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Original Article

TISSUE REACTIONS AFTER INTRAOSSEOUS IMPLANTATION OF THREE RETROFILLING MATERIALS

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Abstract

Bone tissue reactions to EBA, IRM, and cyanoacrylate cement (*Base Liner*) were studied in the rat mandible using an intraosseous implant method. Osseous cavities (1.4 mm in diameter) were surgically created in the mandibles, and materials were implanted in 60 male Wistar rats. Each specimen was evaluated histologically after 4 and 8 weeks. The development of fibrous connective tissue in direct apposition to the material was observed in the EBA and IRM groups at 4 weeks. A slight degree of macrophage infiltration was seen in the EBA group. After the 8-week observation period, IRM and EBA were frequently separated from the bone cavity by a fibrous connective tissue layer (p<0.01). The *Base Liner* appeared to be in direct apposition to the osseous tissue in several areas (p<0.01). These findings indicate that *Base Liner* reacts favorably with osseous tissue, compared with the EBA and IRM materials tested and seems to be a biocompatible material.

Key words: Retrofilling materials—Tissue reaction—Biological evaluation—
               Endodontic surgery—New materials

INTRODUCTION

Endodontic surgery usually consists of root-end resection, retro-preparation, and retro-filling to create a seal the periapical tissues and root canal system[7,9]. The retrofilling material must be suitable not only in terms of physical characteristics but of biocompatibility, meaning that it should not cause tissue irritation or interfere with healing. Important characteristics of the filling material include: 1. Provides a long-term seal after setting; 2. Does not harm soft tissues around the root end at the time filling; 3. Does not dissolve or disintegrate in body fluids after setting; 4. Is radiopaque on X-ray; 5. Is easy to fill; 6. Has little or no environmental influence at the time of setting; 7. Is sufficiently hard for easy

polishing; 8. Adheres well to dentin. Amalgam has historically been the material of choice. However, it has several disadvantages[12,14]. Many alternatives to amalgam have been suggested[1,2,5,6,10]: gutta-percha, Cavit, polycarboxylate cements, composite resins, glass-ionomer cements, zinc-oxide eugenol, and reinforced zinc-oxide eugenol cements. Currently, the recommended materials for retrofilling are the reinforced zinc-oxide eugenol cements and mineral trioxide aggregate[15,16].

Cyanoacrylate has been used in medicine and dentistry for many years as a lining and for pulp capping. Barkhordar *et al.*[4] examined the possibility of using cyanoacrylate as a retrofilling material and found that teeth retrofilled with cyanoacrylate had the least amount of leakage.

The purpose of this study was to compare the tissue reaction of a new cyanoacrylate cement (*Base Liner*: Nissin, Kyoto, Japan) with that of a reinforced zinc-oxide eugenol cement (S-EBA: Bosworth, Skokie IL. IRM: Dentsply, Milford DE) by intraosseous implantation into the rat mandible.

MATERIALS AND METHODS

Sixty male Wistar rats (body weight: 250–300 g) were examined in this study in accordance with the guidelines for the treatment of experimental animals at Tokyo Dental College. Super EBA (hereinafter referred to as EBA) and IRM, which are reinforced zinc-oxide eugenol cement materials, and *Base Liner* (BL), which is a cyanoacrylate cement, were the experimental materials. Each of the materials was used to fill a teflon tube (inner diameter = 1.0 mm) and left one whole day and night to form a cylindrical specimen approximately 1.0 mm in length. The animals were generally anesthetized by intraperitoneal injection of sodium pentobarbital, and the mandibular region was dissected to expose the surface of the bone. Each of the specimens was inserted to an osseous cavity which had been prepared in the central part of the mandible using a round bar (diameter = 1.2 mm)

and closed with cyanoacrylate for medical use. The experimental groups were divided by the three materials, and the animals were observed for 4 or 8 weeks before being sacrificed by administration of lethal dose of sodium pentobarbital. The mandible was fixed in 10% neutral formaldehyde solution, decalcified with planku ryuchlo, sectioned, stained with hematoxylin-eosin, and then histopathologically evaluated under a light microscope. We cataloged the results of the interface of each retrofilling material into four pathological findings: presence of connective tissue or bone formation, presence of macrophage infiltration into the surrounding connective tissue, and thickness of fibrous connective tissue. The thicknesses of the fibrous connective tissue were recorded at five arbitrary points on the circumference of each specimen by two examiners. The Fisher's exact test was used for statistical analysis of the pooled data.

