ABSTRACT

Objective: The aim of the current study is to evaluate the cytotoxic effect of MTA Bioseal root canal sealer (Itena-clinical, Villepinte, France) with AH + epoxy resin-based sealer (Dentsply Sirona, Germany) and ADSEAL resin sealer (Meta Biomed, Korea).

Methods: In sterile test tubes, the tested sealers were consecutively diluted twice. To evaluate the proper concentration where fibroblast cells would survive, extraction media were tempered many times using cell Dulbecco’s Modified Eagle’s Medium (DMEM) following the ISO standards. The cytotoxic effect of concentrations used in the present study (12.5%, 25%, 50%, and 100%) were evaluated by Methyl Thiazol Tetrazolium (MTT) essay after 24, 72 hours, and one week using a human fibroblast cell line. Statistical analysis was performed by using One Way ANOVA test, followed by Tukey’s Post Hoc test for multiple comparisons.

Results: AH Plus and MTA Bioseal showed generally similar behavior. The highest cytotoxicity was recorded for both sealers at conc.100mg/ml in the first 24 hours. Afterwards the mean percentage of cell viability increased progressively with the decrease in concentration. While ADSEAL showed a nearly constant cytotoxicity that is insignificantly affected by the time factor at the four experimental concentrations used in the study.

Conclusion: AH Plus had the lowest cytotoxic effect followed by MTA Bioseal. However, both sealers showed parallel behavior of decreased cytotoxicity with increasing test time period. While ADSEAL had the highest cytotoxicity with statistically insignificant effect of time intervals or concentrations.

KEYWORDS: MTA Bioseal, AH Plus, Cytotoxicity, ADSEAL, Resin sealers.
INTRODUCTION

Three dimensional obturation is considered the cornerstone of root canal treatment. This is essential to generate an intimate, void-free contact between the canal walls and the obturating material. An environment that is conducive for prevention of leakage and/or recurrent infection. Ultimate healing should inevitably be anticipated.\(^1,2\)

Root canal systems are principally obturated by gutta-percha as a core material and different types of sealers. These sealers should possess an antimicrobial and bacteriostatic action.\(^2,3\)

Among the biological requirements of a supreme sealer is to impart excellent sealing properties at the microscopic level so that a complete barrier against bacterial seepage and invasion will be guaranteed. Meanwhile, a negligible cytotoxic effect on the host tissue cells should be there.\(^3\) Selection of the most appropriate or optimum sealer that is suitable to the diverse clinical presentations is a challenge in the modern days.\(^3\)

Nevertheless, the effect of root canal sealers on periodontal fibroblast cells are well documented.\(^4\) Fibroblast cells can conscript inflammatory cells, articulate pro-inflammatory cytokines, growth factors, chemokines, and antimicrobial peptides. Again, a reported immunological qualities was linked to fibroblast.\(^4,5\)

Among the multi-functions of fibroblast cells is its central role in regeneration of a firm link at the root/periodontal ligament interface. Nevertheless, components of some sealers might have cytotoxic action to human fibroblast cells. A delayed wound healing was reported to be an immediate effect that results from extrusion of some unset sealers periapically. This was proofed to occur especially prior to complete sealer setting.\(^6,7,8\)

Abundant types of sealers are present in the market. Among them, eugenol-based sealers, resin-based sealers (AH26 and AH plus), and calcium silicate-based sealers are currently the mostly used sealers.\(^9,10\)

AH Plus resin sealer is taken as a gold standard against which most other sealers are compared irrespective of their different active ingredients. Although it is a modification of the older AH 26, where the formaldehyde was removed; a recent study revealed its presence in the set sealer. This occurred during its setting, and reported once to be in the range of 3.8 ppm.\(^11-14\)

ADSEAL is an epoxy resin sealer that belongs to the resin sealers’ group with a reported biocompatibility added to its very high sealing ability. However, related conflicting results were found in the literature.\(^13-15\)

Recently, there is a growing demand of using bioceramic sealers especially due to their high degree of hydrophilicity, biocompatibility, antimicrobial action,\(^9,10\) and high alkalinity (1). MTA Bioseal is a bioceramic sealer that was reported to serve dual targets of filling as well as sealing\(^16,17\).

