Reactions of Connective Tissue to Amalgam, Intermediate Restorative Material, Mineral Trioxide Aggregate, and Mineral Trioxide Aggregate Mixed With Chlorhexidine

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Abstract

The aim of this study was to histopathologically examine the biocompatibility of the high-copper amalgam, intermediate restorative material (IRM), mineral trioxide aggregate (MTA), and MTA mixed with chlorhexidine (CHX). This study was conducted to observe the rat subcutaneous connective tissue reaction to the implanted tubes filled with amalgam, IRM, MTA, and MTA mixed with CHX. The animals were sacrificed 15, 30, and 60 days after the implantation procedure. The implant sites were excised and prepared for histological evaluation. Sections of 5 to 6 μm thickness were cut by a microtome and stained with hemotoxylin eosin and examined under a light microscope. The inflammatory reactions were categorized as weak (none or few inflammatory cells $\leq$25 cells), moderate ($>$25 cells), and severe (a lot of inflammatory cells not to be counted, giant cells, and granulation tissue). Thickness of fibrous capsules measured five different areas by the digital imaging and the mean values were scored. Amalgam, IRM, and MTA mixed with CHX caused a weak inflammatory response on days 15, 30, and 60. MTA provoked an initial severe inflammatory response that subsided at the 30 and 60 day study period. A clear fibrous capsule was observed beginning from the 15 days in all of the groups. Within the limits of this study, amalgam, IRM, MTA, and MTA mixed with CHX materials were surrounded by fibrous connective tissue indicated that they were well tolerated by the tissues, therefore, MTA/CHX seemed to be biocompatible. *(J Endod 2006;32:1094–1096)*

Key Words

Biocompatibility, endodontic surgery, root-end filling

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Periapical surgery usually consists of root-end resection and root-end filling to seal the communication between the periapical tissues and the root canal system (1). Root-end filling should prevent leakage of microorganisms and provide an effective environment for healing of the periradicular tissues (2). Different root-end filling materials have been used for retrofilling in periapical surgery.

Amalgam was considered the root-end filling material of choice for many years; however, it has a number of disadvantages such as the potential for mercury and other ion release, corrosion and electrolysis, delayed expansion, marginal leakage, and tattoo formation (3). Intermediate restorative material (IRM) has also been advocated as a retrofilling material and was found to be resistant to leakage in in vitro studies (4, 5). Mineral trioxide aggregate (MTA) was introduced to endodontics by Torabinejad et al. in 1993. Studies have reported that MTA is biocompatible with the surrounding tissues (6, 7). It was shown to promote osteoblast activity (8), was less cytotoxic than amalgam, IRM, or SuperEBA (9, 10), and had an antimicrobial effect (11).

Chlorhexidine (CHX) is a cationic antimicrobial agent and it has been used as a root canal irrigant (12) and in the treatment of periodontal diseases. The substitution of 0.12% CHX gluconate for sterile water in tooth-colored MTA has enhanced the antimicrobial effect of the MTA in vitro (13). However, a recent study reported an increased cytotoxicity by the substitution of CHX for sterile water in MTA (14).

The purpose of this study was to histopathologically examine the biocompatibility of the high-copper amalgam, IRM, MTA, and MTA mixed with CHX, by implanting them into the subcutaneous connective tissue of rats for 15, 30, and 60 days.

Materials and Methods

In the study, 30 male, 5 to 6 month-old Wistar Albino rats, each weighing 270 $\pm$ 30 g. were used. Each animal was anesthetized by intraperitoneal injection of pentobarbitone with a dose of approximately 0.6 ml per 250 g weight. The study protocol was approved by the University Institutional Review Board. The following materials were investigated

Group 1: ProRoot, MTA (Dentsply, Tulsa Dental, Tulsa, OK).

Group 2: Oralloy, high-copper amalgam (Coltene AG, Altstatten, Switzerland).

Group 3: IRM (Dentsply, Konstanz, Germany).

Group 4: Proroot MTA mixed with Klorhex, 0.12% CHX Gluconate (Drogsan, İstanbul, Turkey).

