-percha induced a similar tissue reaction to that observed with the fine particles of gutta-percha. In addition, cell remnants were present in association with the material, which indicates an initial toxicity to rosin-chloroform treated gutta-percha. These results showed that the size and surface character of gutta-percha can determine the type of tissue reaction to the material. Moreover the accumulation of macrophages around gutta-percha was considered to be an important factor in the impairment of healing of periapical lesions when teeth were root filled with excess material.
These above-mentioned studies clearly show that biocompatibility needs comprehensive evaluation, since many factors can influence biological response to endodontic filling materials. Theferore, the present study must be considered as a preliminar research on biological properties of a new material, which will undergo further investigation. Nevertheless *in vitro* results are satisfactory: the tested materials (RS and GP) showed similar cytotoxic effects, and no statistically significant difference was noted between them. Since GP is known to exhibit very low toxicity, these preliminar data support the hypothesis of safe clinical use of the new RS material.

REFERENCES

1. Gambarini G, Andreasi-Bassi M, Bolognini G, Testarelli L, Nocca G, Ceccarelli L, Scatena R, Lupi A, Castagnola M. Cytotoxicity of a new endodontic filling materials. *Australian Endodontic Journal 2003;1(29):17-19.*
2. Gambarini G, Tagger M. Sealing ability of a new hydroxiapatite-containing endodontic sealer using lateral condensation and thermatic compaction of gutta-percha, *in vitro. J Endod 1996;22(4):165-7.*
3. Geurtsen W, Leyhausen G. Biological aspects of root canal filling materials-histocompatibility, cytotoxicity, and mutagenicity. *Clin Oral Investig. 1997;1(1):5-11.*
4. Grossman L. Physical properties of root canal cements. *J Endod 1976;2:166-75.*
5. Gutmann JL. Clinical, radiographic and histological perspectives on success and failure in endodontics. *Dent Clin North Am 1992;36:379.*
6. Lee KW, Williams MC, Camps JJ, Pashley DH. Adhesion of endodontic sealers to dentin and gutta-percha. *J Endod 2002;28: 684-8.*
7. Leonardo MR, Utrilla LS, Rothier A, Leonardo RT, Consolaro A. Comparison of subcutaneous connective tissue responses among three different formulations of gutta-percha used in thermatic techniques. *Int Endod J 1990;23(4):211-7.*
8. Orstavik D. Endodontic materials. *Adv Dent Res 1988;2:12-24.*
9. Pascon EA, Spangberg LS. *In vitro* cytotoxicity of root canal filling materials: 1. Gutta-percha. *J Endod 1990;16(9):429-33.*

10. Shipper G, Orstavik D, Teixeira FB, Trope M. An Evaluation of Microbial Leakage in Roots Filled with a Thermoplastic Synthetic Polymer-Based Root Canal Filling Material (Resilon). *J Endod 2004;30(5):342-5*.
11. Sjogren U, Sundqvist G, Nair PN. Tissue reaction to gutta-percha particles of various sizes when implanted subcutaneously in guinea pigs. *Eur J Oral Sci 1995;103(5): 313-21*.
12. Szep S, Grumann L, Ronge K, Schriever A, Schultze M, Heidemann D. *In vitro* cytotoxicity of medicated and nonmedicated gutta-percha points in cultures of gingival fibroblasts. *J Endod 2003; 29(1):36-40*.
13. Tavares T, Soares IJ, Silveira NL. Reaction of rat subcutaneous tissue to implants of gutta-percha for endodontic use. *Endod Dent Traumatol 1994;10(4):174-8*.
14. Torabinejad M, Ung B, Kettering JD. *In vitro* bacterial penetration of coronally unsealed endodontically treated teeth. *J Endod 1990;16:566-9*.
15. Zmener O, Goldberg F, Cabrini RL. Effects of two gutta-percha formulations and one zinc oxide-eugenol and Canada balsam mixture on human blood monocytes and lymphocytes. *Endod Dent Traumatol 1989; 5(2):73-7*.