MTA Bioseal is a root canal sealer with MTA that was recently introduced with claims prevention of bacterial proliferation\(^18\). To our knowledge, the cytotoxic effect of this relatively new MTA Bioseal formulation was deficient and not investigated before.

The aim of the present study was to evaluate -in-vitro- the cytotoxicity of a bioceramic sealer (MTA Bioseal) in comparison with two resin sealers, namely, ADSEAL and AH Plus-the gold standard as a control on human fibroblast cell line.

The tested null hypotheses were no differences in the cytotoxicity among the experimented materials or their set dilutions.

MATERIALS AND METHODS

The present study was approved by the research ethics committee of faculty of dentistry, October 6 University, Giza, Egypt (Approval Number RECO6U/33-2023).
Sample size calculation:

Calculation of the sample size was made following a previous study Nashaat et al. (8). Accordingly, the probability of exposure among controls is 84.7%. If the estimated probability of exposure among cases is 11%, 8 cases will be needed per group with probability (power) of 0.8. A Type I error probability of 0.05 was associated with this test of this null hypothesis. Accordingly, the total sample size was adjusted to 10 subjects per group to compensate the 20% drop out. Sample size was calculated by means of PS power 3.1.6.

Grouping and Cytotoxicity Assay (MTT assay)

Samples were divided into 3 groups of 10 samples each: group (A) ADSEAL (Meta Biomed, Korea) resin sealer, group (B) AH PLUS (Dentsply Sirona, Germany) and group (C) MTA Bioseal (Itena-clinical, Villepinte, France) in three evaluation periods namely (24h, 72h and 1 week).

Cell culture

The cytotoxic effect of the root canal sealers used in this study was evaluated on human fibroblast cell line (2x10^5) this cell line was supplied from Department of Cell Culture at Vacsera-Egypt. Standard protocols were followed in establishing and maintaining the cultures.

All samples of root canal sealers were mixed according to their related manufacturer’s instructions in standard size Teflon molds. Formed discs were allowed to set at 37°C in a 5% CO₂ environment for 24 hours. Careful dismantling of the sealer blocks was done after sealers’ setting confirmation. Samples were exposed to UV light for 24 hours for sterility confirmation. (8)

In sterile test tubes, the tested sealers were consecutively diluted twice. In order to evaluate the proper concentration where fibroblast cells would survive, extraction media were tempered many times using cell Dulbecco’s Modified Eagle’s Medium (DMEM) following the ISO standards (8)

Cytotoxicity and cell viability calculation:

The cytotoxic effect of concentrations used in the present study (12.5%, 25%, 50%, and 100%) were evaluated by Methyl Thiazol Tetrazolium (MTT) essay after 24, 72 hours, and one week using a human fibroblast cell line.

For calculation of cell viability MTT assay was used (9, 10). The percentage of live and healthy cells to the total number of cells in a sample, indicating cell health and functionality.

Statistical analysis

Statistical analysis was performed with SPSS 16® (Statistical Package for Scientific Studies), Graph pad prism & windows excel, while comparison between different groups was performed by using One Way ANOVA test followed by Tukey’s Post Hoc test for multiple comparison. Comparison between different intervals within the same group was performed by using Repetitive One Way ANOVA.

The viability percentage was calculated using the following equation:

Viability percentage = Mean OD of Test Dilution X100/ Mean OD of Neg. Control

RESULTS:

Cytotoxicity at concentration 100mg/ml results are presented in (table 1, figure 1):

At 24 hours: the highest cytotoxicity was detected for ADSEAL, followed by MTA Bioseal then AH plus in a descending order. The differences between the three tested sealers were statistically significant (table 1).

At 72 hours: Significant increase in cytotoxicity was noted for both AH plus and MTA Bioseal. A gradual insignificant increase in the cytotoxicity was noted after 72h for ADSEAL.
After 7 days: cytotoxicity increased for all tested groups. The increase was significant for both AH plus and MTA Bioseal (p<0.0001) and (p<0.0002) respectively as compared to the 24h and 72h. The increase in cytotoxicity was insignificant for ADSEAL group (figure 1).