Group 5: Control Group

The test materials were placed in clean, sterile polyethylene tubes with 1.1 mm inner diameter and 10 mm length, and immediately implanted subcutaneously in the dorsal region of 30 rats. All rats received all five groups. Amalgam, IRM, and MTA were prepared according to the recommendations of the manufacturers. MTA were mixed with Klorhex including 0.12% CHX gluconate. Empty polyethylene tubes were used as controls. Implantation of the materials into the rats was performed as follows: the dorsal skin of animals were shaved and disinfected with a 5% solution of iodine, to prevent interactions of materials the tubes were placed at least 2 cm from each other, and then

TABLE 1. Mean thickness values of fibrous connective tissue capsule forming around the tubes according to the periods

Implant material	Study period		
	15 days	30 days	60 days
Amalgam	2.91 ± 0.58	2.70 ± 0.38	2.86 ± 0.69
IRM	3.90 ± 1.09	2.52 ± 0.63	2.79 ± 0.38
MTA	2.82 ± 1.13	3.01 ± 0.92	3.68 ± 1.19
MTA/CHX	2.21 ± 0.59	2.47 ± 0.36	3.23 ± 0.65
Control	1.96 ± 0.31	2.16 ± 0.34	2.27 ± 0.37

the skin was closed with 3-0 silk suture. The evaluations were made 15, 30, and 60 days after surgical implantation.

In each examination period, 10 animals were sacrificed by administrating high doses of anesthetics. The tubes and surrounding tissues were removed and fixed in buffered 10% formaldehyde solution. Paraffin blocks were prepared after passing through degraded ethyl alcohol and xylol series. Sections of 5 to 6 μm thickness were cut by a microtome and stained with hemotoxylin eosin and examined under a light microscope. The animals were randomly coded to blind the patholog for material identification.

Quantitative evaluations of inflammatory cells were made in five separate areas. The inflammatory reactions were categorized as weak (none or few inflammatory cells ≤25 cells), moderate (>25 cells), and severe (a lot of inflammatory cells not to be counted, giant cells, and granulation tissue). Thickness of fibrous capsules measured five different areas by the digital imaging Nikon E-600 (DS-5M camera and DS-L1 camera control unit) and the mean values were scored.

The statistical analyses of the data were performed by Least Squares Analysis of Variance.

Results

The animals tolerated the surgical procedures well. No apparent adverse events occurred during the observation period of 2 to 8 weeks.

A weak inflammatory response was seen in amalgam, IRM, and MTA mixed with CHX groups on days 15, 30, and 60. Subcutaneous implantation of MTA provoked an initial severe inflammatory response that subsided at the 30 and 60 day study period.

Mean thickness values of fibrous capsule according to the periods are summarized in Table 1. The differences between mean thickness values of fibrous capsule of test materials on 15, 30, and 60 days are shown in Table 2. A clear fibrous capsule was observed beginning from day 15 in all of the groups (Fig. 1).

Discussion

An important characteristic of a root-end filling material is biocompatibility (15), which can be evaluated with subcutaneous implantation tests. The implantation of the materials in subcutaneous connective tissues of small experimental animals in tubes simulates the clinical conditions (16) and provides stabilization of the material in place and standardization of the material-tissue interface (17). Therefore, in this study polyethylene tubes were used. The empty polyethylene tubes, in this study, caused few or no reactions in subcutaneous connective tissues similar to previous studies (18, 19).