RESULTS

The histological findings are summarized in Table 1. Six specimens collected at 4 and 8 weeks were sectioned in such a way that the relationship between the experimental materials and the surrounding tissues could not be evaluated adequately. Data from these specimens were excluded from the study.

Histopathological evaluation revealed the following results for each filling material.

1. Super EBA group

Four weeks after implantation, the EBA was encapsulated by fibrous connective tissue (Fig. 1a). The orientation of the fibers comprising the tissue was parallel to the surface of the material, and macrophages were observed within the tissue (Fig. 1b). New bone formation was observed inside the cortical bone.

Eight weeks after implantation, the opening of the cavity was restored with compact cortical bone with an irregularly stratified structure. The material remaining in the cavity was encapsulated by fibrous connective tissue;

Table 1   Presence of fibrous connective tissue and/or bone formation, inflammation, and thickness of fibrous connective tissue adjacent to the implanted materials

Findings \ Material	4 weeks			8 weeks		
	BL (9)	EBA (6)	IRM (9)	BL (8)	EBA (7)	IRM (9)
Interface						
Connective tissue	3	6	9	2	7	9
Bone formation	6	0	0	6	0	0
Inflammation						
Yes	0	1	0	0	1	0
No	9	5	9	8	6	9
Thickness of connective tissues (μ)						
0	6	0	0	6	0	0
<100	2	1	0	2	1	0
≧101	1	5	9	0	6	9

( ): No. of specimens

direct contact between the material and the osteoid tissue was also observed (Fig. 2).

2. *Base Liner* (BL) group

Four weeks after implantation, the filling material was totally encapsulated by fibrous connective tissue consisting of fibers running parallel to the surface of the experimental material (Fig. 3). No inflammation was observed between the fibers. Eight weeks after implantation, the material was totally encapsulated by fibrous connective tissue with a distinct fibrous structure; no direct bone contact was observed (Fig. 4).

3. IRM group

Four weeks after implantation, fibrous connective tissue was observed sporadically around the material (Fig. 5). Eight weeks after implantation, the bone cavity was totally filled with newly formed bone. The portion inside the bone was in direct contact with the bone tissue, and no connective tissue was observed between the material and bone (Fig. 6).

4. Statistical analysis

There was a statistically significant difference in thickness of connective tissues between BL and the other materials (p<0.01). The differences between EBA and IRM were very small and not statistically different.

DISCUSSION

Direct bone contact in the material-bone interface was observed at four and eight weeks after BL implantation and at eight weeks after EBA implantation. It was particularly notable that bone formation was observed all around the material only four weeks after BL implantation. Thus, the filling material implanted in the bone did not appear to interfere with the restoration process of the surgically created bone defect, and, at the same time, generation of the bone was promoted on both sides of the interface. With IRM, no specimens were observed in direct bone contact, and the material was totally encapsulated by fibrous connective tissue.

The two reinforced zinc-oxide eugenol cements (EBA and IRM) were encapsulated by fibrous connective tissue. These materials were demonstrated to be biocompatible, and neither the formation of granulation tissue nor significant inflammation was observed. Furthermore, because surface collapse of the material may be harmful to the tissues around the implant, the stability of the matrix of the hard set material may affect the long-term
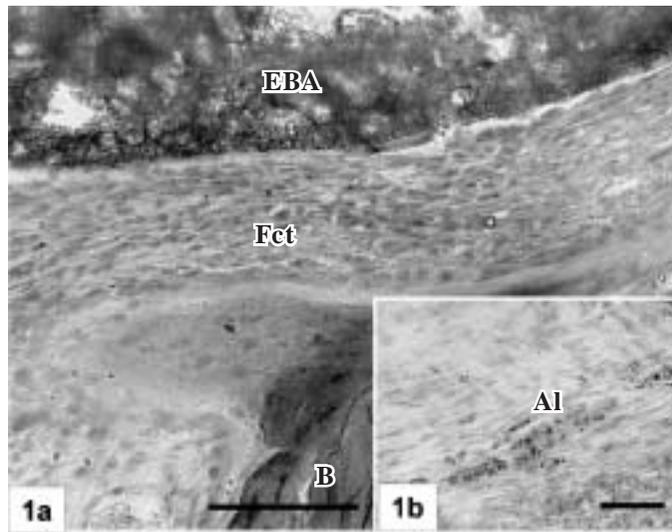
Fig. 1   Four weeks after implantation of EBA   (bar: 10μm)
a. A thin layer of fibrous connective tissue free of inflammatory cells adjacent to EBA is implanted rat mandible.
b. A scattering of particles, which were assumed to be alumina from EBA, was observed.