The intragroup comparison revealed a statistically significant progressive reduction in the percentage of cell viability for AH plus and MTA Bioseal (table 1, figure 1). The ADSEAL showed insignificant increase in cytotoxicity that remained nearly stable with time (figure 1).

**Cytotoxicity at concentration 50 mg/ml results are presented in (table 2, figure 2):**

At 24 hours: the highest percent of cell viability was detected for AH plus; this was followed by MTA Bioseal then ADSEAL in a descending order. The differences between the three tested sealers were statistically significant P<0.0001 (table 2).

At 72 hours: Significant increase in cytotoxicity was noted for both AH plus and MTA Bioseal. A minute insignificant increase in the cytotoxicity was noted after 72h for ADSEAL.

After 7 days: cytotoxicity increased for all the tested groups. The increase was significant for both AH plus and MTA Bioseal (p<0.0001) as compared to the 24h and 72h. The increase in cytotoxicity was insignificant for ADSEAL group (figure 2).

The intra-group comparison revealed a statistically significant progressive reduction in cell viability for AH plus and MTA Bioseal at 24h, 72 h and 7 days P<0.0001 (table 2). For ADSEAL, insignificant reduction in cell viability was found P=0.01 (figure 2).

**Cytotoxicity at concentration 25 mg/ml results are presented in (table 3, figure 3):**

At 24 hours: the least cytotoxicity was detected for AH plus; this was followed by MTA Bioseal then ADSEAL in a descending order. The differences between the three tested sealers were statistically significant P<0.0001 (table 3).

At 72 hours: cytotoxicity remained nearly stationary for AH plus and MTA Bioseal with insignificant difference. While a significant decrease in cytotoxicity was noted for ADSEAL.

After 7 days: a significant marked increase in cytotoxicity was noted for the AH plus group and ADSEAL group. The MTA Bioseal group showed a non-significant decrease in cytotoxicity (table 3 and figure 3).

**TABLE (1) Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 100mg/ml:**

<table>
<thead>
<tr>
<th>Group</th>
<th>24 h</th>
<th>72 h</th>
<th>7 days</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>ADSEAL</td>
<td>9.91&lt;sup&gt;A&lt;/sup&gt; 1.64</td>
<td>9.39&lt;sup&gt;A&lt;/sup&gt; 1.57</td>
<td>8.85&lt;sup&gt;A&lt;/sup&gt; 1.11</td>
<td>0.86 ns</td>
</tr>
<tr>
<td>AH plus</td>
<td>21.73&lt;sup&gt;B&lt;/sup&gt; 1.95</td>
<td>13.20&lt;sup&gt;B&lt;/sup&gt; 1.56</td>
<td>8.97&lt;sup&gt;C&lt;/sup&gt; 2.31</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>MTA Bioseal</td>
<td>17.90&lt;sup&gt;C&lt;/sup&gt; 1.15</td>
<td>10.80&lt;sup&gt;AB&lt;/sup&gt; 2.69</td>
<td>7.54&lt;sup&gt;A&lt;/sup&gt; 2.23</td>
<td>0.0002*</td>
</tr>
</tbody>
</table>

P value: <0.0001* 0.003* 0.29 ns

M: mean  SD: standard deviation  *Significant difference as P<0.05
ns: non-significant difference as P>0.05. Means with different capital letter per column were significantly different as P<0.05.
Means with different small letter per row were significantly different as P<0.05.
CYTOTOXICITY OF BIOCERAMIC AND RESIN BASED ENDODONTIC SEALERS

The intragroup comparison revealed a statistically significant difference in each of the three tested sealers at 24h, 72h and 7 days $P<0.0001$ (table 3, figure 3).

**Cytotoxicity at concentration 12.5 mg/ml results are presented in (table 4, figure 4):**

At 24 hours: A significantly highest percent of viability value was detected for AH plus Sealer (table 4). This was followed by MTA Bioseal and ADSEAL in a descending order ($P<0.0001$).

At 72 hours: a significant increase in cytotoxicity was detected with ADSeal. AH plus remained nearly stationary, while MTA Bioseal showed gradual increase in cytotoxicity which was statistically significant ($P<0.0001$).