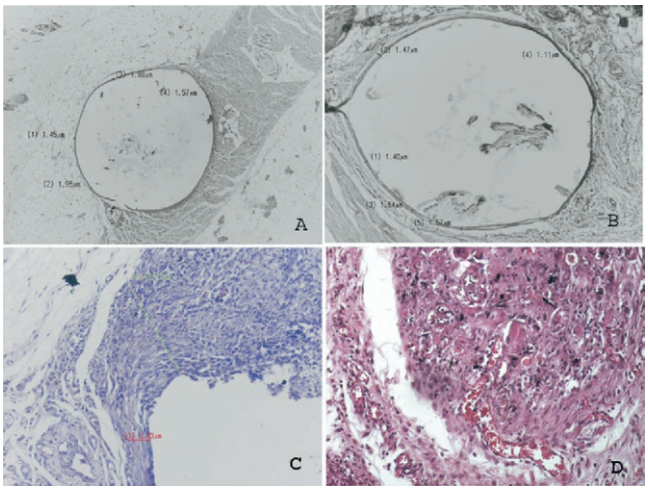

Figure 1. Fibrous capsules of MTA/CHX (*A*) and IRM (*B*) at 15 day showing few inflammatory response (×40 H&E). Fibrous capsule of MTA (*C*) at 15 day (×125 H&E) showing severe inflammatory response (×160 H&E) (*D*).

The results showed that amalgam, IRM, and MTA mixed with CHX materials led to weak inflammatory responses at all periods characterized by the presence of fibrous connective tissue capsule forming around the tube. MTA led to severe inflammatory responses initially, but the response decreased in time with an increasingly thickening fibrous connective tissue capsule forming around the tube. The reactions to MTA in this study were similar to those of Moretton et al. (20) and Yaltirik et al. (17), who found that MTA initially elicited severe reactions that subsided to mostly moderate with time. The initially severe inflammatory response to MTA is most likely multifactorial with high PH, heat generated during setting, and generation of inflammatory cytokines such as interleukin-1 and interleukin-6 contributing to the process (8). When assessing the biocompability of a material, later harmful effects are considered to be more important than its initial effects. Fibrous connective tissue surrounding the materials indicates that they were well tolerated by the tissue (17).

Because IRM contains eugenol, concern has been expressed about possible harmful effects on the periapical tissues (21). Blackman et al.

TABLE 2. The differences between mean thickness values of fibrous capsule of test materials on days 15, 30, and 60

	Amalgam	IRM	MTA	MTA/CHX	Control
Amalgam (day 15)		0.0133*	0.2687	0.0019*	0.0001**
IRM (day 15)	0.0133*		0.0004**	<0.0001**	<0.0001**
MTA (day 15)	0.2687	0.0004**		0.0410	0.0045*
MTA/CHX (day 15)	0.0019*	<.0001**	0.0410		0.4125*
Amalgam (day 30)		0.5424	0.2955	0.4436	0.0728
IRM (day 30)	0.5424		0.0991	0.8749	0.2331
MTA (day 30)	0.2955	0.0991		0.0712	0.0049*
MTA/CHX (day 30)	0.4436	0.8749	0.0712		0.3001
Amalgam (day 60)		0.6393	0.0014*	0.0115*	0.1096
IRM (day 60)	0.6393		0.0003**	0.0029*	0.2560
MTA (day 60)	0.0014*	0.0003**		0.4823	<0.0001**
MTA/CHX (day 60)	0.0115*	0.0029*	0.4823		<0.0001**

*$p < 0.05$.

**$p < 0.001$.

(22) found IRM to be relatively biocompatible and suggested it would be useful for endodontic retrofilling procedures. Silver amalgam has been used as a retrograde filling material and it was biocompatible but it has many disadvantages (3, 19). In this study, amalgam and IRM showed weak inflammatory reactions, characterized by the presence of fibrous tissue interposition.

The addition of the antimicrobial agent CHX to MTA has been evaluated and observed that CHX significantly enhanced the antimicrobial efficacy of MTA (13). However, it was speculated in an in vitro study that MTA/CHX might have an unfavorable effect on the resolution of the periapical periodontitis, and the regeneration of the periodontal connective tissue attachment apparatus (14). Therefore, the potentially beneficial antimicrobial effect of CHX might be accompanied by an increase in the cytotoxicity of the resulting MTA-based material. In the present investigation, subcutaneous implantation of MTA mixed with CHX created weak inflammatory responses at all periods characterized by the presence of a fibrous connective tissue capsule forming around the tube that showed MTA/CHX would be considered to be biocompatible.

Conclusion

Within the limits of this study, amalgam, IRM, MTA, and MTA mixed with CHX materials were surrounded by fibrous connective tissue indicating that they were well tolerated by the tissues. Although MTA/CHX seemed to be biocompatible, further research would be needed before advocating its clinical use.

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