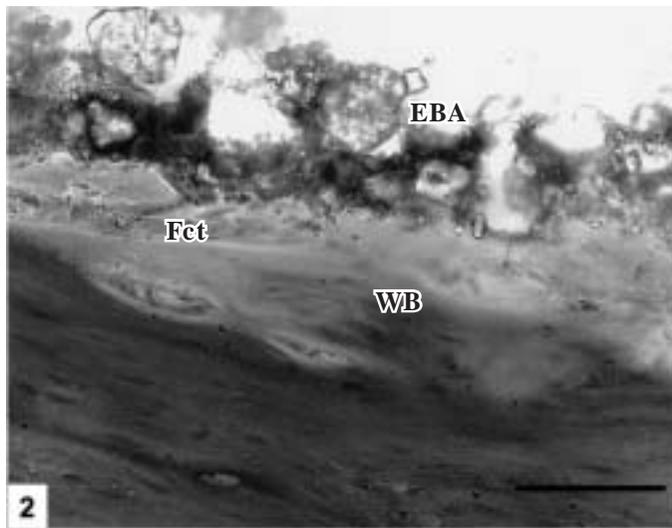material (EBA), fibrous connective tissue (Fct), Bone (B), alumina particles (Al)

Fig. 2   Eight weeks after implantation of EBA   (bar: 10μm)
Direct contact between the material and the osteoid tissue was also observed.
material (EBA), fibrous connective tissue (Fct), woven bone (WB)

sealing of the root canal[2]. After the BL specimen was prepared, lumps were clearly observed, but no dispersion to the surrounding area occurred. Furthermore, IRM and BL were compact and did not exhibit any changes in shape or surface decomposition. Inflammation around filling materials may, result directly from the irritation potential of
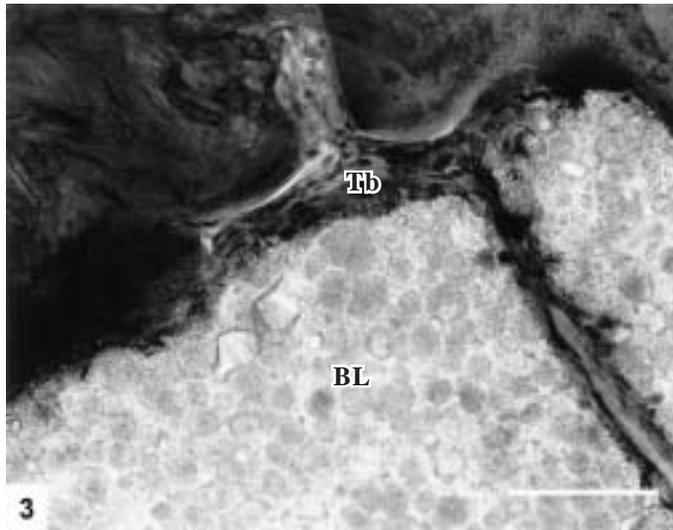
Fig. 3   Four weeks after implantation of *Base Liner* (BL)   (bar: 10μm)

Implanted BL shows a fibrous tissue adjacent to implanted IRM. No inflammation was observed between the fibers.

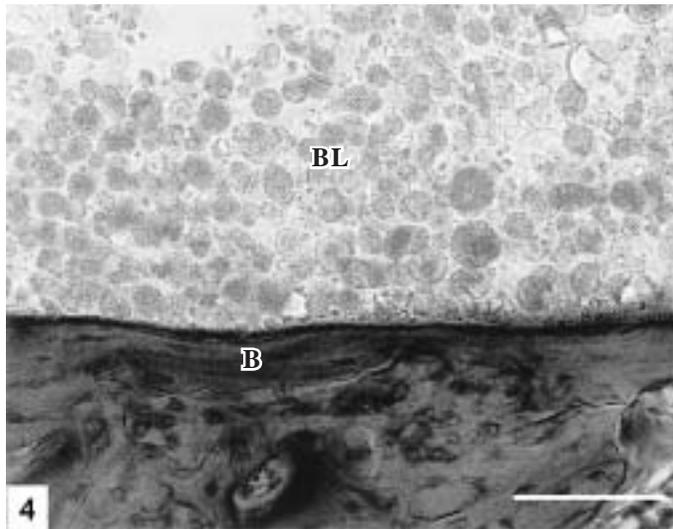material (BL), immature bone trabeculae (Tb)

Fig. 4   Eight weeks after implantation of *Base Liner* (BL)   (bar: 10μm)

BL represents the complete healing with lamellar bone seen adjacent to the material.

material (BL), mature bone (B)

the materials or from foreign body reactions caused by their disintegration[3].