After 7 days: a significant increase in cytotoxicity was noted for the ADSEAL and MTA Bioseal group. The AH plus group showed an insignificant increase in the cytotoxicity (table 4 and figure 4).

The intragroup comparison revealed a statistically significant difference in each of the three tested sealers at 24h, 72h and 7 days $P<0.001$ (table 4, figure 4).

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**TABLE (2) Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 50mg/ml:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Conc. 50 mg/ml</th>
<th>24 h M (SD)</th>
<th>72 h M (SD)</th>
<th>7 days M (SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADSEAL</td>
<td>11.51a</td>
<td>0.56</td>
<td>10.63a</td>
<td>3.18</td>
<td>8.49a</td>
</tr>
<tr>
<td>AH plus</td>
<td>45.01b</td>
<td>4.54</td>
<td>39.96b</td>
<td>3.34</td>
<td>39.47b</td>
</tr>
<tr>
<td>MTA Bioseal</td>
<td>36.72c</td>
<td>3.39</td>
<td>28.78bc</td>
<td>2.24</td>
<td>22.39c</td>
</tr>
</tbody>
</table>

**P value:**

- $<0.0001^*$
- $<0.001^*$
- $<0.001^*$

*Significant difference as $P<0.05$  
ns: non-significant difference as $P>0.05$.

Means with different capital letter per column were significantly different as $P<0.05$.  
Means with different small letter per row were significantly different as $P<0.05$.

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**Fig. (1):** line chart showing Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 100mg/ml.

**Fig. (2):** line chart showing Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 50mg/ml.
TABLE (3): Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 25 mg/ml

<table>
<thead>
<tr>
<th>Group</th>
<th>24 h M</th>
<th>24 h SD</th>
<th>72 h M</th>
<th>72 h SD</th>
<th>7 days M</th>
<th>7 days SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADSEAL</td>
<td>12.76^A</td>
<td>1.40</td>
<td>14.55^A</td>
<td>1.03</td>
<td>10.72^A</td>
<td>3.02</td>
<td>0.03*</td>
</tr>
<tr>
<td>AH plus</td>
<td>84.08^B</td>
<td>3.37</td>
<td>83.95^B</td>
<td>11.66</td>
<td>48.29^B</td>
<td>4.45</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>MTA Bioseal</td>
<td>36.69^C</td>
<td>1.30</td>
<td>36.91^C</td>
<td>2.34</td>
<td>38.29^C</td>
<td>2.94</td>
<td>0.18 ns</td>
</tr>
</tbody>
</table>

M: mean  SD: standard deviation  *Significant difference as P<0.05  ns: non-significant difference as P>0.05.
Means with different capital letter per column were significantly different as P<0.05.
Means with different small letter per row were significantly different as P<0.05

TABLE (4): Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 12.5 mg/ml

<table>
<thead>
<tr>
<th>Group</th>
<th>24 h M</th>
<th>24 h SD</th>
<th>72 h M</th>
<th>72 h SD</th>
<th>7 days M</th>
<th>7 days SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADSEAL</td>
<td>17.16^A</td>
<td>0.90</td>
<td>14.11^A</td>
<td>3.60</td>
<td>12.16^A</td>
<td>1.32</td>
<td>0.01*</td>
</tr>
<tr>
<td>AH plus</td>
<td>101.49^B</td>
<td>1.72</td>
<td>104.68^B</td>
<td>8.00</td>
<td>100.35^A</td>
<td>4.88</td>
<td>0.35 ns</td>
</tr>
<tr>
<td>MTA Bioseal</td>
<td>89.75^C</td>
<td>2.25</td>
<td>81.69^C</td>
<td>4.78</td>
<td>59.76^C</td>
<td>3.93</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

M: mean  SD: standard deviation  *Significant difference as P<0.05  ns: non-significant difference as P>0.05.
Means with different capital letter per column were significantly different as P<0.05.
Means with different small letter per row were significantly different as P<0.05

Fig. (3) Line chart showing Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 25 mg/ml.

Fig. (4) Line chart showing Cytotoxicity test results measured by % of viable cells of all groups at different time intervals for concentration 12.5 mg/ml.
DISCUSSION

Three dimensional obturation of the root canal system necessitates the dual use of core and sealer materials. Selection of a sealer material with good physical, chemical and biological properties is mandatory. This is due to the probability of sealer extrusion periapically. In such cases, even if it is an immune response, possible inflammatory reaction might occur. Fibroblast is considered an immune-regulatory cell, and the principle type of cells of the periodontal ligaments. (20)

Abundant types of sealers are present in the market, each with specific ingredients and chemical composition. These formulations target better canal sealing with superior biocompatibility. (2) Nonetheless, no sealer can fulfill all these requirements. (21)

The shifting in the obturation concept from core based to a sealer based changed the concept and ideal requirements for a multifunctional targeted sealer. (21).

In the present study ADSEAL and MTA Bioseal were investigated for their cytotoxicity effect on fibroblast cell lines. Their behaviors were compared to AH Plus – the gold standard for comparison in most similar researches. (1, 15)(16)(15)(25)(22). In fact, for the MTA Bioseal only one study was found in the literature concerning its physico-chemical properties (23). They recommended further studies concerning its cytotoxicity to add clarification and evidence building.

Dulbecco’s modified eagles medium (DMEM) was selected according to Moore et al. (24) instruction guide. It is a basal medium that supports the growth of many different cells including primary fibroblasts. Again, most similar studies were using this essential medium. So comparison between the different results will be based on standard protocols. (8,9,15,24).

Results showed that cytotoxicity -as a reflection of cell viability- differed for investigated sealer types. Meanwhile, each tested sealer showed different behaviors as related to the different concentrations.

Results of the present study showed that the mean percent of cell viability increased progressively with the decrease in concentration.

The ADSEAL showed a nearly constant cytotoxicity that is insignificantly affected by the time factor at the four experimental concentrations used in the study.

Our results for ADSEAL were in agreement with Mostajeran et al. (25). However, they were in contrary with Kim et al., (26) who stated that ADSEAL leads to an increase in cell viability whether it was set or not due to its fast setting time (27). Previous researches have proved the connection between cytotoxicity and sealer setting time (28,29) where the unset sealers showed an undefined boundary of decolorized zones and severe cytotoxicity.

AH Plus and MTA Bioseal showed generally similar behavior with superior cytotoxicity recorded for the MTA Bioseal. The highest cytotoxicity was recorded for both sealers at conc.100mg/ml in the first 24 hours. Afterwards a progressive decrease in the cytotoxicity with decreasing concentration to 50mg/ml. Similar results were reported by Donnermeyer et al. (9,10). However, our results were in contrary with Canedo et al., (31) who reported that bioceramic sealer has higher biocompatibility than resin sealer. Interestingly, in a mega survey on MTA newer products and assemblies, it was reported that the aluminum percentage of MTA Bioseal was lowered to ≈0.05 wt%. This might explain the findings of our research. (32)

The production of Ca (OH)₂ by the hydration reaction may lead to high alkalinity of the MTA Bioseal. Ca (OH)₂ separates into OH⁻ and Ca²⁺, which, in turn, supports osteogenic potential and antibacterial activity. Nonetheless, the extended alkalinity of the MTA-based sealer solutions may be regarded as a cause of cytotoxicity, resulting in the degradation of proteins and the denaturation of cell membranes by enzymes (33). The increase in Ca
wt% on the sealers’ surfaces may be an indicator for the cytotoxic effect.

Interestingly, the AH Plus had somewhat extraordinary behavior at a concentration of 12.5 mg/ml. At that concentration, the percent of viable cells was found to surpass 100 mg/ml (table 3 and figure 3). Similar recognition was denoted in previous studies (8, 34-37). This was reported to be due to the fact that, some essays depend on a mitochondrial reductase to convert the tetrazole to formazan (32, 34).

CONCLUSIONS

In the present study the degree of cell viability is inversely related to percent concentration.

AH Plus had the lowest cytotoxic effect followed by MTA Bioseal. However, both sealers showed parallel behavior of decreased cytotoxicity with increasing test time period.

While ADSEAL had the highest cytotoxicity with statistically insignificant effect of time intervals or concentrations.

REFERENCES