Our results showed that BL had good intraosseous biocompatibility, with short-term minor reactions. At eight weeks, reactions were still slight, with direct contact between bone and BL occurring more frequently than with EBA.
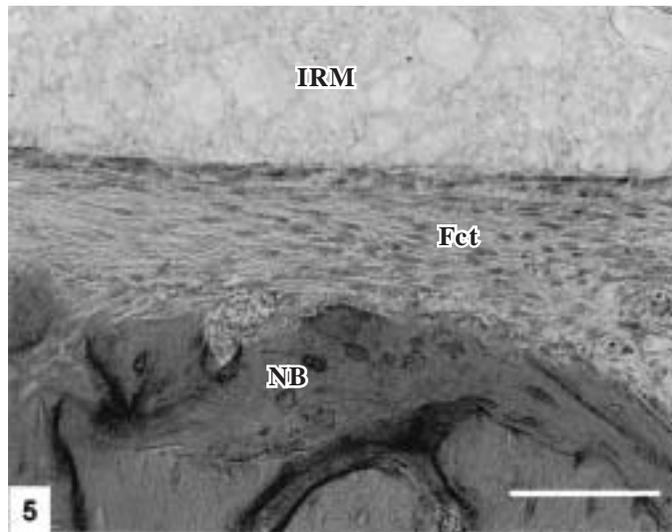
Fig. 5   Four weeks after implantation of IRM   (bar: 10μm)

Implanted IRM shows a fibrous tissue interposition between IRM and new bone. No inflammation was observed between the fibers.

material (IRM), fibrous connective tissue (Fct), newly formed bone (NB)
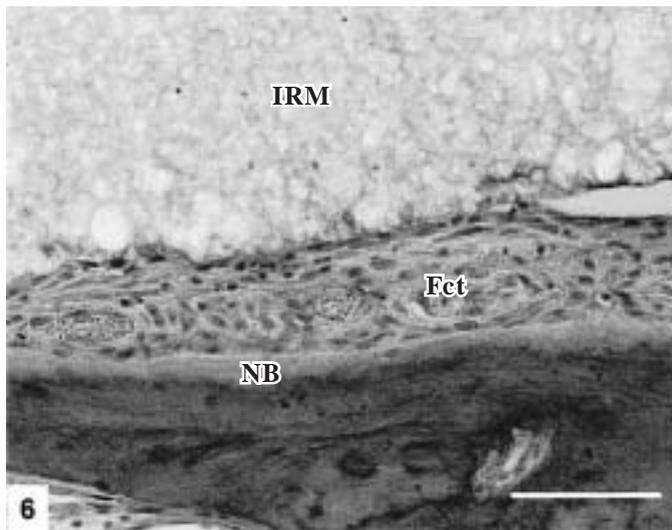

Fig. 6   Eight weeks after implantation of IRM   (bar: 10μm)

The material remaining in the cavity was almost encapsulated by fibrous connective tissue.

material (IRM), fibrous connective tissue (Fct), newly formed bone (NB)

EBA and IRM, although they contain eugenol, did not show any significant tissue irritation potential. Olsen *et al.*[13] have suggested that a significantly shorter response time is related to the release of eugenol, one of the components of EBA. In the present study, no significant inflammation was observed after implantation of any of the materials.

The initial moderate toxicity of EBA may be attributed to the release of eugenol or ethoxybenzoic acid components[11]. In this study, formation of connective and osseous tissue was observed in both ZOE materials within four weeks. Formation of these reparative tissues probably occurred subsequent to a reduction in cytotoxicity of the surface layer. However, some reports have noted that the long-term efficacy of retrofilling is questionable[8]. Of the three materials examined in the present study, BL showed the highest suitability. However, BL, which contains cyanoacrylate, can be hydroxylated or degraded under wet conditions[17]. This may lead to long-term stability problems. These problems may be solved by systematic experimental pathological evaluation and further biological engineering of these materials.

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